JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **35** (Suppl. 3), 2–77, doi: 10.1111/jvp.12004

Plenary lectures, Keynotes and Oral Communications

Session 1: Plenary lectures
Monday 09–07: 13.30–15.30

1.1.PL.
Antibiotic choices and resistance suppression for *Pseudomonas aeruginosa*

G. L. DRUSANO
Institute for Therapeutic Innovation, College of Medicine, University of Florida, FL, USA

Pseudomonas aeruginosa is a serious nosocomial pathogen that is difficult to treat because of the multiplicity of resistance mechanisms that it possesses. Nonetheless, it is possible to identify doses of drug and schedules of administration that counter-select amplification of resistant subpopulations. We have shown this in both murine infection models as well as in our hollow fiber infection model. An important factor regarding resistance suppression that is often overlooked is the variability surrounding effect site penetration in general and into Epithelial Lining Fluid in specific. Because of the variability, attainment of resistance suppression exposure targets in the infection space (e.g. ELF) is often suboptimal. Combination chemotherapy expands spectrum and helps suppress resistance, sometimes by mechanisms that are not directly linked to 'synergistic' drug interaction. For serious *Pseudomonas aeruginosa* infections, combination chemotherapy may be preferred, particularly for resistance suppression.

SUGGESTED READING

1. Drusano, G.L., Bonomo, R.A., Bahniuk, N., Bulitta, J.B., VanScoy, B., DeFigio, H., Fikes, S., Brown, D., Drawz, S.M., Kulawy, R. & Louie, A. (2012) Resistance emergence mechanism and mechanisms of resistance suppression by tobramycin and cefepime for *Pseudomonas aeroginosa*. *Antimicrobial Agents and Chemotherapy*, **56**, 231, DOI 10.1128/AAC.05252-11.
2. Drusano, G.L., Lodise, T.P., Melnick, D., Liu, W., Oliver, A., Mena, A., VanScoy, B. & Louie, A. (2011) Meropenem penetration into epithelial lining fluid in mice and humans and delineation of exposure targets. *Antimicrobial Agents and Chemotherapy*, **55**, 3406–3412.
3. Jumbe, N., Louie, A., Leary, R., Liu, W., Deziel, M.R., Tam, V.H., Bachhawat, R., Freeman, C., Kahn, J.B., Bush, K., Dudley, M.N., Miller, M. H. & Drusano, G.L. (2003) Application of a mathematical model to prevent *in vivo* amplification of antibiotic-resistant bacterial populations during therapy. *The Journal of Clinical Investigation*, **112**, 275–285.

1.2.PL.
Pharmacogenetics in veterinary medicine

K. MEALY
Washington State University, Pullman, USA

Variation in patient response to drug administration is a major challenge for practicing veterinarians, pharmaceutical companies, and regulatory agencies. While there are multiple factors that contribute to individual variability in drug therapy, this review focuses on pharmacogenetics. Pharmacogenetics is the study of genetic variation on drug disposition [pharmacokinetics (PK)] and drug effects [pharmacodynamics (PD)]. Pharmacogenetic variation in PK or PD is a consequence of polymorphisms in drug metabolizing enzymes, drug transporters, or drug receptors. Although veterinary pharmacogenetics lags behind its human counterpart, there are recent discoveries that have altered the way veterinarians treat patients (i.e., drug therapy decisions are made based on a patient's genotype). The next decade will likely witness many clinically significant pharmacogenetic discoveries in veterinary medicine. This brief review will highlight progress in pharmacogenetic research in five veterinary species: *Sheep:* ABCG2 is highly expressed in mammary tissue particularly during lactation. ABCG2 transports nitrofurantoin, danofloxacin and enrofloxacin into milk. Although an ABCG2 polymorphism has not been described in sheep, there are drug interactions that affect secretion of these drugs into milk. *Cattle:* A phylogenetic approach to nomenclature for CYP 1–4 subfamilies was recently described. This will enhance molecular investigations regarding expression and function of cattle CYPs. CYP polymorphisms (i.e., CYP3A28 C994G) have been associated with production parameters in cattle, but their effect on drug disposition has not been investigated. Effects of illicitly used drugs (corticosteroids) on CYP expression have recently been investigated. *Horses:* Comparative studies of CYP enzyme function indicate significant differences in equine CYP activity compared to human CYP activity. CYP-mediated ketamine metabolism has also been investigated from a comparative aspect. *Cats:* Feline ABCG2 has four amino acid differences at conserved sites compared with other mammalian species. Feline ABCG2 exhibits defective ABCG2 transport function relative to that of human ABCG2. These differences are responsible for fluoroquinolone-induced retinal toxicity in cats and may also contribute to that species' sensitivity to acetaminophen. Recently, a polymorphism in the beta-1 adrenergic receptor was identified in cats. This polymorphism alters the amino acid sequence and predicted protein structure but whether or not it produces a pharmacodynamic change is unknown. *Dogs:* Polymorphisms have been described in canine CYP enzymes, including CYP 1A2, CYP 3A12, and CYP 2D15 as well as a suspected polymorphism of CYP 2B11. The most dramatic example involves CYP2D15 and celecoxib metabolism in beagles. Two distinct populations exist with regard to celecoxib clearance, with 45% of beagles characterized by an extensive metabolizer phenotype and 54% by a poor metabolizer phenotype. rCYP2D15d (exon 3 deletion) was unable to metabolize celecoxib. The ABCB1-1Δ mutation primarily affects herding breed dogs and is responsible for life-threatening toxicities when affected dogs receive 'normal' doses of a variety of drugs (antiparasitic, anticancer, antiemetic, and antidiarrheal drugs). Genotyping for ABCB1-1Δ is routinely used

© 2012 The Authors

by veterinarians to direct drug selection and/or drug dosage in canine patients. This truly represents pharmacogenetics in (veterinary) practice.

SUGGESTED READING

1. Antonovic L, Martinez M. Role of the cytochrome P450 enzyme system in veterinary pharmacokinetics: where are we now? Where are we going? Future Med Chem. 2011 3(7):855–79.
2. Mosher CM, Court MH. Comparative and veterinary pharmacogenomics. Handbook of Experimental Pharmacology, 2010 1(199):49–77.
3. Martinez M, et al. The pharmacogenomics of P-glycoprotein and its role in veterinary medicine. Journal of Veterinary Pharmacology and Therapeutics, 2008 31(4);285–300.
4. Mealey KL. ABCG2 transporter: therapeutic and physiologic implications in veterinary species. Journal of Veterinary Pharmacology and Therapeutics, 2012 35(2);105–112.

© 2012 The Authors

Session 2: Antimicrobials
Monday 09-07: 16.00–18.00

2.1.
Pharmacodynamic/pharmacokinetic analysis of marbofloxacin activity in plasma and in intestines of swine

A. FERRAN[1], D. BIBBAL[1], T. PELLET[2], M. LAURENTIE[2], M. GICQUEL-BRUNEAU[2], P. SANDERS[2], M. SCHNEIDER[3], P.-L. TOUTAIN[1] & A. BOUSQUET-MÉLOU[1]
[1]*INRA, UMR1331, Toxalim, ENVT, Toulouse, France;* [2]*Anses, Laboratoire de Fougères, Fougères, France;* [3]*Vetoquinol Research and Development, Lure, France*

INTRODUCTION
Classical PK/PD studies for antimicrobial agents are usually based on the assessment of drug plasma concentrations and MIC. These studies often are very predictive of the activity of the drug at infection sites closely connected to circulating blood, but not on digestive bacteria. Thus, it is quite impossible to assess the activity of a drug regimen initially optimized for targeting systemic infections on digestive pathogens, or on digestive commensal bacteria which should be maximally preserved from drug activity to avoid selection of antimicrobial resistance. The aim of this study was to assess the impact of marbofloxacin at both systemic [lung infection (3DOTS)] and intestinal level after a parenteral administration to swine by an original PK/PD approach including intestinal concentrations and intestinal activity of marbofloxacin.

MATERIALS AND METHODS
Plasma and intestinal marbofloxacin concentrations were assessed at different times after an intramuscular administration of 8 mg kg^{-1} marbofloxacin in swine (n = 26). In parallel, by performing killing curves, we *in vitro* modelled the relationship between marbofloxacin concentrations and its activity assessed by the ability to reduce the initial inoculum by 3 log. Marbofloxacin activity was assessed on low versus high inoculum sizes (10^5 and 10^8 CFU ml^{-1}) of *P. multocida* and *E. coli* and in Mueller-Hinton broth (MHB) versus sterilized intestinal contents.

RESULTS
After an intramuscular administration of marbofloxacin, intestinal exposure of swine, estimated by the AUC, was 3–8-fold higher than plasma exposure. However, by modelling concentration – bactericidal effect relationship, we showed that marbofloxacin activity on *E. coli* was reduced 5–11-fold in the digestive contents compared to MHB probably due to binding to intestinal fluid matrix. We also showed that marbofloxacin activity was approximately the same on similar inoculum size of *P. multocida* and *E. coli* but was 4–7-fold lower on high than on low inoculum sizes.

CONCLUSIONS
In swine, the marbofloxacin dose of 8 mg kg^{-1} used to target respiratory pathogens would be able to eradicate a population of the same size of pathogenic or commensal *E. coli* with similar MIC located in small intestines. However, by targeting a low pathogenic inoculum by early treatments, we showed that the marbofloxacin concentrations eradicating the pathogen would slightly affect the aerobic commensal flora of the ileum and so, limit the selective pressure for resistance in zoonotic bacteria such as *Salmonella*.

2.2.
Chromosome-encoded quinolone resistance in *E. coli* isolated from animals

M. CENGIZ, E. BUYUKCANGAZ, S. SONAL, A. SEN, E. ARSLAN, P. SAHINTURK & B. MAT
Uludag University, Bursa, Turkey

INTRODUCTION
In parallel with extensive consumption of fluoroquinolones (FQ) in veterinary medicine, the rapid dissemination of FQ-resistant *E. coli* isolates has become a major problem in infection control and treatment worldwide. In Gram-negative bacteria, resistance to FQs primarily occurs from chromosomal mutations in the quinolone resistance determining region (QRDR) of the genes encoding the drug target enzymes (DNA gyrase and topoisomerase IV). Moreover, the multiple antibiotic locus (*mar*) controls multidrug resistance (MDR) and the susceptibility of *E. coli* strains to many structurally unrelated antibiotics, including *β*-lactams and aminoglycosides.

MATERIALS AND METHODS
EUCAST and CLSI disc diffusion and minimum inhibitory concentration tests, respectively, were used to determine FQ resistance and molecular methods were used to detect the mutations. Representative 28 *E. coli* isolates were selected in order to evaluate correlation between phenotype and genotype of chromosomal quinolone resistance.

RESULTS AND CONCLUSION
The FQ-resistant *E. coli* isolates presented an alteration in *gyrA* (Ser-83Leu, Asp-87Asn) and *parC* (Ser-80Ile). *E. coli* isolates with mutations in *gyrA* and *parC* were more resistant to enrofloxacin and danofloxacin than *E. coli* isolates that had only one mutation in either *gyrA* or *parC*. The MICs of enrofloxacin for isolates with a single mutation in *gyrA* are lower compared to isolates with two mutations, one in *gyrA* and a second in *parC*, and three mutations, two in *gyrA* and one in *parC*. In this study, *E. coli* isolates with a MIC of ≥ 2 mg l^{-1} had mutation at Ser-80 in *parC* in addition to mutations at Ser-83, Asp-87 or both in *gyrA*. Overall, 22 of 28 (79%) of selected *E. coli* isolates showed point mutation in *marR* (Ser-3Asn, Ala-53Glu, Gly-103Ser, Tyr-137His). All of the 22 *E. coli* isolates had amino acid changes from glycine (Gly) to serin (Ser) and from tyrosine (Tyr) to histidine (His). In addition, among the mutant *E. coli* isolates one had amino acid change from serin (Ser) to asparagine (Asn) and from alanine (Ala) to glutamic acid (Glu). Nine of *E. coli* isolates with a *marR* mutation were susceptible to enrofloxacin and danofloxacin. The observed *marR*

© 2012 The Authors

sequences in this study are seen in strains with wild-type levels of expression of *marA* and represent a variant genotype of *E. coli* without loss of MarR function. Quinolone-resistant *E. coli* isolated from healthy animals and food-producing animals is an important public health issue and can create a high risk for the treatment of infectious diseases at the recommended available dosage regimens.

REFERENCES
1. Hopkins, K.L., Davies, R.H. & Threlfall, E.J. (2005) Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Ag* **25**, 358–373.
2. Oethinger, M., Podglajen, I., Kern, W.V. & Levy, S.B. (1998) Overexpression of the *marA* or *soxS* regulatory gene in clinical topoisomerase mutants of *Escherichia coli*. *Antimicrob Agents Ch* **42**(8), 2089–2094.
3. Weber, M. & Piddock, L.J.V. (2001) Quinolone resistance in *Escherichia coli*. *Vet Res* **32**, 275–284.

2.3.
Plasmid-mediated quinolone resistance in *Escherichia coli* isolated from animals

E. ARSLAN, M. CENGIZ, E. BUYUKCANGAZ, S. SONAL, A. SEN, P. SAHINTURK & B. MAT
Uludag University, Bursa, Turkey

INTRODUCTION
E. coli is an important Gram-negative pathogen of animals and humans causing gastro-intestinal, pulmonary infections and septicaemia. Due to their excellent *in vitro* activity flouroquinolones (FQs) are commonly used in the treatment of animal infections worldwide. Transfer of genetic material which encodes FQ resistance is an important factor for dissemination of FQ resistant *E. coli*. Two major plasmid-mediated quinolone resistance (PMQR) genes are *qnr* and *aac (6′)-Ib-cr*. To date, at least five major groups of *qnrs*, including *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS*, have been identified. These pentapeptide repeat proteins (QnrA, QnrB and QnrS) increase MIC of FQs for *E. coli* by altering the molecular structure of DNA gyrase and protecting it from inhibition by FQs. *aac (6′)-Ib-cr* gene encodes aminoglycoside acetyltransferase enzyme which is capable of modifying FQs and reducing their activity via acetylation.

MATERIALS AND METHODS
EUCAST and CLSI disc diffusion and minimum inhibitory concentration (MIC) tests, respectively, were used to determine FQ resistance and molecular methods were used to detect the PMQR determinants. Two hundred forty-six *E. coli* isolates from infected and healty animals were used in this study.

RESULTS AND CONCLUSION
Only five isolates (2.03%) contained *qnrA* and *qnrS*; *qnrB* was not detected in any of *the E. coli* isolates. The MICs of enrofloxacin and danofloxacin for the *qnr*-containing *E. coli* ranged from 32 to 256 mg l^{-1}. Two *E. coli* isolates from healthy cattle also contained *qnrA* and *qnrS*. Of interest, the MICs of enrofloxacin and danofloxacin for the *E. coli* isolated from healthy cattle were the highest (256 mg l^{-1}) of those in this study. Thirty-six isolates (14.06%) contained *aac (6′)-Ib* gene. The MICs of enrofloxacin and danofloxacin for the *aac (6′)-Ib*-containing *E. coli* ranged from 0.032 to 256 mg l^{-1}. Three isolates contained both *qnrS* and *aac (6′)-Ib* genes. MIC values for those isolates are from 0.032 to 256 mg l^{-1}. The 33 of 47 *E. coli* isolates from the same farm were positive for *aac (6′)-Ib* gene (76.6%). Both *qnr* and *aac (6′)-Ib-cr* genes play an important role in emergence and dissemination of PMQR. PMQR determinants present in *E. coli* isolated from healthy animals and food-producing animals is an important public health concern and may cause risks for the treatment and control of infectious diseases with recommended dosage regimens.

REFERENCES
1. Strahilevitz, J., Jacoby, G., Hooper, D. & Robicsek, A. (2009) Plasmid-mediated quinolone resistance: a multifaceted threat. *Clinical Microbiology Reviews*, **22**, 664–689.
2. Veldman, K., Cavaco, L., Mevius, D., Battisti, A., Franco, A., Bottledoorn, N., Bruneau, M., Guyomard, A., Cerny, T., Escobar, C., Guerra, B., Schroeter, A., Gutierrez, M., Hopkins, K., Myllyniemi, A., Sunde, M., Wasyl, D. & Aarestrup, F. (2011) International collaborative study on the occurance in *Salmonella enterica* and *Escherichia coli* isolated from animals, food and the environment in 13 European countries. *Journal of Antimicrobial Chemotheraphy*, **66**, 1278–1286.

2.4.
PK/PD and clinical relationships of amoxicillin and clavulanic acid administered to weaned pigs for the treatment of *Escherichia coli*

D. G. S. BURCH[1] & U. KLEIN[2]
[1]*Octagon Services Ltd, Old Windsor, UK;* [2]*Novartis Animal Health Inc., Basel, Switzerland*

OBJECTIVES
The objective of the report was to correlate the modelled pharmacokinetics (PK) in the small intestine of amoxicillin (AMX) and clavulanic acid (CA) (Strenzen® – Novartis) administered to pigs via the drinking water with the pharmacodynamics (PD) of the combination against *Escherichia coli* (EC) and compare with the results from an artificial infection study.

MATERIALS AND METHODS
Pharmacokinetics: The concentration in the mid small intestine (jejunum) was estimated using the model of Burch (2007), as no actual data was available. The model is mainly used for in-feed products but can be adapted for any oral daily dosing programme and gives a concentration steady-state (Css) figure that can be expected. A dose of 20 mg AMX kg^{-1} b.w. is equivalent to 400 ppm in feed, The concentration in the colon contents (CCC) is 514 $\mu g\ ml^{-1}$, which is 1.67 times (feed: faeces ratio) less bioavailability of 23%. The concentration in the jejunum is approximately 20.1% of the CCC or 103 $\mu g\ ml^{-1}$. *Pharmacodynamics:* Minimum inhibitory concentration (MIC) data of AMX alone and in combination with CA were determined against 152 EC isolates from Europe (Felmingham, 2009). *Challenge trial:* Recently weaned pigs were challenged orally with an isolate of EC with an MIC of 4.0 $\mu g\ ml^{-1}$ of AMX/CA (Banting, 2003) and after 2 h one group of pigs was treated orally with 10 mg AMX & 2.5 mg CA kg^{-1} b.w. twice daily for 5 days and another remained untreated and there was an uninfected, untreated control. The pigs were monitored for mortality and were scored daily for clinical signs of infection

© 2012 The Authors

including, body temperature, faecal (diarrhoea) score and clinical appearance. After 5 days following treatment, the pigs were necropsied.

RESULTS

Pharmacokinetics: A Css of 103 $\mu g\ ml^{-1}$ of amoxicillin was determined for the small intestine, taking into account the bioavailability of the drug but it did not include any breakdown of the active compound binding to intestinal contents, which was expected to be low. *Pharmacodynamics:* The MIC_{50} was 4.0 $\mu g\ ml^{-1}$, MIC_{90} was 8.0 $\mu g\ ml^{-1}$ and range 1.0–32 $\mu g\ ml^{-1}$. Resistance to AMX alone was 43%, with MICs of $\leq$ 128 $\mu g\ ml^{-1}$ but none to AMX + CA, suggesting the resistance was primarily caused by beta-lactamases. *Challenge trial:* There was a high level of mortality following challenge, prior to the start of treatment. Following treatment there was no more mortality in the treated group, or in the uninfected untreated controls but one further pig in the infected controls. All groups were showing signs of diarrhoea at the start of the trial and the treated pigs responded well with mean clinical signs being equal to the uninfected control by day 5 (see Figure 1).

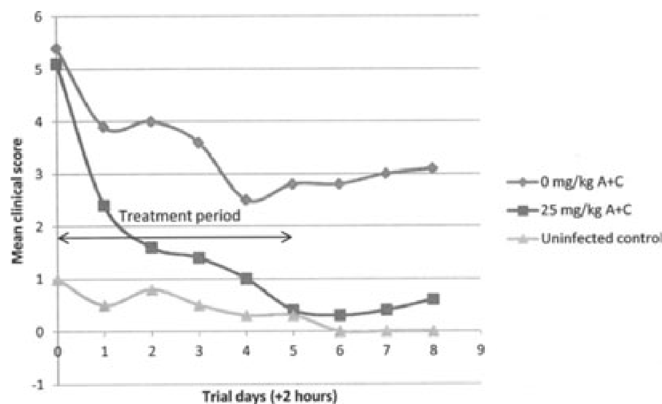


Figure 1. Mean clinical scores of untreated and treated infected pigs and uninfected untreated controls.

CONCLUSIONS

The product appeared to work well in a severe challenge study at 20 + 5 mg kg^{-1} of AMX + CA with an EC isolate with an MIC of 4.0 $\mu g\ ml^{-1}$. A high level of AMX resistance was determined at 43% associated with beta-lactamase production.

REFERENCES

1. Banting, A. (2003) Report to Lek 'Treatment of induced acute colibacillosis in pigs post weaning.
2. Burch, D.G.S. (2007) *Pig Journal* **59**, 91–111.
3. Felmingham, D. (2009) VETPATH II (2004–2006) collection of bacterial pathogens.

2.5.
PK/PD and clinical relationships of amoxicillin and clavulanic acid administered to weaned pigs for the treatment of *Actinobacillus pleuropneuminiae*

D. G. S. BURCH[1] & U. KLEIN[2]
[1]*Octagon Services Ltd, Old Windsor, UK;* [2]*Basel, Novartis Animal Health Inc.*

INTRODUCTION

The objective of the report was to correlate the pharmacokinetics (PK) of amoxicillin (AMX) and clavulanic acid (CA) (Strenzen® – Novartis AH) administered to pigs via the drinking water with the pharmacodynamics (PD) of the combination against *Actinobacillus pleuropneumoniae* (APP) and compare with the results from an artificial infection study.

MATERIALS AND METHODS

Pharmacokinetics: Pigs were given the product via the drinking water at a daily dose of 20 mg AMX and 5 mg CA kg^{-1} bodyweight for 5 consecutive days (Ross, 2004). Blood samples were taken every 3 h during day 1 and 5 to see if there was any accumulation and the plasma was assayed using an HPLC means. *Pharmacodynamics:* Minimum inhibitory concentration (MIC) data of AMX alone and in combination with CA were determined against 129 APP isolates from Europe (Felmingham, 2009). *Challenge trial:* Pigs were challenged intranasally with an isolate of APP with an MIC of 2.0 $\mu g\ ml^{-1}$ of AMX/CA (Banting, 2004). Clinical signs developed rapidly after 2 h and one group of pigs was treated orally with 10 mg AMX & 2.5 mg CA kg^{-1} bwt twice daily for 5 days and the other remained untreated. They were scored daily for clinical signs of infection including, body temperature, respiration rate and clinical appearance. After 7 days following treatment, the pigs were necropsied and the lung lesions scored, weighed and cultured for APP.

RESULTS

Pharmacokinetics: The AMX results were similar on day 1 and 5 with the C_{max} being 0.83 and 1.06 $\mu g\ ml^{-1}$; $AUC_{24\ h}$ 8.09 and 7.43 $\mu g\ h\ ml^{-1}$ and Css ($AUC_{24\ h}$/24) calculated at 0.34 and 0.32 $\mu g\ ml^{-1}$, respectively. Plasma protein binding was 24% (Agerso & Friis, 1998) giving an effective plasma Css of 0.26 and 0.24 $\mu g\ ml^{-1}$, respectively, for day 1 and 5. Pharmacodynamics: The MIC_{50} for AMX + CA was 0.25 $\mu g\ ml^{-1}$, MIC_{90} was 0.5 $\mu g\ ml^{-1}$ and range was 0.06–1.0 $\mu g\ ml^{-1}$. Only 4.7% of isolates were resistant to AMX alone but these were susceptible to AMX + CA. Challenge trial: The pigs responded well to AMX + CA treatment and were clinically normal from day 3 (see Figure 1) and were significantly better than untreated controls by 24 h. Lung scores and weights were significantly better and APP was only isolated from 15.4% of lungs of treated pigs, in comparison with 84.6% of untreated controls.

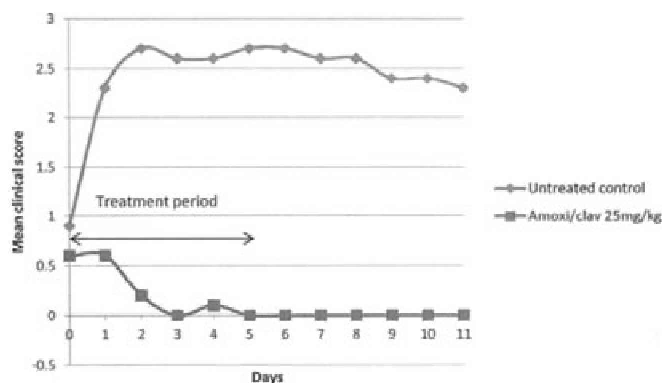


Figure 1. Mean clinical scores of untreated controls and treated pigs challenged with APP.

CONCLUSION

The product appeared to be highly effective clinically, in the treatment of an artificial infection with an APP isolate of 2.0 μg ml^{-1}, well above the MIC$_{90}$ of 0.5 μg ml^{-1}. The average Css result of 0.25 μg ml^{-1} seems to under represent the effective concentration that can be achieved when administered orally.

REFERENCES

1. Agerso, H. & Friis, C. (1998) *Research in Veterinary Science*, **64**, 245–250.
2. Banting, A. (2004) Report to Lek (Novartis) 'Treatment of induced acute Actinobacillus in pigs.
3. Felmingham, D. (2009) VETPATH II (2004–2006) collection of bacterial pathogens.
4. Ross, V. (2004) Report to Lek Pharmacokinetic study in pigs.

2.6.
A new technique to evaluate antimicrobial concentrations in lower respiratory airways in pigs

N. VILLARINO[1], S.P. LESMAN[2], D. GARCIA TAPIA[2], M. LUCAS[2], S. BROWN[2] & T. MARTIN JIMENEZ[1]

[1]*Knoxville, The University of Tennessee, Knoxville, TN, USA;* [2]*Pfizer Animal Health, Kalamazoo, MI, USA*

INTRODUCTION

Bronchial micro-sampling (BMS) (BC-402C; Olympus; Tokyo, Japan) probes have been used experimentally in human setting to harvest bronchial epithelial lining fluid (BELF). These probes would be a valuable tool to obtain fluid samples from the lower respiratory airways of pigs. The objective of this study was to evaluate the use of BMS probes (BC-402C; Olympus; Tokyo, Japan) for quantification of tulathromycin in BELF of pigs.

MATERIAL AND METHODS

Female Duroc × Landrace pigs (n = 3) were dosed with tulathromycin at 2.5 mg kg^{-1} (Draxxin™ Injectable Solution; Pfizer Animal Health, New York, NY, USA). Afterwards, animals were euthanized 24 h after the administration of tulathromycin by captive bolt stunning followed by exsanguination. BELF was gathered from each animal using BMS probes and a fiber-optic flexible endoscope (Olympus, model XP-10). The BMS probe were inserted into the trachea and directed into the middle lobe following the bronchi until resistance was met. Three independent samples were taken from a secondary bronchus at the right and left middle lobes of each experimental animal (6 bronchial samples/animal). The fluid volume gathered from each probe was estimated by weight as previously reported (Kikuchi *et al.*, 2008). Tulathromycin content in samples were determined by LC-MS/MS.

RESULTS

Tulathromycin extraction from BMS probes was > 90%. Tulathromycin concentrations were notably high in the BELF (Mean ± SD, ng ml^{-1}) 2710 ± 885 and 2520 ± 881 for the left and right bronchus, respectively. Consistent drug concentrations were obtained from equivalent anatomical sites from the same animal (P > 0.8).

CONCLUSIONS

The use of BMS probes represents a fast and simple technique for sampling BELF for quantitation of drugs in pigs. This technique does not require terminal sampling and it might allow taking multiple samples from the same the animal. The use of BMS probes for sampling epithelial lining fluid of pig or other species should be evaluated at a larger scales study.

REFERENCES

1. Kikuchi, E., Yamazaki, K., Kikuchi, J., Hasegawa, N., Hashimoto, S., Ishizaka, A. & Nishimura, M. (2008) Pharmacokinetics of clarithromycin in bronchial epithelial lining fluid. *Respirology*, **13**, 221–226.

2.7.
Pharmacokinetics of tulathromycin in healthy and pneumonic swine: lung homogenate

N. VILLARINO[1], S.P. LESMAN[2], A. FIELDER[2], D. GARCIA TAPIA[2], S. COX[2], J. ROBINSON[2], S. BROWN[2] & T. MARTIN JIMENEZ[1]

[1]*The University of Tennessee, Knoxville, Knoxville, TN, USA;* [2]*Pfizer Animal Health, Kalamazoo, MI, USA*

INTRODUCTION

Tulathromycin (Draxxin™) is a triamilide antimicrobial effective in treating and controlling bovine and swine respiratory disease. The objective of the study was to assess the pharmacokinetics of tulathromycin in lung tissue homogenate (LT) and plasma from healthy and lipopolysaccharide (LPS)-challenged pigs.

MATERIALS AND METHODS

Clinically healthy pigs were allocated to two dosing groups of 36 animals each (group 1 and 2). All animals were treated with tulathromycin (2.5 mg kg^{-1}). Animals in group 2 were also challenged intra-tracheally with LPS from *Escherichia coli* (LPS-Ec) 3 h prior to tulathromycin administration. Blood and LT samples were obtained from all animals during 17 days post-tulathromycin administration. For LT, one sample from the middle (ML) and caudal lobes (CL) were taken. The wet to dry ratio was determined to assess changes in the water content in the LT samples. Drug concentrations were determined by UPLC-MS-MS.

RESULTS

The administration of LPS caused macroscopic lesions associated with acute pneumonia. The concentration versus time profile of the drug in the ML in animals challenged with LPS-Ec (group 2) differs (lower concentrations) from the concentration versus time of tulathromycin from animals from group 1 (P < 0.02). These differences are due to a change in the concentration time versus of the drug between 0 and 72 h post-tulathromycin administration (at 6 h (P = 0.03) and 72 h (P < 0.01) post-tulathromycin administration). Overall for the CL there were not statistical differences but there were differences at 24 (P < 0.02) and 72 h (P = 0.04) post-tulathromycin administration. Despite the lower concentrations obtained at some sampling times in LT of LPS-challenged animals, the overall exposure to tulathromycin in both lung lobes was relatively high (AUC$_{0–408}$/AUC$_{0–408}$ plasma was > 78 fold). Also, high drug concentrations persisted for a long period of time, with the LS mean concentration in LT > 300 times the plasma concentration 17 days after drug

© 2012 The Authors

administration. The lower concentration of tulathromycin in LPS-treated animals cannot be attributed to edema in lung tissue since there were no statistical differences in the wet-to-dry ratio between both groups. Lung homogenate samples limit the direct interpretation of the results in terms of antimicrobial effect. However, the rational to measure total concentration in lung homogenate samples is related to the evaluation of impact a pulmonary acute inflammatory response on the accumulation and kinetics of the drug in LT.

CONCLUSIONS

In conclusion, the drug distributed extensively and rapidly into the lungs in both groups. It also persisted in lung tissue at relatively high levels for 17 days post-administration. The concentration versus time profile of the drug in the middle and caudal lobe in animals challenged with LPS-Ec was influenced by the intra-tracheal administration of LPS-Ec. The clinical significance of these findings is unknown.

2.8.
Pharmacokinetics of tulathromycin in healthy and pneumonic swine: intra-airways compartment

N. VILLARINO[1], S.P. LESMAN[2], A. FIELDER[2], D. GARCIA TAPIA[2], S. COX[2], M. LUCAS[2], J. ROBINSON[2], S. BROWN[2] & T. MARTIN JIMENEZ[1]

[1]*The University of Tennessee, Knoxville, Knoxville, TN, USA;* [2]*Pfizer Animal Health, Kalamazoo, MI, USA*

INTRODUCTION

Knowledge of drug concentration in the intra-airway compartments can provide valuable information for treatment. The objective of this study was to assess the pharmacokinetics of tulathromycin in pulmonary and bronchial epithelial lining fluid (PELF and BELF) from pigs.

MATERIALS AND METHODS

Clinically healthy Duroc × Landrace pigs were allocated to two groups of 36 animals each. All animals were treated with tulathromycin (2.5 mg kg^{-1}). Animals in group 2 were also challenged intra-tracheally with lipopolysaccharide from *Escherichia coli* 3 h prior to tulathromycin administration. PELF and BELF were harvested using broncho-alveolar lavage fluid (BALF) and bronchial micro-sampling (BMS) probes (BC-402C; Olympus; Tokyo, Japan), respectively. BMS probes were used with a fiber-optic flexible endoscope (Olympus, model XP-10). Samples were taken for 17 days post-tulathromycin administration. Drug concentrations were determined by UPLC-MS-MS.

RESULTS

The administration of LPS resulted in a large accumulation of inflammatory cells (macrophages and neutrophils) in the intra-airways compartment, in addition to abnormal macroscopic changes in the middle lobe consistent with an acute inflammation. No statistical differences in the concentration of tulathromycin were observed in PELF between groups. The concentration versus time profile in BELF was evaluated only in Group 1. Tulathromycin distributed rapidly and extensively into the airway compartments of both groups. The AUC_{0-408} ratio of PELF-plasma and BELF-plasma was 90 and 223, respectively. The drug penetrated rapidly into the airways, but concentration declined slowly. Also, the drug concentration ratio PELF:plasma and BELF:plasma change in a time and concentration dependent manner following drug administration (ratio range 29.6–271 and 2.69–1615 for PELF:plasma and BELF:plasma, respectively from 0 to 17 days post-tulathromycin administration). This is the first study that describes the intra-pulmonary pharmacokinetics of tulathromycin in pigs both in healthy and pneumonic animals.

CONCLUSIONS

The successful clinical outcome of the drug after a single administration may be explained by the magnitude of drug concentration and the long persistence of the drug in the intra-airway compartments. It is necessary to emphasize that if the BALF technique is not used properly could lead to overestimation of the drug concentration (Kiem & Schentag 2008). In this study, it were minimized those factors that could lead to drug concentration overestimation. Studies are necessary to define if the ELF represents the biophase in pulmonary infections. In conclusion, tulathromycin not only distributed rapidly into intra-airway compartments at relatively high concentrations but also resided in the airway lining fluid for a long time (> 4 days) both in healthy animals and pigs with pulmonary acute inflammatory response.

REFERENCES

1. Kiem, S. & Schentag, J.J. (2008) Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrobial Agents and Chemotherapy*, **52**(1), 24–36.

2.9.
Influence of dexamethasone and gamithromycine on the acute phase response in lipopolysaccharide-challenged calves

E. PLESSERS, A. WATTEYN, H. WYNS, B. PARDON, P. DE BACKER & S. CROUBELS

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

INTRODUCTION

Lipopolysaccharide (LPS) is a potent inducer of the bovine acute phase response and has been widely used in research to provoke acute inflammation. An intravenous challenge with LPS elicits the endogenous synthesis and release of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6). These cytokines initiate fever and stimulate the hepatic production of acute phase proteins, such as Serum Amyloid A (SAA). Regarding the fact that immunomodulating drugs are able to influence this acute phase response, the aim of the present research was to study the potentials of dexamethasone and gamithromycin in a standardized LPS-inflammation model. Dexamethasone was applied as a positive control, due to its major anti-inflammatory effects. The novel azalide gamithromycin on the other hand, was selected since macrolide antibiotics have been reported to exert immunomodulatory effects. Furthermore, the combination of both drugs was studied for possible additive and/or synergistic effects.

MATERIALS AND METHODS

A standardized and reproducible inflammation model was developed by challenging 12 4-week-old calves intravenously

© 2012 The Authors

with a single dose of LPS [*E. coli* serotype O111:B4, 0.5 μg kg^{-1} body weight (BW)]. Three control animals on the other hand received an equivalent volume of 0.9% NaCl. Rectal body temperature was measured and plasma samples were collected at several points in time until 72 h p.a. These samples were analyzed using ELISAs for TNF-α, IL-6 and SAA. As part of the immunomodulation study, 18 different calves were randomly divided in three groups, each group consisting of six calves. The groups received a single bolus of respectively 0.3 mg kg^{-1} BW dexamethasone i.m. (Dexa 0.2%®; Kela), 6 mg kg^{-1} BW gamithromycin s.c. (Zactran®; Merial) and the combination of both drugs. At T_{max} of the drug (time at which the maximum plasma concentration is reached) the LPS-bolus was administered, followed by a similar experimental design as for the inflammation model.

RESULTS AND CONCLUSIONS

In comparison with the results obtained in LPS-administered animals which did not receive any treatment, dexamethasone and the combination of dexamethasone and gamithromycin significantly inhibited the release of TNF-α, IL-6 and SAA after an LPS-challenge. The administration of gamithromycin solely did not affect the cytokine and acute phase protein concentrations. Regarding the course of the body temperature, neither dexamethasone, nor the combination had a major influence, while gamithromycin alone induced a remarkable delay of the maximum body temperature. In other words, these results demonstrate the possible additive effect of a combined administration of an antibiotic with a corticosteroid in the acute phase of a bacterial infection, which could contribute to a better clinical condition of the animal.

© 2012 The Authors

Session 3: Biotransformation and Drug Transporters
Monday 09-07: 16.00–18.00

3.1.

Equine CYP2B6: genomic annotation, heterologous expression and its role in the metabolism of ketamine

A. SCHMITZ[1], L. PETERS[2], S. DEMMEL[2], B. HAASE[3], J. BUTERS[4], W. THORMANN[5], T. LEEB[3] & M. MEVISSEN[2]
[1]*University of Bern, Bern, Switzerland;* [2]*Division Veterinary Pharmacology and Toxicology, Vetsuisse Faculty, Uni Bern, Bern, Switzerland;* [3]*Institute of Genetics, Vetsuisse Faculty, Uni Bern, Bern, Switzerland;* [4]*ZAUM – Center of Allergy and Environment, Helmholtz Zentrum München/TU München, München, Germany;* [5]*Clinical Pharmacology Laboratory, Institute for Infectious Diseases, Uni Bern, Bern, Switzerland*

INTRODUCTION
Cytochrome P450 enzymes (CYPs) are predominant in the phase I metabolism of drugs and other xenobiotics. They have been thoroughly examined in humans and laboratory animals, amongst others due to their involvement in adverse drug reactions. Although the horse is subject to very refined multi-drug treatments today, little is known about equine CYPs. Substrate specificities and inhibitors can differ between orthologous CYPs of different species. Therefore, the characterization of equine CYPs is essential for a better understanding of drug metabolism in horses. We report genomic annotation, cloning and heterologous expression of the equine CYP2B6 and *in vitro* investigations on the metabolism of racemic ketamine by this CYP.

MATERIALS AND METHODS
After computational annotation of the genes of the CYP2B subfamily, the coding sequence (CDS) of equine *CYP2B6* was amplified by RT-PCR from horse liver total RNA. Alignment to human *CYP2B6* revealed an amino acid sequence identity of 77.2%. Comparison to the equine reference sequence (EquCab2.0) displayed three single nucleotide poly-morphisms (SNPs) in the CDS of the equine *CYP2B6*. Minor allele frequencies for these SNPs were determined in a group of 96 Franches-Montagnes horses. CYP2B6-transfected cells were incubated with racemic ketamine in order to characterize metabolites. V_{max} and K_m for ketamine *N*-demethylation to norketamine were calculated and the known inhibitor clopidogrel was used to examine inhibition of ketamine *N*-demethylation in the equine CYP2B6. The recombinant equine CYP2B6 *N*-demethylated racemic ketamine to norketamine in a non-stereoselective way and metabolized norketamine to further metabolites.

RESULTS
Ketamine *N*-demethylation could be effectively inhibited by clopidogrel, a known inhibitor for human CYP2B6. Knowledge of substrate specificity, turnover rates and polymorphisms of individual CYPs provides a basis for avoiding drug-drug interactions, explaining adverse drug reactions, and it will facilitate a more individualized pharmacological therapy in horses in the future.

CONCLUSIONS
The impact of the detected polymorphisms on CYP function can be estimated by computational 3D structural modeling.

3.2.

Genotyping and preliminary phenotyping of cytochrome P450, 2D50, in the horse

C. CORADO, A. YOUNG & H. KNYCH
University of California, Davis, Davis, CA, USA

INTRODUCTION
Human cytochrome P450 2D6 has been well characterized and is known to be responsible for the metabolism of approximately 20–25% of commonly prescribed therapeutic agents. It is widely accepted that CYP2D6 is highly polymorphic, with over 80 mutations identified and proven responsible for widely differing levels of metabolic activity. The use of probe drugs has allowed for categorization into several phenotypes; poor, intermediate, extensive and ultra-rapid (Neafsey *et al.*, 2009). Recently, a homologue to CYP2D6, CYP2D50, has been identified and characterized in the horse, and it has been proposed that this gene may be equally as prone to polymorphism (DiMaio Knych & Stanley, 2008). The results of this study identified polymorphisms in CYP2D50 and suggest a distribution of metabolic phenotypes in the horse.

MATERIALS AND METHODS
DNA sequences for CYP2D50 were obtained from the UCSC Genome Browser. Blood samples were obtained from over 100 horses. PCR reactions were carried out using a long amplification system to obtain the entire 6 kb gene. The gene was cloned and sequenced in pieces using eleven separate primers. Full length sequences were assembled and analyzed for possible SNPS, deletions, or insertions using Vector NTI software. Pharmacokinetic data from a study of the administration of a single dose of the analgesic Tramadol in nine horses was used to calculate metabolic ratios in a manner described previously to phenotype CYP2D6 in humans (Garcia-Quetglas *et al.*, 2007). Sequences for the 2D50 protein were obtained by translating mRNA sequences from GenBank in Vector NTI.

RESULTS
Alignment of the full length sequences of over 100 horses showed a distribution into three distinct haplotypes which may correspond to phenotypes previously described in the human. A number of SNPs were discovered which may interfere with protein function, as determined by SIFT analysis of the protein sequence. Probit analysis of the metabolic ratio of Tramadol/o-desmethyltramadol in nine horses administered an oral dose of Tramadol revealed what may be two or three phenotypes. One horse in particular appeared to be much more efficient at metabolizing Tramadol with respect to production of *O*-desmethyltramadol.

© 2012 The Authors

CONCLUSIONS

Although the sample size was very small, and additional phenotyping studies are necessary to confirm these results, the data seems to support reports of phenotypic distribution similar to humans.

REFERENCES

1. DiMaio Knych, H.K. & Stanley, S.D. (2008) Complementary DNA cloning, functional expression and characterization of a novel cytochrome P450, CYP2D50, from equine liver. *Biochemical Pharmacology*, **76**, 904–911.
2. Garcia-Quetglas, E., Azanza, J.R., Sadaba, B., Munoz, M.J., Gil, I. & Campanero, M.A. (2007) Pharmacokinetics of tramadol enantiomers and their respective phase I metabolites in relation to CYP2D6 phenotype. *Pharmacological Research*, **55**, 122–130.3.
3. Neafsey, P., Ginsburg, G., Hattis, D. & Sonawane, B. (2009) Genetic polymorphism in cytochrome P450 2D6 (CYP2D6): population distribution of CYP2D6 activity. *Journal of Toxicology and Environmental Health, Part B*, **12**(5–6), 334–361.

3.3.
Aryl hydrocarbon receptor-mediated regulation of the bovine breast cancer resistance protein

S. HALWACHS, L. WASSERMANN & W. HONSCHA
Universität Leipzig, Leipzig, Germany

INTRODUCTION

The efflux transporter Breast Cancer Resistance Protein (BCRP/ABCG2) is expressed at the apical membrane of alveolar epithelial cells of the bovine mammary gland and is induced during lactation. Despite its major role in active secretion of drugs into milk including enrofloxacin or carcinogenic substances like aflatoxin B1, no information is as yet available on regulation of BCRP. As the mammary BCRP promotor region exhibits a dioxin responsive element (DRE, EU570105) we have investigated the influence of the environmental pollutant 2,3,7,8-tetrachlordibenzo-*p*-dioxin (TCDD) and the imidazole fungicide prochloraz on functional BCRP activity. Both substances are known to regulate expression of target genes via the nuclear aryl hydrocarbon receptor (AhR, [1]).

MATERIALS AND METHODS

Functional BCRP activity was assessed in primary mammary epithelial cells (PBMEC) obtained at slaughter from lactating Holstein cows using the H33342 accumulation assay. Generally, cells were incubated with 0.1, 1, and 10 nM TCDD or 1, 10, and 100 nM prochloraz over 1, 3 as well as 5 days. Activation of the AhR signalling cascade was confirmed by 7-ethoxyresorufin-?-deethylase (EROD) activity and indirect immunofluorescence. BCRP expression was investigated by quantitative RT-PCR and Western blot analysis. To confirm AhR dependence of carrier gene regulation, a 5′ bovine BCRP reporter construct was generated and promoter activity was assessed using a reporter assay.

RESULTS

Treatment of PBMECs with TCDD or prochloraz significantly induced BCRP efflux activity. This effect was totally abolished in the presence of the specific BCRP inhibitor Ko143. TCDD and prochloraz caused a significant time- and dose-dependent induction of AhR-mediated EROD activity involving translocation of AhR into the nucleus. In further mechanistic studies we demonstrated that induction of BCRP efflux activity by TCDD or prochloraz was due to binding of the activated receptor to DRE motifs in the BCRP promoter. This AhR binding was significantly reduced in the presence of the specific AhR antagonist salicylamide. Induction of AhR finally resulted in a time- and dose-dependent induction of BCRP gene expression and elevated carrier protein levels.

CONCLUSIONS

Altogether, this study shows that BCRP transport activity in the bovine mammary gland is regulated via AhR at the transcriptional level. As BCRP plays a crucial role in active secretion of xenobiotics into milk our results suggest that exposure of dairy cows with food contaminants including dioxins like TCDD or pesticides such as prochloraz increase the risk of relevant drug residues and toxins in milk production. Thus, our data contribute to the understanding of carrier-mediated drug transport into milk and thereby may help to improve the health of suckling animals as well as the protection of the consumer.

REFERENCE

1. Long, M., Laier, P., Vinggaard, A.M., Andersen, H.R., Lynggaard, J., Bonefeld-Jorgensen, E.C. (2003) Effects of currently used pesticides in the AhR-CALUX assay: comparison between the human TV101L and the rat H4IIE cell line. *Toxicology*, **194**, 77–93.

3.4.
Tissue distribution and phenobarbital induction of target SLC- and ABC-transporters in cattle

V. ZANCANELLA[1], M. GIANTIN[2], R. M. LOPPARELLI[2], C. NEBBIA[3] & M. DACASTO[2]
[1]*Università degli Studi di Padova, Legnaro, Padova, Italy;* [2]*Dip Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, Legnaro, Padova, Italy;* [3]*Dipartimento di Patologia Animale, Università degli Studi di Torino, Grugliasco, Torino, Italy*

INTRODUCTION

Phenobarbital (PB) is a known prototypical cytochrome P450 inducer that transcriptionally affect some drug transporters (DTs) in humans and rodents. In cattle, little is known about DTs expression and regulation phenomena, including their response to prototypical enzyme inducers such as PB. In this study, the transcriptional effects of PB on a number of PB-responsive genes in human and rodents were investigated, for the first time, in liver and several extra-hepatic tissues of cattle. Target genes were represented by the solute carrier transporter family 1, member 3 (SLCO1B3), SLCO2B1 and SLC10A1 as well as the ATP-binding cassette transporter B1 (ABCB1), ABCB11, ABCC2 and ABCG2.

MATERIALS AND METHODS

Seven male Friesian cattle (10 months old) were used; four of them received PB by oral gavage (18 mg kg^{-1} body weight day^{-1} for 7 days), while the remaining ones were used as controls. At the slaughterhouse aliquots of liver, duodenum, kidney, lung, testis, adrenal, and muscle were collected,

© 2012 The Authors

immediately snap frozen in liquid nitrogen and stored at −80°C until use. Target genes tissue distribution and PB transcriptional effects were measured by using a quantitative Real Time PCR, and amplification data analyzed with the 2^{-Ct} method (1). According to published literature (2), three internal control genes (ICGs) useful for the normalization of data from several tissues were identified by using the geNormPLUS algorithm (3).

RESULTS
All target DTs were expressed in the liver. Only two out of the seven PB-responsive DTs (SLCO1B3 and SLC10A1) were not constitutively expressed in extra-hepatic tissues. Apart from the liver, the greatest number of quantifiable DTs (SLCO2B1, ABCB1, ABCC2, ABCG2) were noticed in intestine and testis, followed by adrenal gland (SLCO2B1, ABCB1, ABCG2), lung (ABCB1, ABCG2), kidney and skeletal muscle (ABCG2). Phenobarbital administration never altered DTs mRNA levels, except for an increase of hepatic ABCC2 mRNA and a down-regulation of renal ABCG2.

CONCLUSIONS
Present data only partially confirm those obtained in humans and laboratory species, and should be considered a preliminary step for further molecular investigations about species-differences in DTs gene expression, regulation and function.

REFERENCES

1. Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. *Methods*, **25**, 402–408.
2. Zhang, J., Tang, Z., Wang, N., Long, L. & Li, K. (2012) Evaluating a set of reference genes for expression normalization in multiple tissues and skeletal muscle at different development stages in pigs using quantitative real-time polymerase chain reaction. *DNA and Cell Biology*, **31**, 106–113.
3. Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. & Speleman, F. (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, **3**, 34.

3.5.
Interactions of a veterinary tyrosine kinase inhibitor with canine drug transporters

J. A. SCHRICKX[1], M. M. J. M. ZANDVLIET[2], E. TESKE[3] & J. FINK-GREMMELS[4]
[1]*Utrecht University, Utrecht, The Netherlands;* [2]*Utrecht Univesity, Faculty of Veterinary Medicine, Utrecht, The Netherlands;* [3]*Department of Clinical Sciences of Companion Animals, Utrecht, The Netherlands;* [4]*IRAS Veterinary Pharmacology, Pharmacotherapy and Toxicology, Utrecht University, Utrecht, The Netherlands*

INTRODUCTION
The Tyrosine kinase inhibitor (TKI) Masitinib-mesylate (1) has been approved for use in canine patients with non-resectable mastcel tumors. Combinations of TKI's in human medicine with the classical chemotherapeutic agents have been studied for their potential in overcoming multidrug resistance in tumour cells since many TKI's modulate the function of human ABC-transporters and thereby inhibit the efflux of cytotoxic drugs (2). Based on this mechanism we have characterized the *in vitro* inhibitory potential of Masitinib-mesylate on canine ABC-transporters and the antiproliferative effect of the combination of masitinib-mesylate and doxorubicin on canine lymphoma cell-lines.

MATERIALS AND METHODS
The canine lymphoma cell line GL-40 that highly expresses the ABC-transporter P-gp (ABCB1) was incubated with fluorescent substrates and the modulation of efflux and uptake of fluorescent dyes by Masitinib-mesylate was analysed by Fluorescence Associated Cell Sorting (FACS). The antiproliferative effect was assessed with the Cell Counting Kit-8 (Dojindo Molecular Technologies, USA) that contains a soluble tetrazolium salt that is intracellular reduced to a soluble formazan product and quantifiable by light absorbance.

RESULTS
Our data indicate that Masitinib-mesylate inhibits the function of P-gp. Masitinib-mesylate decreased the P-gp mediated efflux of Rh123 at micromolar concentrations and increased the uptake of Rh123 and Calcein-AM by the GL-40 cells. Masitinib-mesylate did not have an effect on the uptake of CFDA. Masitinib-mesylate potentiated the cytotoxic effects of doxorubicin on the GL-40 cells as could be demonstrated by decreases in the IC50 values of doxorubicin.

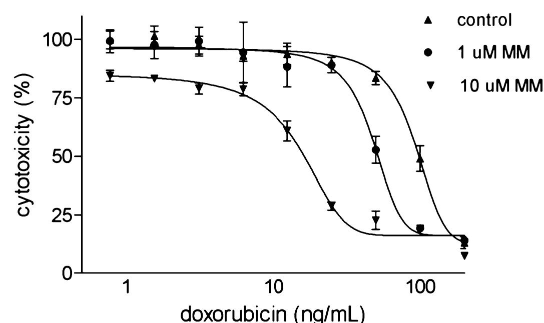


Figure 1. Cytotoxicity of doxorubicin on GL-40 cells and the combination of doxorubicin and Masitinib-mesylate (MM) at the concentrations of 1 and 10 mi.

CONCLUSIONS
The presented results indicate that Masitinib-mesylate inhibits the function of the efflux transporter P-gp and potentiates the cytotoxicity of doxorubicin on the lymphoma cell-line. Further studies using Masitinib-mesylate in combination with classical cytotoxic antitumor agents used in veterinary medicine should be conducted to investigate whether or not the observed effects can be used to overcome multi-drug resistance under clinical conditions (Shukla 2008).

REFERENCES

1. Hahn, K.A., Oglivie, G. & Rusk, T. (2008) Masitinib is safe and effective for the treatment of canine mast cell tumors. *Journal of Veterinary Internal Medicine*, **22**, 1301–1309.
2. Shukla, S., Wu, C.P. & Ambudkar S.V. (2008) Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges. *Expert Opinion on Drug Metabolism and Toxicology*, **4**(2), 205–223.

© 2012 The Authors

3.6.
Cytochromes P450 and ABC-transporters mRNA expression in canine mast cell and mammary tumours

M. GIANTIN[1], A. GRANATO[2], E. M. MORELLO[3], R. M. LOPPARELLI[1], P. BURACCO[3], M. VASCELLARI[2], F. MUTINELLI[2] & M. DACASTO[1]

[1]*Dip Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, Legnaro, Padova, Italy;* [2]*Instituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy;* [3]*Dipartimento di Patologia Animale, Università degli Studi di Torino, Grugliasco, Torino, Italy*

INTRODUCTION

Cytochromes P450 (CYPs) and ABC-transporters (ABCTs) are involved in anticancer drugs and carcinogens biotransformation and multidrug resistance phenomena, respectively. Limited information are actually available about CYPs and ABCTs expression in canine tumours. The aim of this study was to measure mRNA levels of foremost CYPs and ABCTs in canine mast cell and mammary tumours.

MATERIALS AND METHODS

Biopsies of 70 mast cell tumours (MCTs, 32 G1 and 38 G2 + G3) and 33 mammary tumours (MTs, 12 benign and 21 malignant) were collected during surgery. Quantitative Real Time RT-PCR assays for canine CYP1A1, 1A2, 1B1, 2A13, 2A25, 2B11, 2C21, 2D15, 2E1, 3A12, MDR1, MRP1, -3, -5, -6, -7 as well as two internal control genes (ICGs) were set up and validated. Differences between benign and malignant tumours in target gene expression profiles in term of relative quantification were evaluated by using the arithmetic mean of selected ICGs and the 2^{-Ct} method (Livak *et al.*, 2001).

RESULTS

Among the whole set of candidate genes, CYP2A13, 2A25, 2B11 (only in MTs), 2E1 and MRP6 mRNAs were never detected in MCTs and MTs. Most of undifferentiated MCTs showed highest amounts of MRP7 mRNA compared to differentiated ones ($P < 0.05$). In contrast, CYP1B1 and BCRP were significantly inhibited in malignant versus benign MTs ($P < 0.05$).

CONCLUSIONS

Cytochromes P450 mostly involved in anticancer drugs metabolism were shown to be expressed in MCTs and MTs and independently from tumour malignancy. The absence of CYP2B11 mRNA in MTs would confirm the potential usefulness of the gene pro-drug therapy approach envisaged in humans (i.e., for cyclophosphamide: Chen *et al.*, 2007). Likewise to humans, CYP1B1 mRNA amount was higher in pre-neoplastic than malignant MTs (Yang *et al.*, 2008). Finally, ABCTs gene expression profiles might be important, in perspective, to detect multidrug resistance phenomena.

REFERENCES

1. Livak & Schmittgen (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. *Methods*, **25**, 402–408.
2. Chen, C.S., Jounaidi, Y., Su, T. & Waxman, D.J. (2007) Enhancement of intratumoral cyclophosphamide pharmacokinetics and antitumor activity in a P450 2B11-based cancer gene therapy model. *Cancer Gene Therapy*, **14**, 935–944.
3. Yang, X., Solomon, S., Fraser, L.R., Trombino, A.F., Liu, D., Sonenshein, G.E., Hestermann, E.V. & Sherr, D.H. (2008) Constitutive regulation of CYP1B1 by the aryl hydrocarbon receptor (AhR) in pre-malignant and malignant mammary tissue. *Journal of Cell Biochemistry*, **104**, 402–417.

3.7.
The feline hypersensitivity against Acetaminophen toxicity is beyond the glucuronyl transferase deficiency: primary evidence for involvement of MRP2

H. MALEKINEJAD, S. VARASTEH & F. RAGMANI

Urmia University, Urmia, Iran

INTRODUCTION

This study was carried out to clarify any possible links between acetaminophen hepatotoxicity in cats and the expression of the efflux transporter MRP2 in the liver.

MATERIALS AND METHODS

Twelve mixed breed male cats and three mixed breed male dogs were used. The objective for including dogs in the current study was to compare the MRP2 expression in the liver of the two species. The cats were assigned to four groups with three cats in each group. The individual groups were denoted as control, which received normal saline and group L, M and H receiving low (2 mg kg^{-1}), medium (10 mg kg^{-1}) and high (50 mg kg^{-1}) dose levels of acetaminophen, respectively, for 14 days. To evaluate the acetaminophen-induced hepatotoxicity, body weight gain and serum levels of the hepatic enzymes (ALT, ALP) were assessed. Additionally, the acetaminophen-induced oxidative/nitrosative stresses in the liver and histopathological injuries were examined. To determine the lipid peroxidation rate, the malondialdehyde (MDA) content of the liver samples was measured using the thiobarbituric acid (TBA) (1). The carbonyl content of the tissue homogenates was measured by performing the reaction between 2, 4-dinitrophenylhydrazine (DNPH) and protein carbonyls (2). To show any links between acetaminophen hepatotoxicity and MRP2 transporter, the expression level of MRP2 in the liver was determined by RT-PCR.

RESULTS

A dose-dependent and significant ($P < 0.05$) reduction in body weight gain and an increase in the serum levels of ALP and ALT in acetaminophen-treated cats were recorded at the end of experiment. Moreover, a significant ($P < 0.05$) elevation in lipid peroxidation, protein oxidation, and NO content of the liver of acetaminophen-treated animals in comparison with the control group was measured, while a remarkable reduction in the total thiol molecules (TTM) level of the liver in test groups was found. Histopathological examination showed dose-dependent cloudy swelling, bile retention, lipid degeneration, pyknotic nuclei and hepatocellular necrosis in the test groups. Interestingly, we found that unlike to the intact dogs, the expression of MRP2 was not detectable in control cats. Our PCR analyses also revealed that the MRP2 gene was expressed in a dose-dependent fashion at mRNA level in the cats which received the medium and high dose, but not the low dose of acetaminophen.

© 2012 The Authors

CONCLUSIONS

Our data suggest that in addition of the hepatic TTM depletion, lipid and protein oxidation also play role in acetaminophen-induced hepatotoxicity in cats. The dose-dependent increase in the expression of MRP2 in the acetaminophen-treated cats may indicate a compensatory reaction of hepatocytes to efflux the toxic metabolites of acetaminophen.

REFERENCES

1. Niehaus, W.G. Jr & Samuelsson, B. (1968) Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem*, **6**, 126–130.
2. Levine, R.L., Williams, J.A., Stadtman, E.R. *et al.* (1994) Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol*, **233**, 346–357.

3.8. Veterinary pharmacogenetics on dogs: CYP1A2, CYP2C41, MDR1 and others

J. GEYER

Institute of Pharmacology and Toxicology, Giessen, Germany

Pharmacogenetics is the study of the impact of genetic variants on drug pharmacokinetics and pharmacodynamics and has increasingly been recognised in veterinary medicine in the last two decades. This was particularly fostered by the complete genome sequences that became available for many veterinary species. This sequence information can be used to discover novel sequence variants in candidate genes associated with altered drug response. In dogs, prominent examples that affect cytochrome P450 (CYP) mediated drug metabolism include the CYP1A2 1117C>T single nucleotide polymorphism and the CYP2C41 gene deletion polymorphism, that both result in the complete loss of metabolic enzyme function in affected canine individuals. As a further example, a 4-bp gene deletion mutation is present the multidrug resistance *MDR1* gene that normally is coding for the drug efflux carrier P-glycoprotein at the blood-brain barrier. This carrier restricts the entry of many drugs and toxins into the brain and dogs affected by this *MDR1* mutation are prone to neurological toxicity after application of many drugs, particularly from the macrocyclic lactone class. Considering these genetic variants in dogs, certain variabilities in drug pharmacokinetics and drug response can be explained and can even be predicted based on the genotype of the dog. In the future, more clinical studies are needed to correlate some of the already known genetic polymorphisms with a particular clinical phenotype and to elucidate further genetic variants in groups of different drug response.

© 2012 The Authors

Session 4: Contaminants in the Food Chain
Monday 09-07: 16.00–18.00

4.1.K.
Regulation of contaminants in feed and food: from risk assessment to risk management
F. VERSTRAETE
Unit E3: Chemicals, Contaminants and Pesticides, Brussels, Belgium

The EU legislation on contaminants in feed and food fulfils two essential objectives: the protection of animal and public health and removal of internal barriers to trade within the EU. Following the principles and objectives of the General Food Law, feed and food safety legislation shall pursue a high level of animal and human health protection. To achieve this objective legislation shall be based upon risk analysis. Risk assessment shall be based on the available scientific evidence and undertaken in an independent, objective and transparent manner. Risk management shall take into account the results of risk assessment, other factors legitimate to the matter under consideration and the precautionary principle where appropriate. When international standards exist or their completion is imminent, they shall be taken into consideration in the development of any standard at EU level.

4.2.K.
Recent advances in analytical techniques: mycotoxins as an example
S. DE SAEGER
Laboratory of Food Analysis, Ghent University, Ghent, Belgium

This presentation on recent advances in analytical techniques will focus on screening as well as confirmatory techniques. Applications in mycotoxin analysis will be given as an example. For commercial and governmental laboratories the need to use validated methods is ever increasing to ensure quality assurance of results. Besides, the increasing number of sample matrices and analytes calls for more rapid techniques. Two main approaches exist: immunochemical rapid screening tests and multi-analyte LC-MS/MS methods for screening and confirmation. For the first approach so-called 'typical' immunoassays such as lateral flow devices will be discussed. Also biosensors will be presented since these are reusable, can be miniaturized and more commercial developments are to be expected. Antibodies are the most popular and best established affinity tool in diagnostics, however, alternatives such as molecularly imprinted polymers and aptamers become more and more widespread.

SUGGESTED READING
1. De Saeger, S. (Ed). (2011) Determining mycotoxins and mycotoxigenic fungi in food and feed. Woodhead Publishing Series in *Food Science, Technology and Nutrition: Number 203*. 427p.
2. Ruigrok, V.J.B., Levisson, M., Eppink, M.H.M., Smidt, H. & van der Oost, J. (2011) Alternative affinity tools: more attractive than antibodies? *Biochemistry Journal*, **436**, 1–13.
3. Shephard, G.S., Berthiller, F., Burdaspal, P.A., Crews, C., Jonker, M.A., Krska, R., MacDonald, S., Malone, R.J., Maragos, C., Sabino, M., Solfrizzo, M., Van Egmond, H.P. & Whitaker, T.B. (2012) Developments in mycotoxin analysis: an update for 2010–2011. *World Mycotoxin Journal*, **5**, 3–30.

© 2012 The Authors

Session 5: Population Kinetics & Bioequivalence
Tuesday 10-07: 9.00–10.30

5.1.K.

How population PK/PD improves the evaluation and clinical use of veterinary drugs

C. M. LAFFONT

INRA, UMR1331, Toxalim, Research Center in Food Toxicology, Toulouse, France and Université de Toulouse, Ecole Nationale Vétérinaire, Toulouse, France

Inter-individual variability is a key issue in veterinary medicine that needs to be documented to ensure adequate coverage of the population in terms of drug effects and exposure (especially for food animals) and to individualize dosing regimen in fields like oncology (especially for pets). Food safety is a very important aspect with issues around the prevention of antimicrobial resistances susceptible to affect human health and the proper determination of drug withdrawal times. This inter-individual variability can only be evaluated through the setting of well-organized field trials and the use of appropriate data analysis methods like 'population PK/PD' methods. This lecture illustrates with several examples the need and benefit of applying population PK/PD methodology for the evaluation of veterinary drugs.

5.2.K.

Bioequivalence: accomplishments, on-going initiatives, and remaining challenges.

M. N. M. MARTINEZ

US Food and Drug Administration, Center for Veterinary Medicine, Office of New Animal Drug Evaluation, Rockville, MD, USA

Despite the many years of applying bioequivalence concepts, there remain numerous issues that have yet to be resolved. Identifying potential solutions to these complex challenges is particularly difficult in veterinary medicine where we encounter dosage forms multiple animal species approvals, and biological limitations that render it difficult to generate straightforward profile assessments. This presentation will provide a summary of accomplishments to date, points to consider, ongoing initiatives, and areas where additional research is needed.

SUGGESTED READING

1. Bioequivalence. Journal of Veterinary Pharmacology and Therapeutics, **35**(Suppl. 1), 1–44.

Session 6: Recent Advances in Pharmacotherapy (Oncology) Tuesday 10-07: 9.00–10.30

6.1.K.

Adjuvant endocrine therapy in human and canine breast cancer treatment

M. B. M. VAN DUURSEN[1], E. E. J. W. SMEETS[1], S. M. NIJMEIJER[1], P. C. DE JONG[2] & M. VAN DEN BERG[1]
[1]*Endocrine Toxicology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands;* [2]*Department of Internal Medicine, St Antonius Hospital, Nieuwegein, The Netherlands*

Breast cancer is the most common cancer in women worldwide, both in developed and developing countries. In humans, treatment and chemoprevention of (oestrogen receptor-positive) breast cancer is based on drugs that interact with the oestrogen-signalling pathway and are used to deprive the cancer cells of oestrogens. This type of hormonal adjuvant therapy comprise of selective oestrogen receptor modulators (SERMs) that interfere with endogenous oestrogens on the ER in tumour cells or selective oestrogen enzyme modulators (SEERs) that target enzymes involved in the biosynthesis of steroid hormones. SERMs and SEERs target different cells in a breast tumour. Breast tumours are composed of not only tumour epithelial cells, but also other cell types such as fibroblasts, adipocytes, blood vessels and immune and inflammatory cells, which are important in the regulation of epithelial breast cell growth. Over the past years, it has become clear that the interaction between stromal fibroblasts and epithelial breast tumour cells is pivotal in tumour development and progression. The fibroblasts contain the aromatase enzyme and provide the ER+ epithelial tumour cells with estradiol and stimulate proliferation. The tumour cells in turn secrete growth factors and cytokines, such as interleukin-8 (IL-8) and prostaglandin E2 (PGE2) that can induce aromatase expression in the surrounding fibroblasts. Local oestrogen production by aromatase is differentially regulated in disease-free and tumorigenic breast tissue. In disease-free mammary fibroblasts, aromatase expression is low, but in the presence of a tumor expression is elevated 3–4-fold and a promoter switch in aromatase regulation takes place. Similar to cases in humans, ~40–60% of canine mammary cancers are oestrogen receptor (ER)-positive and canine breast cancer are being considered a spontaneous model of human breast cancer (Marinelli *et al.* 2004). This implies that canine tissue might be useful as models for human carcinogenesis. But also, that drugs that are effective in human breast cancer may be useful in the treatment of breast tumours in dogs. In our lab, we have set up a breast cancer model that consists of a co-culture system of MCF-7 cells and primary mammary fibroblasts. We have used both human breast adipose fibroblasts (BAFs), canine BAFs and carcinoma-associated fibroblasts (CAFs) from a canine breast tumour. Using this model, we can study interactions of e.g. dietary compounds with SERMs and SEERs and subsequent effects on breast tumour proliferation. In this lecture, the therapeutic use of aromatase inhibitors in human clinical practice and potential relevance for the veterinary clinic will be addressed. Further, concerns about the adverse interactions with soy-based phytoestrogens and SEERs/SERMs will be discussed.

SUGGESTED READING

1. Marinelli, L. Gabai, G., Wolfswinkel, J. & Mol, J.A. (2004) Mammary steroid metabolizing enzymes in relation to hyperplasia and tumorigenesis in the dog. *The Journal of Steroid Biochemistry and Molecular Biology* **92**(3), 167–173.
2. van Duursen, M. B., Nijmeijer, S.N., de Morree, E.S., de Jong, P.C., & van den Berg, M. (2011) Genistein induces breast cancer-associated aromatase and stimulates estrogen-dependent tumor cell growth in *in vitro* breast cancer model. *Toxicology*, **289**(2–3), 67–73.

6.2.

Population pharmacokinetics of carboplatin in dogs

T. MARTIN-JIMENEZ, N. VILLARINO & A. LEGENDRE
University of Tennessee, Maryville, TN, USA

INTRODUCTION

Precise dosing of anticancer drugs is difficult due to the proximity between effective and toxic doses. Variability among dogs in drug elimination is a major reason why the response to chemotherapy is more closely related to the concentration of drug in blood and tissues than to the administered dose. Carboplatin is an anticancer drug commonly used in the treatment of a variety of solid tumours in dogs. The objective of this study was to explore the pharmacokinetics (PK) of this drug in a population of canine oncology patients, with particular attention to the size and sources of PK variability.

MATERIALS AND METHODS

This prospective study included 82 cases of dogs treated at our Veterinary Teaching Hospital for a variety of malignancies. Doses were administered by a 20 min CRI at 200–300 mg m^{-2} (1–12.5 mg kg^{-1}). Blood samples were collected on heparin-vacutainer tubes at the start of the infusion and then at several times during the following 8 h, averaging five samples per dog. Samples were analysed by HPLC using a method developed and validated in our laboratory (Villarino, 2009). Clinical and demographical covariates were recorded from each case. Data were analysed using Monolix 4.1.2 Software (Lixoft SAS, Orsay, France).

RESULTS

Data were best fit by a monocompartmental model with zero order input and proportional intra- and inter-individual variability. Population parameter and variability estimates of Vd and Cl were 3.4 l (64% CV) and 57 ml min^{-1} (56% CV), respectively. Residual CV was 24.5%. Clearance was correlated with body weight (BW), body surface area (BSA) and serum creatinine, but not with age or gender. Volume was correlated with BW and

© 2012 The Authors

BSA. PK parameter values were more closely correlated with BW than with BSA. When introduced separately in the model as co-variables for Cl and Vd, BW decreased the objective function by 113 points and inter-individual variability by almost 50%, while BSA decreased the objective function by 33 points and inter-individual variability by 17%.

CONCLUSIONS

The results of our study confirmed large inter-individual variability for carboplatin in dogs and a larger improvement in the fit associated to BW than to BSA. The latter is in agreement with previous reports of carboplatin in children and doxorubicin in dogs. These results provide an important argument in favour of the use of BW instead of BSA in the calculation of canine doses, which is further supported by the fact that BW is usually more accurately estimated than BSA. The model obtained in this study allows predicting carboplatin clearance in dogs, and hence the doses required to achieve target AUC values. Further prospective PKPD studies are needed to define appropriate AUC targets.

6.3.
Expression and functionality of TRPV1 receptor in human and canine mammary cancer cells: evaluation of the role of vanilloid system

C. VERCELLI, R. BARBERO, B. CUNIBERTI & G. RE
Faculty of Veterinary Medicine of Turin, Grugliasco-Torino, Italy

INTRODUCTION

Breast cancer is a common leading cause of cancer associated death in women (Authier, 2010). Canine mammary tumours are similar to human ones and they have been proposed as animal models for human breast cancer research. The Transient Receptor Potential Vanilloid receptor 1(TRPV1) has been identified in both species and some authors have speculated on the key role of the TRPV1 in the regulation of cell proliferation.

MATERIALS AND METHODS

The first step of the present study was to verify the presence of TRPV1 receptor on MCF-7 cells performing binding assay with labeled resiniferatoxin (^{3}[H]RTX) and Western Blot using a purified goat polyclonal antibody. Afterwards, displacement (in presence of ^{3}[H]RTX 1.2 M) and functionality assays (in presence of 1 μCi[$^{45}Ca^{2+}$] ml^{-1}) were performed using decreasing concentrations of agonists (Capsaicin, RTX, Anandamide) and antagonists (Capsazepine, Sb-366791, 5-I-RTX) in order to measure the affinity and the efficacy of the different compounds on the TRPV1, respectively. The last part of the study was to compare MCF-7 cells to CF.41 cells (derived from canine mammary adenocarcinoma) during proliferation assays. Experiments were performed by plating 5000 cell per well in 96-well plates and administering the same agonists and antagonists used in the above assays. At different experimental times (24, 48 and 72 h), 20 μl of MTT solution (4 mg ml^{-1} in PBS) was added to each well and after 4 h of incubation the plates were read at λ = 570 nm.

RESULTS

The results of the binding assay showed a B_{max} value of 1492 ± 192 fmol mg^{-1}, K_d value of 0.03 ± 0.004 mM and $r < 0.9$. The images obtained from Western Blot permitted to identify a signal corresponding to TRPV1 receptor (100 kDa). The results of displacement and of functionality studies are shown in Table 1. SB-966791 7.5*10^{-10} > 0.9 95.11. The results of proliferation assays were analysed using Kruskal–Wallis test and Dunn's multiple comparison test ($P < 0.05$) (Graph Pad Prism 4 Software). The statistical analyses allowed to create different graphs (data not shown) indicating dose-response and time-response effects of the tested drugs on MCF-7 and CF.41 cells.

Table 1

		Displacement assay			Functionality assay		
	Drug	Ki (M)	R^2	E_{max} (%)	EC_{50}	R^2	E_{max} (cpm)
Agonist	Anandamide	2.8×10^{-11}	> 0.9	92.01	1.1×10^{-7}	> 0.9	557.0
	Capsaicin	1.5×10^{-11}	> 0.9	102.20	1.5×10^{-7}	> 0.9	478.9
	RTX	1.3×10^{-8}	> 0.9	82.40	2.2×10^{-9}	> 0.9	777.0
Antagonist	5-I-RTX	5.6×10^{-11}	> 0.9	94.97	5.6×10^{-11}	> 0.9	108.3
	Capsazepine	7.9×10^{-11}	> 0.9	93.00	7.9×10^{-11}	> 0.9	104.0
	SB-966791	7.3×10^{-10}	> 0.9	95.11			

CONCLUSIONS

Data obtained by binding assays and by Western Blot confirm the presence of TRPV1 receptors on MCF-7 cells. The results obtained by competition and functionality assays permitted to rank all drugs as follows: – Agonists' affinity: Anandamide > Capsaicin > RTX – Antagonists' affinity: 5-I-RTX > Capsazepine > SB – 366791 – Agonists' efficacy: RTX > Anandamide > Capsaicin – Antagonists' efficacy: 5-I-RTX > Capsazepine The proliferation assays permitted to establish that both partial (Capsaicin and Capsazepine respectively) and high affinity (RTX and 5-I-RTX) agonists and antagonists are able to inhibit MCF-7 cells proliferation (from 50 to 70% at every time point and for all concentrations). The results concerning the CF.41 cells demonstrate that Capsaicin and Capsazepine seem to stimulate the cell proliferation (80% more than control) while RTX and 5-I-RTX seem to inhibit the cell growth (50% less than control).

© 2012 The Authors

Session 7: Receptors and Signal Transduction
Monday 10-07: 9.00–10.30

7.1.K.
G-protein-coupled receptors: Their therapeutic implications and signal transduction

G. ABRAHAM
Faculty of Veterinary, University of Leipzig, Leipzig, Germany

G protein-coupled receptors (GPCRs) constitute the largest family among integral cell membrane receptor proteins involved in signal transmission. GPCRs are involved in the control of every aspect of our behaviour and physiology in man and animals. Examples are receptors for hormones such as calcitonin, luteinizing hormones or neurotransmitters and catecholamines such as noradrenaline, serotonin and dopamine. GPCRs have a common body plan with seven transmembrane helices connected by alternating extracellular and intracellular loops, with the *N* terminus extracellular and the C terminus intracellular. The intracellular loops that connect these helices form the G protein-binding domain. How do GPCRs activate G proteins and cause such specific responses in cells? What are the triggering changes in GPCRs on agonist binding and diseases? How do they fold, and what causes misfolding in so many genetic diseases? This is not surprising as many signaling systems rely on this class of receptors to convert external and internal stimuli to intracellular responses. Binding of specific ligands/drugs, such as hormones, neurotransmitters, chemokines, lipids, and glycoproteins, activates GPCRs by inducing or stabilizing a new conformation in the receptor. Activated receptors (R^*) can then activate heterotrimeric G proteins (composed of a, b, and g subunits) on the inner surface of the cell membrane. Since almost all known physiological processes are regulated by GPCRs, it is easy to appreciate that dysfunctions in these signaling pathways will lead to various pathological states including cardiovascular, allergic and mental disorders, retinal degeneration, cancer and several others. Since the discovery of the first naturally occurring mutation in GPCRs causing human disease, the list for diseases caused by GPCR mutations are expanding. In addition to diseases caused by the dysfunctional mutations in GPCRs, dysfunction in basal activity can also cause diseases. GPCRs represent thus targets for more than half of all active and approved compounds presently used as therapeutic agents and either activate or inactivate them. Here, a constitutive activation of GPCRs and disease states caused by either constitutive activation or loss of constitutive activity and receptor dysfunctions will be presented. Selected examples of human disorders and animal disease models related to GPCR dysfunction and constitutive activation will be related.

7.2.
β-adrenergic receptor-mediated growth and proliferation of equine bronchial fibroblasts

J. FRANKE, B. ABS, I. HOCHHEIM, M. WIECZOREK & G. ABRAHAM
Institute for Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

INTRODUCTION

Abnormal growth and proliferation of airway fibroblasts and the resultant thickening of the airway walls may produce narrowing of airway caliber and thereby contributing to decreased bronchodilation in equine recurrent airway obstruction (RAO). Such airway remodeling as a result of fibrotic processes might be characterized by an exaggerated deposition of extracellular matrix (ECM) components such as collagen and enhanced fibroblast transformation to myofibroblasts characterized by an increased α-smooth muscle actin (α-sma) formation. In the present study, we established equine bronchial fibroblast (EBF) cultures and examined (i) TGF-β-stimulated fibrotic changes by measuring α-sma and collagen content and (ii) the effect of β2-adrenergic agonists and glucocorticoids on EBF growth and proliferation.

MATERIALS AND METHODS

EBFs were isolated from bronchial segments by trypsin digestion and cultured in DMEM. β-adrenergic receptors were identified and classified by radioligand binding studies using [125I]-cyanopindolol in EBFs (Abraham, 2011). Cell proliferation was assessed by quantitative [3H]-thymidine incorporation assay in the presence or absence of different concentrations of β-receptor agonists and antagonists as well as with dexamethasone for 24 h. Moreover, the effect of these drugs on cell transformation rate of TGF-β pre-treated cells was studied. Here, α-sma was determined by immune-staining as a marker.

RESULTS

EBFs express β2-adrenergic receptors. The total receptor number decreased to 75%, 61% and to 39% after treatment with isoproterenol, salbutamol and clenbuterol, respectively, after 24 h. Treatment of EBFs with isoproterenol, salbutamol, clenbuterol as well as dexamethasone reduced significantly the proliferation capacity of these cells in a concentration-dependent manner. These β-adrenergic agonist effects were antagonized by (DL)-propranolol and ICI 118 551, but not by CGP 20712A ($P < 0.001$; Fig. 1). TGF-β markedly enhanced α-sma expression and cells were more spindle-shaped indicating the presence of transformed myofibroblasts. Salbutamol did not markedly alter TGF-β-induced α-sma formation while dexamethasone decreased this protein expression.

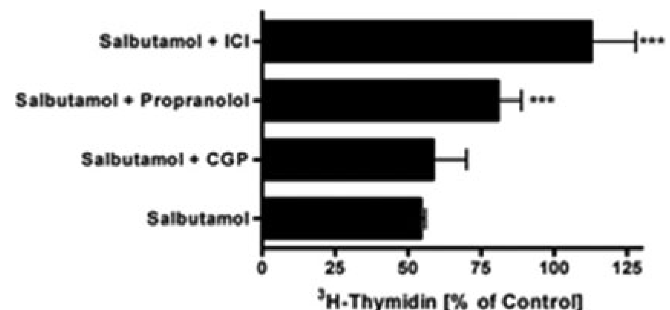


Figure 1. β-agonists and -antagonists effect on EBF proliferation.

© 2012 The Authors

DISCUSSION

These results suggest that stimulation of the β2-adrenergic receptors in equine bronchial fibroblasts and consequent production of cyclic AMP inhibit cell proliferation. Also, mesenchymal transition can be blocked by stimulation of glucocorticoid-receptors. Since RAO can be associated with increased mesenchymal proliferation and transformation, it can be suggested that β2-agonists and glucocorticoids would inhibit the airway remodeling process.

REFERENCES

1. Abraham, G., Shibeshi, W. & Ungemach, F.R. (2011) Identification and characterization of β-adrenergic receptors in isolated primary equine tracheal epithelial cells. *Pulmonary Pharmacology Therapeutics*, **24**(1), 174–181.

7.3.
Contractile effect of endothelin-1 in the isolated bovine digital vein

H. ZERPA, S. FLORES, J. ROJAS, S. COMERMA-STEFFENSEN, A. RODRIGUEZ, D. ARRIETA & A. RUIZ
Central University of Venezuela, Maracay, Venezuela

INTRODUCTION

Laminitis predisposes the bovine digits to develop other pathologies resulting in lameness. The equine digital vasculature has been widely studied; however, limited research has been conducted in other ungulates. Endothelin-1 has been proposed as a mediator of digital vasoconstriction in equine laminitis. The aim was to investigate the contractile response of isolated bovine digital veins (BDV) to endothelin-1 and to characterize the subtype of endothelin-receptor involved.

MATERIALS AND METHODS

BDV were obtained from animals killed in a local abattoir. Isometric tension responses of endothelium-intact and -denuded BDV rings to endothelin-1 were studied (1). Pairs of BDV rings ($n = 7$–8) from the same crossbred bull (Bos indicus) were studied simultaneously. Cumulative concentration response curves (CRC) to endothelin-1 (0.01–0.1 μM) were constructed at 37°C. The maximum contraction ($n = 8$) to a single concentration of endothelin-1 (0.01 μM) was evaluated in endothelium-denuded BDV in the presence and absence of the selective ETA antagonist (BQ-123 (cyclo(D-Trp-D-Asp-Pro-D-Val-Leu): 0.1 μM), selective ETB antagonist (BQ-788 (*N*-[(cis-2,6-Dimethyl-1-piperidinyl) carbonyl]-4-methyl-L-leucyl-1-(methoxycarbonyl)-D-tryptophyl-D-norleucine sodium salt): 1 μM) or the combination of both. Data are presented as the increase in tension expressed as a percentage of the maximal response to a depolarizing Krebs solution (DKS; 118 mM KCl). CRC were fitted using Graphpad Prism 5.01. Statistical differences between treatments were evaluated by paired *t*-test and significance was accepted at $P \le 0.05$.

RESULTS

The presence of the endothelium did not change the efficacy (E_{max} [% DKS]; endothelium-intact: 115.8 ± 21.7 versus endothelium-denuded: 110.6 ± 17.8) or the potency [pD2 (−log EC50); endothelium-intact: 7.6 (7.5–7.70 versus endothelium-denuded: 7.5 (7.3–7.8)] of endothelin-1. The maximal contraction elicited by endothelin-1 (0.01 μM) was significantly ($P < 0.05$) reduced by the selective ETA receptor antagonist (% DKS; control: 84.2 ± 10.8 versus BQ-123: 56.9 ± 7.2). However, the selective ETB receptor antagonist failed to reduce the endothelin-1 effect (% DKS; control: 84.2 ± 10.8 versus BQ-788: 99.5 ± 10.6). The combination of both antagonists caused a significant ($P < 0.05$) reduction of the endothelin-1 response (% DKS; control: 84.2 ± 10.8 versus BQ-123 + BQ-788: 58.8 ± 10.6). The effect of the combination of antagonists was similar to the effect of BQ-123 on its own.

CONCLUSIONS

The potency of endothelin-1 in BDV is similar to that reported in equine digital veins (2-3) The subtype ETA is the main receptor mediating endothelin-1 contraction in the BDV, as has been published in equine tissues and in other vascular beds of ruminants (2-3-4). The efficacy and potency of endothelin-1 in the BDV suggests that this peptide could contribute to digital venoconstriction, as has been hypothesized during bovine laminitis (5). Whether the contraction evoked by endothelin-1 in the BDV could be modified by the gender or the metabolic condition of the animal, requires further analysis. Endothelin-1 caused a potent endothelium-independent contraction in BDV. The ETA receptor is the main functional receptor involved.

REFERENCES

1. Risso *et al.* (2012) *Res Vet Science*, **92**, 299–305.
2. Katz *et al.* (2003) *Am J Vet Res*, **64**, 1438–1443.
3. Stokes *et al.* (2006) *Can J Vet Res*, **70**, 197–205.
4. Lohsiriwat *et al.* (2011) *Pharmacol Res*, **63**, 490–495.
5. Christmann *et al.* (2002) 12th Int. Symp. Bovine Lammeness in Ruminants. 165–166.

7.4.
5-HT-mediated vasoconstriction in bovine digital vein

S. PUNZI[1], M. Y. MALLEM[2], J.-C. DESFONTIS[2] & C. BELLOLI[1]
[1]*Faculty of Veterinary Medicine, University of Bari, Valenzano, Italy;* [2]*LUNAM Université, Oniris, Nantes, France*

INTRODUCTION

A substantial contribution of 5-hydroxytryptamine (5-HT) in the contractile response of bovine digital artery (BDA), as well as short- and longer-term changes in basal reactivity to the amine induced by endothelium removal, have been described in recent work (1). Vasomotor reactivity to 5-HT was investigated in bovine digital vein (BDV) in order to evaluate possible differential control by this amine in the two sides of vascular bed.

MATERIALS AND METHODS

BDVs were collected from healthy adult mixed-breed animals of both sexes slaughtered at a local abattoir. Vessel rings were prepared for isometric tension recording as previously described (2). Responses to cumulative concentrations of 5-HT (10^{-10}–10^{-4} M) were recorded in fresh vessels with (Fe^{+}) or without (Fe^{-}) endothelium and overnight-incubated vessels with endothelium removed before ($Ie^{-}b$) or after ($Ie^{-}a$) incubation. E_{max} (g tension per g tissue wet weight) and pD_2 (−log EC_{50}) best fit values were derived from cumulative concentration-response

curves by a computerized non-linear regression procedure (GraphPad Prism 5.0). Statistical comparisons were performed by a Student *t*-test.

RESULTS

Results are presented in Table 1. No changes in vascular reactivity to 5-HT were recorded in vessels challenged immediately after the endothelium removal. A significant increase of 5-HT efficacy and potency was recorded in Ie⁻b vessels as compared to Ie⁻a.

Table 1 Efficacy (E_{max}) and Potency (pD2) of 5-HT in bovine digital veins. Mean ± SEM of (n) subjects.

	Fe+	Fe−	Ie⁻a	Ie⁻b
E_{max}	180.47 ± 26.67 (10)	194.56 ± 23.71 (14)	13.09 ± 19.35[a] (11)	441.37 ± 63.03[a] (14)
pD2	6.99 ± 0.10 (10)	7.12 ± 0.10 (14)	6.98 ± 0.10[b] (11)	7.34 ± 0.09[b] (14)

Ie⁻b versus Ie⁻a: [a]$P < 0.001$; [b]$P < 0.01$.

CONCLUSIONS

Three main differences in BDA and BDV vascular reactivity to 5-HT were observed. (i) 5-HT induces more effective and potent contractile effects in BDV than in BDA; (ii) the endothelium removal induces short-term increased reactivity to 5-HT in BDA but not in BDV suggesting different distribution of 5-HT endothelial receptors; (iii) significant but opposite long-term changes in contractile reactivity to 5-HT were recorded in Ie-b BDV (hyper-reactivity) and BDA (hypo-reactivity). This last finding suggests that different vasomotor pathways in smooth muscle layer are triggered by endothelium damage in BDV compared to BDA and the release of vasoconstrictive mediators resulting from endothelium damage may support a relevant role of digital venoconstriction in the pathophysiology of bovine laminitis. Finally, the disclosed differential arterial/venous vasomotor control of 5-HT may contribute to the physiological haemodynamic regulation of the bovine foot as well as to the establishment of poor blood flow leading to laminitis.

REFERENCES

1. Zizzadoro, C., Caruso, M., Punzi, S., Ormas, P., Crescenzo, G., Zongoli, F. & Belloli, C. (2012) Reactivity of isolated equine and bovine digital arteries to vasoactive agents: a comparative study with relevance to laminitis. *Research in Veterinary Science* (under revision).
2. Mallem, M.Y., Gogny, M., Gautier, F., Bucas, V. & Desfontis, J.-C. (2003) Evaluation of beta3-adrenoceptor-mediated relaxation in intact and endotoxin-treated equine digital veins. *American Journal Veterinary Research*, **64**, 708–714.

7.5.
Increased Rho-kinase signaling in endothelium damaged bovine digital vein

S. PUNZI[1], J.-C. DESFONTES[2], C. BELLOLI[1] & M. Y. MALLEM[2]
[1]*Faculty of Veterinary Medicine, University of Bari, Valenzano, Italy;* [2]*LUNAM Université, Oniris, Nantes, France*

INTRODUCTION

In a previous study long-term developing selective augmentation of bovine digital vein (BDV) smooth muscle (SM) reactivity to 5-hydroxytryptamine (5-HT) was observed after endothelium removal (1). Among different intracellular mechanisms involved in the control of the myogenic properties of the vascular SM tone, Rho-kinase pathway is considered a key process in G protein-mediated Ca^{2+} sensitization for SM contraction (2). Based on these assumptions, the possible involvement of Rho-kinase signaling pathway in the enhanced functional response of BDV-SM to 5-HT was investigated.

MATERIALS AND METHODS

BDVs were collected from healthy adult mixed-breed animals of both sexes, slaughtered at a local abattoir. Vessel rings were prepared for isometric tension recording as previously described (3). Responses to cumulative concentration of 5-HT (10^{-10}–10^{-4} M) in fresh (F) or overnight-incubated (I) (DMEM 30°C, 5% CO_2) pairs (control and treated) of vascular rings from the same endothelium denuded BDV, were studied simultaneously. Treated samples were assayed after 30 min incubation with the selective Rho-kinase inhibitor, fasudil (1 μM). E_{max} (g tension per g tissue wet weight) and pD_2 (−log EC_{50}) best fit values were derived from cumulative concentration-response curves by a computerized non-linear regression procedure (GraphPad Prism 5.0). Statistical comparisons were performed by two-way ANOVA and Student's t-test in a top down fashion.

RESULTS

As shown in Figure 1, incubated BDVs exhibited significantly enhanced contractile reactivity to 5-HT as compared to the fresh ones ($F = 153.6 \pm 64.75$; $I = 344.94 \pm 53.21$ g tension per g tissue wet weight – $P < 0.01$). Fasudil suppressed the augmented BDV-SM reactivity to 5-HT in incubated samples (210.24 ± 27.77 g tension per g tissue wet weight – $P < 0.01$) but did not modify the contractility of fresh samples. Similar changes were evident for pD_2 but these did not reach statistical significance.

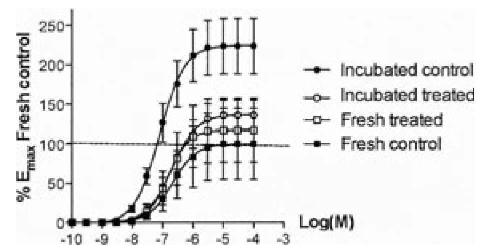


Figure 1. Effect of Fasudil on contractile response of fresh or incubated endothelium denuded bovine digital veins to 5-HT. Mean ± SEM, $n = 8$.

CONCLUSIONS

The results strongly suggest that the expression of Rho-kinase could be up-regulated in BDV-SM after endothelium damage thus shifting the vascular reactivity towards higher degree of vasoconstriction. The mechanisms to which endothelium controls the Rho-kinase function and/or expression in vascular SM remains to be established. However, due to the possible endothelium damage occurring in digital vessels of laminitic cows, the results here described offer some insight in the mechanisms underlying the hemodynamic disturbances observed during laminitis and suggest novel therapeutic approaches.

© 2012 The Authors

REFERENCES

1. Punzi, S., Mallem, M.Y., Desfontis, J.-C. & Belloli, C. (2012) 5-HT-mediated Vasoconstriction in Bovine Digital Vein. *Proceeding of 12th EAVPT Congress* – July 8–12, 2012.
2. de Godoy, M.A.F & Rattan, S. (2011) Role of rho kinase in the functional and dysfunctional tonic smooth muscles. *Trends in Pharmacological Sciences*, **32**(7), 384–393.
3. Mallem, M.Y., Gogny, M., Gautier, F., Bucas, V. & Desfontis, J.-C. (2003) Evaluation of beta3-adrenoceptor-mediated relaxation in intact and endotoxin-treated equine digital veins. *American Journal of Veterinary Research*, **64**, 708–714.

7.6.

Seasonal variation in maintenance of agonist-induced tone in isolated equine digital arteries under hypoxic or normoxic conditions *in vitro*

K. BORER[1], S. BAILEY[2], P. HARRIS[3] & J. ELLIOTT[1]

[1]*Royal Veterinary College, Hatfield, UK;* [2]*Melbourne, University of Melbourne;* [3]*Waltham on the Wolds, Waltham Centre for Pet Nutrition, UK*

INTRODUCTION

The underlying pathophysiological triggers for equine acute laminitis are unknown, although digital vasoconstriction, ischaemia, hypoxia and reperfusion injury may be involved. Pasture-associated laminitis has a seasonal incidence, peaking in spring and summer, associated with sunshine hours and pasture carbohydrate content. Direct seasonal influences on digital vessels have not been previously investigated. This study assessed seasonal variation in maintenance of phenylephrine (PHE)-induced tone in isolated equine digital arteries (EDAs) under hypoxic or normoxic conditions. The null hypothesis was that there would be no seasonal variation in maintenance of tone.

MATERIALS AND METHODS

During autumn, winter and spring, EDAs harvested from the hindlimb pastern of normal horses (n = 6–9 for each season) euthanized for non-research purposes were studied acutely (up to 3 h) under either normoxic (95% oxygen, 5% CO_2) or hypoxic (95% nitrogen, 5% CO_2) conditions in organ baths. After assessing viability, PHE (10^{-6} M) was added to contract vessels, following which EDAs were observed for up to 2 h at which point they were discarded. Percentage contraction or relaxation from the initial plateau observed was calculated. Statistical comparisons using Wilcoxon's signed rank test were performed 1 h after reaching plateau, to assess percentage contraction or relaxation using zero (0% change) to represent maintenance of tone. Percentage contraction or relaxation was compared between different treatments and seasons using a Kruskal–Wallis test with Dunn's *post-hoc* test. Significance was set at $P < 0.05$. Results are presented as median (inter-quartile range).

RESULTS

Normoxic EDAs in all seasons maintained PHE-induced tone (no significant difference from 0% change). In autumn, hypoxic EDAs relaxed to 59% (44–77%) below plateau (significantly less than zero) whereas in winter, hypoxic vessels only relaxed to 18% (0–28%) of plateau (no significant difference from zero). In spring, hypoxic vessels contracted to 65% (20–192%) above plateau (significantly greater than zero). In hypoxic EDAs, percentage change from plateau in spring was significantly greater than in autumn and winter.

CONCLUSION

EDAs under normoxic conditions were able to maintain PHE-induced tone; this did not vary seasonally. In hypoxic vessels, relaxation of PHE-induced tone occurred in autumn, compared to further contraction in spring and no change from plateau in winter. Seasonal variation in maintenance of agonist-induced tone under hypoxic conditions may influence the seasonality of laminitis in horses and ponies which have been exposed to digital hypoxia.

© 2012 The Authors

Workshops: Tuesday 10-07: 11.00–12.30

Workshop 1: Antibiotic Dose Regimens

Coordinator: P.-L. Toutain

W-1.1.
Antibiotic dosage regimen for a sustainable use of antibiotics in veterinary medicine

P.-L. TOUTAIN
Ecole Nationale Vétérinaire, Université de Toulouse, Toulouse, France

The sustainable use of antibiotic drugs in veterinary medicine necessitates optimizing dosage regimens to minimize the development of resistance not only among the target pathogens (an animal health issue), but also to prevent or minimize the impact of antibiotic resistance on non-target bacteria including zoonotic pathogens (*Salmonella, Campylobacter, E. coli*, etc), on animal commensal flora (mainly gut flora) and also on environmental flora. The selection of the most appropriate antibiotic in terms of pharmacokinetics and pharmacodynamics, the value and limits of susceptibility testing in the selection of an antibiotic, routes of administration (general or local), early or late treatments, low or high doses, duration of treatment, etc will be briefly presented to fuel discussion. In addition, the urgent need for pharmaceutical innovation i.e. antibiotics that fit public health expectations, will be addressed. After a keynote lecture aiming at highlighting these different critical items, the exchange format of this workshop will be via a series of questions with which the speaker will challenge the participants for answers and to elicit discussion.

© 2012 The Authors

Workshop 2: Clinical Pharmacology Feline Practice

Coordinators: L. Reeve-Johnson & O. Vainio

W-2.1.

The medical consultation: comparing emphasis on history taking and physical examination in human and veterinary therapeutics

L. G. REEVE-JOHNSON

Goyd Project Solutions/Pacific Animal Consulting and Agribusiness, Brisbane, Australia

INTRODUCTION

There are fundamental differences in the way initial diagnosis are made by the primary care general practitioner in veterinary or human medical professions.

MATERIALS AND METHODS

A linear scoring method based upon direct observation of medical and veterinary first opinion consultations was implemented in Australia and UK. Scores were allocated in 10 percentile ranges according to the extent to which the practitioner relied upon communication or physical examination to initially arrive at a diagnosis that was subsequently shown to be correct. The study involved 25 medical general practitioners and 50 veterinarians in 500 primary care consultations. Eight categories of medical problem were assessed over a 2 year period.

RESULTS

The most obvious differences were the greater extent to which medical practitioners relied upon oral communication to establish a correct diagnosis (Figure 1) and that veterinarians placed far greater emphasis on physical examinations to arrive at correct diagnoses (Figure 2).

Figure 1: The extent to which oral communication led to correct diagnosis

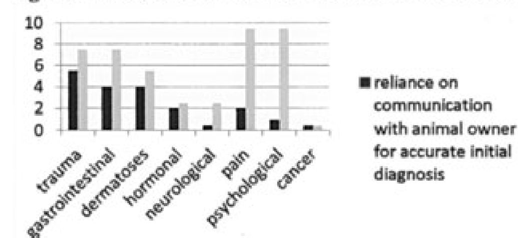
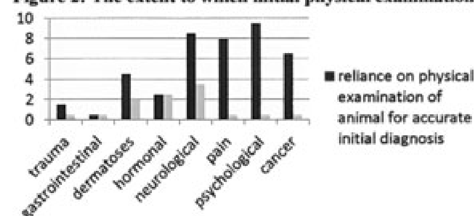


Figure 2: The extent to which initial physical examination led to correct diagnosis



CONCLUSION

Clinical history provided to veterinarians is a collateral account. The emphasis placed upon owner observation was consistently less than that placed upon history elicited by medical practitioners, even when taken from a third party. By contrast, veterinary practitioners relied to a far greater extent on physical examination and were more confident in basing diagnosis upon examination findings.

REFERENCES

1. Kumar, P. & Clark, M. (2011) *Clinical Medicine*, 7th edn. Elsevier.
2. Talley, N.J. & O'Connor, S. (2010) *Clinical Examination*, 6th edn. Churchill Livingstone.
3. Wagner, M.M. *et al.* (1989) The diagnostic importance of the history and physical examination as determined by the use of a medical decision support system. National Library of Medicine.

W-2.2.

Difficulties in oral medication of pet cats in Finland

M. SAVOLAINEN, S. K. RÄNTILÄ, M. RAEKALLIO, O. M. VAINIO, M. SIVEN, S. AIRAKSINEN & A. M. JUPPO

University of Helsinki, Helsinki, Finland

INTRODUCTION

The purpose of this study was to find out what problems cat owners encounter when medicating their cats with orally administered drugs at home. Similar studies have not previously been done, and only a few studies assessed the palatability and administration of a drug in cats, when determining drug efficacy (1, 2). We hypothesized, that a marked number of owners have difficulties in giving drugs to their cats.

MATERIALS AND METHODS

The study was carried out as an open Internet questionnaire survey. The survey was addressed to cat owners who had medicated their cats during January–March 2010 in Finland.

RESULTS

A total of 46 completed questionnaires were included in the study. Forty-six cats received 67 orally administered drugs. Most of the drugs were registered for cats by the European Medicines Agency (54%), and there were also off-label drugs registered for human medication (36%) and canine medication (7.4%), and ex tempore drugs (3.0%). The owners were unable to give all the doses to their cats in 16/67 (24%) of the medications. The free choice acceptance of the drug was excellent in 10% of the medications and good in 12% of the medications. The owners suspected that some of the drugs caused adverse effects in their cats, because 33% of the drugs caused increased salivation, and 18% of the drugs caused vomiting or gagging in the cats. The owners had difficulties to provide a correct single dose from the

© 2012 The Authors

package in 25% of the medications. Thirty-three percent of the owners would have preferred a tablet or a capsule formulation and only 6% would have chosen a semi solid or a liquid formulation.

CONCLUSIONS
In our study, approximately a quarter of all treatment regimens was not completed successfully, which could have affected the outcome of the treatment. Our results suggest that there is a need for more palatable and easily to administer drugs for cats.

REFERENCES
1. Litster, A., Moss, S., Honnery, M., Rees, B., Edingloh, M. & Trott, D. (2007) Clinical efficacy and palatability of pradofloxacin 2.5% oral suspension for the treatment of bacterial lower urinary tract infections in cats. *Journal of Veterinary Internal Medication*, **21**, 990–995.
2. Gunew, M.N., Menrath, V.H. & Marshall, R.D. (2008) Long-term safety, efficacy and palatability of oral meloxicam at 0.01–0.03 mg kg^{-1} for treatment of osteoarthritic pain in cats. *Journal of Feline Medicine and Surgery*, **10**, 235–241.

W-2.3.

Is mycotoxin-contaminated pet food responsible for reduced fertility and for stillbirth?

A. PFOHL-LESZKOWICZ[1], K. HADJEBA-MEDJDOUB[1], V. FAUCET-MARQUIS[1], M. TOZLOVANU[1] & I. POLISENSKA[2]
[1]*Institut National Polytechnique Toulouse, Auzeville-Tolosane, France;* [2]*Agrotest Fyto, Kromeriz, Czech Republic*

INTRODUCTION
As cereal grains and nuts are often used as ingredients in industrial pet food, companion animals such as cats and dogs may be exposed to mycotoxins. Some owners and breeder of cats and dogs observed a decrease of fertility and increase in stillbirth. As all other causes such as viral infections could be ruled out, it has been hypothesised that feed contaminants may be involved. This hypothesis was supported by the observation that replacement of incremented feed by another batch without cereals, avoided the problems.

MATERIAL AND METHODS
The aim of this study was to analyse the occurrence of mycotoxins in feed which has induced problems. In parallel mycotoxins were analysed in tissues (blood of mother and in liver, kidney, intestine, brain of litters). Ochratoxin A (OTA), Aflatoxins (AF), fumonisins (FB), zearalenone (ZEA), citrinin (CIT) and deoxynivalenol (DON) after purification by solvent extraction or immunoaffinity have been analysed, by specific HPLC method with fluorimetric detection as described by Molinié *et al.*, (2005).

RESULTS
Some cats gave birth to monstrous litters with intestines outside the abdomen, cleft lips without fur and wasted legs (Figure 1). All the pet food leading to reproductive problems were contaminated by several mycotoxins: OTA (1 $\mu g\ kg^{-1}$), AFB_1 (1.5 $\mu g\ kg^{-1}$ + other metabolites AFB_2, AG_1, AFG_2), CIT (0.5 $\mu g\ kg^{-1}$), ZEA (50–170 $\mu g\ kg^{-1}$), FB_1 (ranged 90–290 $\mu g\ kg^{-1}$, often presence in addition of FB_2), DON (ranged 200–320 $\mu g\ kg^{-1}$). The pet food without cereal contained less mycotoxins and enabled normal birth. Large amount of OTA, ZEA, AF and their metabolites and FBs were found in blood, kidney, liver, intestine, brain and placenta from monstrous kittens. Specific DNA adducts (related to OTA and/or ZEA) were detected in the tissue of these little cats.

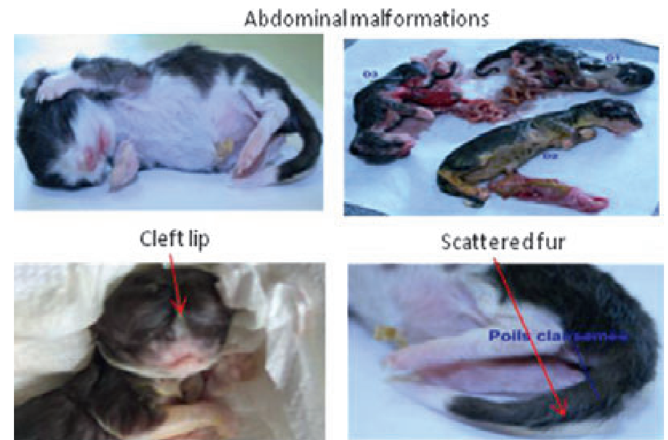


Figure 1. Monstrous kittens.

CONCLUSIONS
Although the amounts of individual mycotoxins seems to be low, the concomitant occurrence of these mycotoxins in pet food and their possible synergistic effects seem to be responsible of the reduced fertility and still birth.

REFERENCES
1. Molinié, A., Faucet, V., Castegnaro, M. & Pfohl-Leszkowicz, A. (2005) Analysis of some breakfast cereals collected on the French market for their content in ochratoxin A, citrinin and fumonisin B1. Development of a new method for simultaneous extraction of ochratoxin A and citrinin. *Food Chemistry*, **92**, 391–400.
2. Pfohl-Leszkowicz, A. & Castegnaro, M. (2005) Further arguments in favour of direct covalent binding of Ochratoxin A (OTA) after metabolic biotransformation. *Food Additives & Contaminants Supp*, **1**, 75–87

W-2.4.

Oral intoxications in small animals in the Finnish veterinary teaching hospital at the University of Helsinki from 2009 to 2010 (R1)

M. H. VAINIONPÄÄ, E. M. VALTONEN, M. RAEKALLIO & O.M. VAINIO
University of Helsinki, University of Helsinki, Helsinki, Finland

INTRODUCTION
Currently there is no instance to collect animal poisoning in Finland.

OBJECTIVE
The aim of this retrospective study was to find out the number, common causes and outcome of intoxications in small animals in Veterinary Teaching Hospital in years 2009–2010.

© 2012 The Authors

MATERIALS AND METHODS

Patient data was collected from the patient database of the Veterinary teaching hospital in the years 2009–2010. All acute oral intoxications were included. From each patient following data was collected: suspected cause, clinical status when admitted to the hospital, certainty of the intoxication, first aid given by owner of referring veterinarian and whether the patient survived. Collected data was processed by cross tabulation, and data from dogs was analysed by Chi-Square Tests, Independent-Samples Kruskal–Wallis Test.

RESULTS

Four hundred and ninety-four (0.03% of total patient load) patients were admitted to the Veterinary Teaching hospital due to suspected intoxications. Four hundred and fifty-four of the suspected intoxication patients were dogs (0.03% of total canine patients), 37 cats (0.009% of total feline patients), two guinea pigs and a rabbit. The most common intoxications in dogs were non-steroidal anti-inflammatory drugs ($n = 128$), xylitol ($n = 119$), chocolate ($n = 53$), other pharmaceuticals ($n = 48$) and rodenticide ($n = 39$) followed by mushrooms ($n = 14$), nicotine ($n = 11$), plants ($n = 7$), ethylene glycol ($n = 4$), blue algae ($n = 3$), grapes/raisins ($n = 2$) and unknown cause ($n = 18$). In cats the most common intoxications were pharmaceuticals other than non-steroidals ($n = 8$), non-steroidal anti-inflammatory drugs ($n = 7$), plants ($n = 7$) and unknown cause ($n = 6$). Clinical status when admitted to the hospital had a tendency ($P = 0.077$) to differ between the most common intoxications in dogs. The inclination of owners and referring veterinarians differed significantly ($P = 0.016$) between suspected intoxications. Owners or referring veterinarians seemed to be more likely to give first aid when the dog was suspected to have a rodenticide or xylitol intoxication compared to an intoxication by non-steroidal anti-inflammatory drugs or a chocolate intoxication.

CONCLUSIONS

The results suggest that many companion animal owners are quite aware of the possible risks of intoxications to their animals. There seems to be a relatively high amount of negligence in keeping those toxic substances out of reach of the animals. Similar remarks have been made earlier (Oehme 1977). The most common causes of intoxications seemed to vary between countries and continents. As in Finland, also in Brazil the most common intoxications were non-steroidal anti-inflammatory drugs (Xavier 2002). In Italy the most common cause of intoxication was pesticides (Caloni 2012). Domestic animal intoxications can be assumed to be quite common globally (Xavier 2002, Caloni 2012) indicating that client education is needed, and veterinarians play an important role in treating these patients.

REFERENCES

1. Caloni, F., Cortinovis, C., Rivolta, M. & Davanzo, F. (2012) Animal poisoning in Italy: 10 years of epidemiological data from the Poison Control Centre of Milan. *The Veterinary Record*, **22**, 255–259.
2. Oehme, F.W. (1977) Veterinary toxicology: the epidemiology of poisonings in domestic animals. *Journal of Clinical Toxicology*, **10**(1), 1–21.
3. Xavier, F.G., Kogika, M.M. & de, S. (2002) Common causes of poisoning in dogs and cats in a Brazilian veterinary teaching hospital from 1998 to 2000. *Veterinary and Human Toxicology*, **44**(2), 115–116.

© 2012 The Authors

Workshop 3: Globalization in Teaching: Needs and Opportunities

Coordinators: P. Mills & V. Fajt

Contributions from Australia (P. Mills), America (V. Fajt), and Europe (L. Schipper)

W-3.1.

Globalization in teaching: needs and opportunities

P. MILLS

The University of Queensland; Gatton Campus. Australia

The discipline of veterinary pharmacology, as this congress will ably demonstrate, is advancing at a tremendous rate. Many of the attendees of this congress are involved in teaching veterinary pharmacology to students, providing continuing education and/or employing veterinarians in roles where there knowledge and application of veterinary pharmacology is essential. The question arises: What do we want our graduates to know and be able to apply in veterinary pharmacology? This workshop will showcase three initiatives from different parts of the globe addressing these issues, emphasising the need for collaborative approaches to both significantly increase the resources available to all and to set the platform for benchmarking and exchange. An expert panel will then convene to elicit audience input and work towards firm resolutions to make the teaching and learning of veterinary pharmacology truly global.

© 2012 The Authors

Session 8: Drug Residues
Tuesday 10-07: 13.30–15.00

8.1.K.
Towards the determination of realistic tissue withdrawal times taking into account population variability and disease

J. E. RIVIERE
Food Animal Residue Avoidance and Depletion (FARAD) Program, Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC, USA

The determination of tissue withdrawal times for drugs in food producing animals is performed in control groups of healthy animals. Although the pharmacometric approaches to the calculation of such parameters in various regulatory jurisdictions may differ, the experimental design of such trials is similar. Once approved, drugs are then used in natural clinical populations where diseases processes for which the drug is labeled to treat are present, and concomitant medications are also often administered. This has resulted in a regulatory system with a reasonable degree of reproducibility relative to determination of the withdrawal time metric, but a lack of direct relationship to drug disposition processes seen in clinical populations of animals. This issue has been raised multiple times in the past. Various authors have amply reviewed the impact of disease processes on the primary drug pharmacokinetic parameters, with focus being on drug elimination and distribution pathways and processes as they affect blood concentration-time profiles as a function of drug efficacy. However, the effect of such factors on very low residue-level concentrations of drugs and their metabolites in target tissues has rarely been addressed or even considered. Similarly, residue depletion trials are often conducted in homogeneous groups of animals in order to reduce animal numbers while still arriving at a statistical solution to the withdrawal time algorithm; yet variability in the actual treated populations relative to breed and production factors alone easily violates this assumption. This is particularly true when the withdrawal time algorithm is attempting to estimate behaviour in 1–5% of the population with 95% confidence. The focus of this presentation is twofold: (i) to define mechanism where disease and population factors may alter disposition of drugs and marker residues, and (ii) to present modern pharmacokinetic and pharmacometric approaches where these can be defined or potentially taken into account during clinical drug trials. Situations where concern arises include when the disease process alters the normal ratio of parent drug to marker residue produced by altered biotransformation processes, when a product of the disease process binds to and modifies the drug residue depletion profile in a target tissue, or when disposition processes fundamentally alter pharmacokinetic patterns. New advances in population (mixed effect) pharmacokinetics and physiological based pharmacokinetic (PBPK) modeling open up approaches to study, model and predict these factors; in many cases directly based on the mechanism of the interaction. Both of these modeling approaches allow disease related and potentially pharmacogenomic factors to be directly estimated and modeled. Such considerations become increasingly important if global regulatory jurisdictions set residue tolerances based on the limits of analytical detection, which of late continually drops to levels where minor interactions at the molecular tissue level become important for model predictions and violations of tissue tolerances, but are likely irrelevant as they relate to toxicological impact on human food safety.

8.2.
Antimicrobial residue persistence in stomach tissue from commercial swine operations

R. BAYNES, H. WU, D. LINDQUIST & D. YEATTS
North Carolina State University, Raleigh, NC, USA

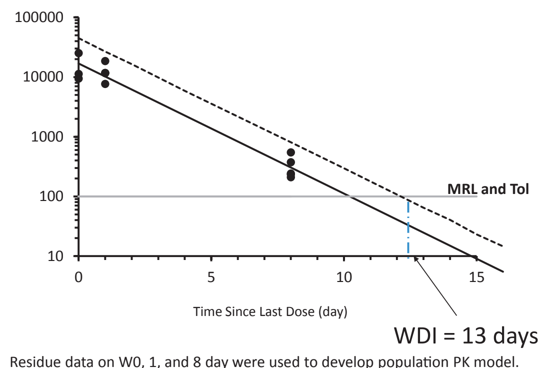
INTRODUCTION
Tetracycline and sulfamethazine are approved for therapeutic use in drinking water of commercial swine operations. There are concerns that these water additives may cause tissue residues even though the drug was administered according to label with adherence to the approved withdrawal time. This has impacted US exports of pork in recent years with a more recent concern for violative residues in stomach tissue. EU MRLs and US tolerances for the sulfonamides are similar, but not for the tetracylines. Furthermore, drug depletion in stomach tissue has not been adequately addressed in the literature which makes it difficult to estimate a safe withdrawal time.

MATERIALS AND METHODS
Our study utilized 25 pigs in each age class (weanling and finisher pigs) that were treated with tetracycline or sulfamethazine via water medication to steady state concentrations according to label. Blood samples were collected in the morning and after the 5-day water medication and tissue samples were collected up to 1–2 time points beyond the label withdrawal times for both drugs in pigs. All blood and tissue samples were processed and then analyzed by HPLC-UV and data analyzed by population pharmacokinetics (Phoenix, Pharsight Corp., Mountain View, CA, USA) to conduct all of population PK analyses and U.S. tolerance limit method for withdrawal interval (WDI) estimation.

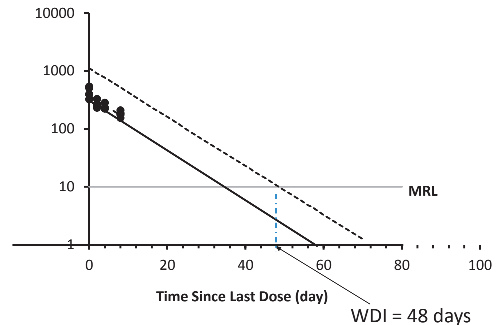
RESULTS
Steady state plasma concentrations with predicted diurnal patterns were observed and drug depletion of both drugs in plasma was similar to that reported in the literature. The half-life of tetracycline in weanling pigs was 10.3 ± 2.9 h ($n = 22$), while there may be pharmacokinetic differences between weanling and finisher pigs with regards to the clearance of sulfamethazine. Tetracycline residues persisted at levels greater than MRLs in stomach tissues 4 days beyond the U.S. 4-day label withdrawal time in both age classes. Sulfamethazine residues in stomach tissues were at or greater than the MRL of 100 ppb in several pigs in both age classes at the U.S. labeled 15 day withdrawal time.

© 2012 The Authors

The figures below depict withdrawal interval (WDI) estimates for tetracycline and sulfamethazine in finisher pigs.



Residue data on W0, 1, and 8 day were used to develop population PK model.



All data were used to develop population PK model.

CONCLUSIONS

The high drug residue levels in stomach tissue at or after the label withdrawal time could explain why stomach meat tested in Russia and Europe are positive even though the meat passed inspection in the United States prior to export. The 14-day tetracycline withdrawal time recommended by the pork producers for export markets may be inadequate based on our estimates. Our laboratory is completing analyses for these drugs in several other target tissues to further ascertain differences in depletion across various edible tissues.

8.3.
The effect of dietary sodium butyrate on the pharmacokinetics of erythromycin in broiler chickens

G. CSIKÓ, G. NAGY, G. MÁTIS, Z. NEOGRADY & P. GÁLFI
Faculty of Veterinary Science, Szent István University, Budapest, Hungary

INTRODUCTION

Sodium *n*-butyrate is used as growth promoter in the poultry and pig industry (Le Gall *et al.*, 2009; Zhang *et al.*, 2011). Due to its inhibitory effect on histone deacetylases butyrate is known as an epigenetically active molecule (Davie, 2003). As a consequence of this, butyrate may influence the expression of drug-metabolizing microsomal cytochrome P450 (CYP) monooxygenases. The aim of our study was to investigate, whether the orally added butyrate does influence the pharmacokinetics of erythromycin *in vivo*.

MATERIALS AND METHODS

Twenty one-day-old Ross 308 broilers (n = 10/group) were fed a normal diet with or without sodium butyrate supplementation (1.5 g kg^{-1} diet) for 6 weeks. At the end of the feeding period chickens were treated with a single IM dose (30 mg kg^{-1} bw., breast musculature) of erythromycin (Gallimycin®) injection. After the injection blood samples were collected at 0.5, 1, 1.5, 2, 3, 4, 8 and 12 h. Plasma levels of erythromycin were measured with validated HPLC method. Pharmacokinetic parameters were calculated (Kinetica 4.4.1; Thermo Electron) and compared statistically (R-2.14.1).

RESULTS

In butyrate-treated chickens the absorption half-life ($T_{half\text{-}abs}$) and T_{max} values were significantly higher, however the plasma elimination half-life was significantly lower (c.f. Table). The maximum concentration (C_{max}) and the AUC tended to be higher, the MRT lower, comparing to controls, but these changes were not significant. Based on AUCs the two groups were bioequivalent, but not according to C_{max} values.

Table 1 The mean pharmacokinetic parameters of erythromycin following 30 mg kg^{-1} bw IM injection (mean + SEM; *P < 0.05)

Group	$t_{half\text{-}abs}$	C_{max}	T_{max}	$t_{half\text{-}el}$	AUC (mg l h^{-1})	MRT (h)
Control	0.39 ± 0.07	3.30 ± 0.5	1.62 ± 0.21	13.82 ± 2.40	33.53 ± 4.67	8.48 ± 1.19
Butyrate	0.56 ± 0.15*	3.93 ± 0.83	1.98 ± 0.32	11.15 ± 2.06*	37.18 ± 5.41	7.35 ± 1.23

CONCLUSIONS

The application of sodium butyrate in chicken feed may interact with drug metabolism, and this could alter the major pharmacokinetic parameters. However, based on our results these changes do not influence either the therapeutic activity of erythromycin, or the terminal elimination of the drug from the body significantly. This latter is an important aspect from a food safety point of view.

REFERENCES

1. Davie, J. R. (2003) Inhibition of histone deacetylase activity by butyrate. *The Journal of Nutrition*, **133**, 2485S–2493S.
2. Le Gall, M., Gallois, M., Sève, B., Louveau, I., Holst, J. J., Oswald, I. P., Lallès, J. P. & Guilloteau, P. (2009) Comparative effect of orally administered sodium butyrate before or after weaning on growth and several indices of gastrointestinal biology of piglets. *British Journal of Nutrition*, **102**, 1285–1296.
3. Zhang, W. H., Jiang, Y., Zhu, Q. F., Gao, F., Dai, S. F., Chen, J. & Zhou, G. H. (2011) Sodium butyrate maintains growth performance by regulating the immune response in broiler chickens. *British Poultry Science*, **52**, 292–301.

© 2012 The Authors

8.4.

A biochip array-based immunoassay for detection of six groups of antimicrobials in oral fluids, milk, urine, meat, and feed samples

H. H. ORUC[1], W. K. RUMBEIHA[2], D. E. SCHRUNK[2], C. OLSEN[2] & S. ENSLEY[2]
[1]*Uludag University, Iowa State University (Visiting Scholar), Bursa, Turkey;* [2]*Iowa State University, Ames, IA, USA*

INTRODUCTION

Antimicrobial residues are of food safety concern. Therefore, it is necessary to test pigs for potential antimicrobial residues on the farm before they are put on the market. Simultaneous detection of antibiotics with different structures in biological matrices is difficult. Here we describe a biochip array-based immunoassay designed for the simultaneous detection of six different classes of antimicrobials in biological matrices and in feed samples. The platform is used to perform simultaneous quantitative analysis of multiple antimicrobial analytes in milk, urine, meat, honey, and feed samples. We investigated the use of this technology for analysis of antimicrobials in porcine oral fluids. Oral fluid is not a common diagnostic medium for antimicrobials in domestic animals, but is ideal for premarket drug residue monitoring. We then used the assay to measure these antimicrobials in milk, urine, meat, and feed samples under experimental conditions to evaluate advantages and disadvantages of this assay method.

MATERIALS AND METHODS

Anti Microbial Array II (AM II) test (Evidence Investigator™, EV 3524, Randox Laboratories Ltd, Crumlin, UK) was used for simultaneous detection of norfloxacin, ceftiofur, florfenicol, streptomycin, tylosin, and tetracyclines in clean and dirty porcine oral fluid samples, dairy milk, pig urine, pig tissue and pig feed. Since there was no extraction method for these antibiotics in oral fluids of domestic animals; we developed novel extraction methods for this purpose. Whole and skimmed dairy milk, urine, meat and pig feed samples were analysed according to the AM II Kit method provided by Randox.

RESULTS

Norfloxacin, ceftiofur, florfenicol, streptomycin, and tylosin could be were detected in both clean and dirty oral fluid samples; tetracycline could not be optimally detected in dirty pig oral fluid samples. The six antibiotics were also efficiently recovered from spiked milk, urine, meat, and feed samples under experimental conditions. The method was successfully used to analyse field oral fluids, urines and feed samples collected from a pig farm.

CONCLUSIONS

This method has the advantage that it is very sensitive and able to detect various classes of antimicrobials at ppb concentrations. A major limitation is the detectable concentrations ranges are narrow.

8.5.

Determination of the transition kinetics of florfenicol to eggs

A. FILAZI, U. T. SIRELI, B. YURDAKOK, F. G. AYDIN & A. G. KÜÇÜKOSMANOGLU
Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

INTRODUCTION

The aim of this study was to develop a suitable method for florfenicol and florfenicol amine analysis in chicken eggs and to determine the transition of florfenicol to eggs following different administration routes.

MATERIALS AND METHODS

Florfenicol, florfenicol amine and the internal standard chloramphenicol were extracted from the egg yolk, white and homogenized egg by phosphate buffer (pH = 7) and ethyl acetate. Following the purification, samples were applied to HPLC with photodiode array detector (PDA) performed by C18 column and the methods were validated according to ICH guidelines. Fifty laying hens were divided into five groups; a single dose of 20 mg kg^{-1} b.w. florfenicol was administered orally by gavage to Group 1, intramuscular to Group 2 and subcutaneous to Group 3; and as repeated dose applications, 20 mg kg^{-1} orally by gavage for 3 days to Group 4 and 5 days to Group 5.

RESULTS

The retention time of florfenicol, florfenicol amine and the internal standard chloramphenicol were found to be 20.98; 13.37 and 23.28 respectively in the spiked samples. The limit of detection (LOD) and the limit of quantitation (LOQ) values were found to be 1.94 and 6.45 ppb for florfenicol and 0.48 and 1.58 ppb for florfenicol amine, respectively. Mean recovery values for florfenicol and florfenicol amine in the spiked egg yolk, egg white and homogenized egg samples were found to be 86.6%; 86.5%; 88.5%; 87.4%; 90.0%; and 90.9% respectively. Relative standard deviation (RSD) values (< 11%) for intra-day and inter-day variation confirmed the accuracy of the method for analysing florfenicol and florfenicol amine in eggs. From the first day of both oral and parenteral administration, florfenicol and florfenicol amine were detected in eggs at a level of 0.1% and 0.08% respectively regardless the route of application. Total detection time of the drug for Group 1, 2 and 3 were found to reach 7 days, for Group 4 8 days and for Group 5 9 days.

CONCLUSIONS

In order to set safe withdrawal periods for eggs, residue depletion studies should be performed. These data is expected to provide a basis for further kinetic modelling and residue depletion of other antibiotics in eggs; since the data counters the typical assumption of residue concentrations in the yolk peaks later than the albumen.

© 2012 The Authors

Session 9: Advances in Pharmacology
Tuesday 10-07: 13.30–15.00

9.1.

Capturing the diurnal changes in renin activity and blood pressure to streamline drug therapy of renin-angiotensin-aldosterone-related disorders in dogs

J. P. M. MOCHEL[1,3], M. F. FINK[1], M. PEYROU[2], J. GIRAUDEL[2], C. D. DESEVAUX[2], M. D. DEURINCK[2] & M. DANHOF[3]
[1]*Novartis DMPK & Bioanalytics, Basel, Switzerland;* [2]*Novartis Animal Health, Saint Aubin, Switzerland;* [3]*Leiden University, Leiden, The Netherlands*

INTRODUCTION
The role of Renin-Angiotensin-Aldosterone (RAA) activation in the development of congestive heart failure and hypertension has long been recognized. Though daily variations in renin activity (RA) and blood pressure (BP) have been extensively characterized in humans, little is actually known about the periodicity of these variables in dogs. Investigations in human patients have shown differences in magnitude and duration of efficacy depending on the administration time of drugs interacting with the RAA system. We hypothesized that similar to humans, RA and BP oscillated with a circadian periodicity, indicating that administration time might also influence efficacy in dogs.

MATERIALS AND METHODS
Blood specimens for RA determination were collected once every 2 h over a 24-h span in 18 healthy beagle dogs, while systolic and diastolic BP were recorded continuously from six healthy telemetered individuals. To investigate the effect of feeding time on the periodicity of RA and BP dogs were either fed in the morning (07.00 AM), or in the evening (07.00 PM). They were exposed to natural daylight, in addition to fluorescent light from 06.00 AM to 06.00 PM. The area under the curve of day ($AUC_{[7.00–19.00]}$) versus night ($AUC_{[19.00–07.00]}$) observations were derived from individual time course profiles, averaged and compared by analysis of variance. The rhythmicity of RA and BP was further characterized by 24-h period cosine functions, using a nonlinear mixed effect model.

RESULTS
When dogs were fed in the morning RA showed a pattern very similar to that of diastolic and systolic BP, exhibiting trough values in the morning followed by a substantial rise in the afternoon and a peak activity at late evening. $AUCs_{[19.00–07.00]}$ were 91%, 9% and 8% higher than $AUCs_{[7.00–19.00]}$ for RA (*P*: 0.0001), diastolic (*P*: 0.02) and systolic BP (*P*: 0.003), respectively. No significant changes in RA and BP were reported when dogs were fed in the evening.

CONCLUSIONS
Our data demonstrate that RA, diastolic and systolic BP oscillate in parallel along the 24-h span, under strong influence of feeding time. These information should serve as a working basis to determine whether it is possible to improve drug therapy of RAA-related disorders in dogs by selecting the appropriate time of treatment.

9.2.

Population PK/PD modeling of benazepril-induced RAAS inhibition using nonlinear mixed effects

J. P. M. MOCHEL[1,3], M. FINK[1], M. PEYROU[2], J. GIRAUDEL[2] & M. DANHOF[3]
[1]*Novartis DMPK & Bioanalytics, Basel, Switzerland;* [2]*Novartis Animal Health, Saint Aubin, Switzerland;* [3]*Leiden University, Leiden, The Netherlands*

INTRODUCTION
Angiotensin converting enzyme inhibitors (ACEIs) e.g. benazepril are one of the lead therapeutic options to offset activation of the Renin-Angiotensin-Aldosterone System (RAAS) in canine heart failure. Most literature on the effect of ACEIs on systemic RAAS peptides reports plasma concentrations without information on the dynamics of RAAS inhibition, thereby providing limited knowledge on the actual level of inhibition as this is time-dependent. We assumed that benazepril influenced RAAS dynamics in dogs and used population PK/PD modeling to provide quantitative insights into the effect of benazepril on renin activity (RA), angiotensin I, II (AI, AII) and aldosterone (ALD).

MATERIALS AND METHODS
Blood specimens were collected from a group of 12 healthy beagle dogs fed a low-sodium diet as an experimental model of RAAS activation. RA, AI and AII levels were determined using immunoassay-based methods. Benazeprilat and ALD concentrations were quantified in plasma by mass spectrometry. Population PK/PD models were developed using nonlinear mixed effects (NONMEM version 7.1.2). Covariate search was performed using the stepwise covariate model building tool of Perl-speaks-NONMEM. Standard goodness-of-fit diagnostics, normalized prediction distribution errors (NPDEs), as well as posterior predictive checks and bootstraps were performed to assess the adequacy of selected models.

RESULTS
A nonlinear binding model was found to best describe the pharmacokinetic disposition of benazeprilat. Cosine functions were identified to fit the periodic nature of RA, AI, AII and ALD well, and served as baseline for the further development of PK/PD models to characterize the effects of benazepril on the RAAS. Moving down from the 'top' of the renin cascade, benazepril evoked a substantial increase in RA and AI, while decreasing AII and ALD. Bodyweight, gender and sodium intake proved the most significant covariates to explain part of the between-subject variability.

CONCLUSIONS
Our data show that benazepril markedly influences RAAS dynamics in dogs. Nonlinear mixed effect modeling helped integrating information on benazepril-induced RAAS inhibition over time and identifying the main determinants of between-animal variability.

© 2012 The Authors

9.3.

Rationale of a combination of spironolactone and benazepril for treatment of heart failure in dog

J. ELLIOTT[1], A. GENETEAU[2], J. GUYONNET[2] & V. KALTSATOS[2]
[1]*The Royal Veterinary College, London, UK;* [2]*CEVA Santé Animale, Libourne, France*

INTRODUCTION

CARDALIS® is a product containing spironolactone, a mineralocorticoid receptor antagonist and benazepril, an angiotensin converting enzyme inhibitor (ACEi). The rationale for using spironolactone with ACEi stems from understanding aldosterone's chronic cardiovascular effects (Ovaert, 2010). After starting ACEi therapy, circulating aldosterone concentrations reduce initially but tend to increase over time ('aldosterone escape'; Bomback, 2007). This justifies combined blockade of the RAS and mineralocorticoid receptors (Häggström, 1996). This study aimed to determine the pharmacodynamic consequences of combining spironolactone and benazepril therapy.

MATERIALS AND METHODS

Eighteen experimental dogs (7.46–8.76 kg) were included in a three period cross-over study, each consisting of 10 days where dogs received either PRILACTONE® alone (2 mg spironolactone kg^{-1} per day orally), FORTEKOR® alone (0.5 mg benazepril kg^{-1} per day orally) or spironolactone plus benazepril at the same dose rates. Aldosterone was administered intramuscularly (3 mg kg^{-1}; day 1 and 10 of each period) and urine was then collected for 6 h to determine urinary sodium and potassium excretion. Plasma samples were collected before dosing and at multiple time points on days 1 to days 10 of each period. The primary efficacy measure of spironolactone was the log ($[Na^+]_{urinary}$x10/$[K^+]_{urinary}$) ratio and of benazepril was *ex vivo* plasma ACE activity. The absolute values were compared between each phase using analysis of variance.

RESULTS

The data showed that hyperaldosteronism was induced [peak (aldosterone) 500 pg ml^{-1} 30 min after administration]. In a previous study this dose of aldosterone reduced the ($[Na^+]_{urinary x10/[K^+]urinary}$) ratio from 1.0 ± 0.18 to 0.7 ± 0.22 in placebo treated dogs (Guyonnet, 2009). In the present study, the ($[Na^+]_{urinary}$x10/$[K^+]_{urinary}$) ratio when spironolactone alone (1.11 ± 0.14 and 0.97 ± 0.12 on days 1 and 10) and when spironolactone with benazepril (1.19 ± 0.22 and 1.04 ± 0.21 on days 1 and 10) were given were virtually identical and the criterion of no interaction was met. This efficacy parameter was significantly different in both spironolactone groups when compared to benazepril alone (1.00 ± 0.20 and 0.81 ± 0.19 on days 1 and 10). Spironolactone alone had no effect on plasma ACE whereas when administered with benazepril, the effect on plasma ACE was not different to benazepril alone (AUEC of 323 h*mU ml^{-1} versus 361 h*mU ml^{-1}).

CONCLUSIONS

The surrogate PD measures for both spironolactone and benazepril are not affected by co-administering the two drugs, suggesting that neither interferes with the action of the other. The benefit of adding spironolactone to conventional treatment of degenerative valvular heart disease has been demonstrated (Berney, 2010). Combining these two drugs within the same tablet would improve compliance and animal welfare through reduced restraint for dosing. Such a fixed ratio combined product is acceptable based on the wide safety margins of each active ingredient.

REFERENCES

1 Ovaert, P. *et al.* (2010) *J Vet Pharmacol Ther*, **33**(2), 109–172
2. Bomback, A.S. *et al.* (2007) *Nat Clin Prac Nephrol*, **3**, 486–923.
3. Häggström, J. *et al.* (1996) *Am J Vet Res*, **57**, 1645–524.
4. Guyonnet, J. *et al.* (2009) *J Vet Pharmacol Ther*, **33**, 260–275.
5. Bernay, F. *et al.* (2010) *J Vet Int Med*, **24**, 331–341.

9.4.

Cisplatin-induced emesis in dogs: evaluation of arginine vasopressin and cortisol as biomarkers of nausea

L. KENWARD[1], L. PELLIGAND[1], F. D. TATTERSALL[2], K. SAVARY-BATAILLE[3] & J. ELLIOTT[1]
[1]*The Royal Veterinary College, Hatfield, UK;* [2]*Pfizer Ltd, Ramsgate, UK;* [3]*Pfizer Animal Health, Paris, France*

INTRODUCTION

Nausea is a subjective sensation, not easily quantified in non-verbal veterinary species. Identification of a biomarker of nausea would allow for greater clinical recognition of nausea, assessment of the efficacy of current anti-emetic medicines against nausea, development of novel anti-nausea drugs and testing the nausiogenic potential of novel compounds.

OBJECTIVE

Potential biomarkers arginine vasopressin (AVP) and cortisol were selected from evidence in the literature where correlation with nausea has been previously reported in humans (Cubeddu *et al.*, 1990; Morrow *et al.*, 2002).

MATERIALS AND METHODS

A two period placebo controlled *cross*-over design was implemented with a high dose (70 mg m^{-2}, first period, $n = 4$) and low dose (15 mg m^{-2}, second period, $n = 4$) of intravenous cisplatin to induce nausea and vomiting in healthy dogs. The number of emetic events was recorded and nausea behaviour assessed at 15 min intervals by an observer blind to the treatment using a visual analogue scale (VAS) for 6–7 h following cisplatin administration. Blood samples were collected at pre-determined time points to measure plasma concentrations of AVP and cortisol. Data are reported as mean ± SD, $P < 0.05$ was considered significant.

RESULTS

Nausea VAS scores were significantly increased for cisplatin compared to placebo treated animals for both the high and low doses of cisplatin. Peak VAS response were 71.3 ± 12.6 and 48.2 ± 3.9 mm for the high and low doses of cisplatin respectively, on a scale where 100 mm represents the 'worst possible nausea', placebo treated animals scored an average VAS of 1.5 ± 0.3 mm. The latency to the peak nausea VAS response was greater for the low dose, compared with the high dose of cisplatin. Plasma AVP and cortisol were significantly increased compared to placebo following both doses of cisplatin. Plasma AVP peaked at 3.5 h (19.8 ± 5.8 pM) for the high dose and 6 h

© 2012 The Authors

(4.18 ± 1.9 pM) for the low dose. Peak cortisol plasma concentrations of 465.5 ± 108.5 nM occured at 3.5 h for high dose cisplatin and 268.3 ± 11.3 nM cortisol 5 h post cisplatin for low dose cisplatin.

CONCLUSIONS

Both plasma AVP and cortisol are increased in response to treatment with cisplatin. The increases in plasma concentrations of both hormones appear to be dose dependent with greater increases seen when the high dose of cisplatin is administered. This corresponds with the behavioural nausea scores for the two doses of cisplatin. AVP and cortisol appear to be suitable candidate biomarkers of nausea for cisplatin-induced emesis in dogs.

REFERENCES

1. Cubeddu, L.X., Lindley, C.M., Wetsel, W., Carl, P.L. & Negro-Vilar, A. (1990) Role of angiotensin II and vasopressin in cisplatin-induced emesis. *Life Sci*, **46**(10), 699–705.
2. Morrow, G.R., Hickok, J.T., Andrews, P.L. & Stern, R.M. (2002) Reduction in serum cortisol after platinum based chemotherapy for cancer: a role for the HPA axis in treatment-related nausea? *Psychophysiology*, **39**(4), 491–495.

9.5.
Target animal safety studies of Cerenia® (maropitant citrate) in cats

M. J. KRAUTMANN, R. K. FRANK, T. C. BERG, J. F. KING, S. P. BOUCHER, S. P. LEMAN & M. R. STEGEMANN
Pfizer, Kalamazoo, MI, USA

INTRODUCTION

Cerenia® (maropitant citrate) blocks the binding of Substance P to the Neurokinin-1 (NK1) receptor, thereby inhibiting the vomiting reflex. Its effectiveness has been demonstrated against vomiting due to a variety of causes. Two laboratory studies that complied with national animal welfare regulations demonstrated the safety of maropitant injectable solution administered subcutaneously to cats.

MATERIALS AND METHODS

Each study used equal numbers of healthy, intact 16-week-old male and female purpose-bred laboratory cats (eight per group). Endpoints included clinical pathology, veterinary examinations, body weight, food consumption, response to dosing, and toxicokinetic sampling. The safety margin (Study A) was assessed for 15 days at 0, 1, 3, or 5 mg kg^{-1} by subcutaneous injection. Each cat was humanely euthanized, a complete necropsy examination was performed, and a full set of tissues was collected and evaluated microscopically. A second study (Study B) demonstrated safety at exposures (AUC) corresponding to 3× in older, adult cats. Thus, cats received 0 or 4 mg kg^{-1} twice daily for 5 days, followed by an 8-day recovery phase. Pharmacokinetic sampling was designed to permit comparison to pharmacokinetics in adult cats.

RESULTS

In Study A, maropitant citrate was well tolerated at all dosages but produced clinically significant discomfort associated with injection in some cats. At 1 and 3 mg kg^{-1}, approximately 6–10% of responses to injection included attempted biting or scratching, marked vocalization, or persistent attention to the injection site. All other changes were not clinically relevant. An observed 10% decrease in food intake (5 mg kg^{-1}) did not produce a decrease in body weight. One and two cats, respectively, at the 3 and 5 mg kg^{-1} doses had clinical signs suggestive of dehydration but without correlating findings. One cat (5 mg kg^{-1}) had lethargy on 3 days. A minor increase in amylase and decrease in albumin were noted (3 and/or 5 mg kg^{-1}). At necropsy, thymus weights were decreased (3 and 5 mg kg^{-1}). Microscopically, injection sites (all doses) showed mild to moderate irritation and progressed normally toward resolution. Other findings were unrelated to treatment. In Study B, maropitant was well-tolerated and achieved systemic exposures (AUC) sufficient to demonstrate a 3-fold safety margin for adult cats. Clinically insignificant changes were observed in clinical chemistry. Recovery-phase diarrhea in four cats either self-corrected (two cats) or responded to supportive subcutaneous fluids and supplementary canned food.

CONCLUSIONS

Maropitant citrate injectable solution administered subcutaneously at 1 mg kg^{-1} for up to 5 days was well-tolerated and demonstrated a good safety margin in cats 16 weeks and older. Some cats experienced discomfort associated with injection.

© 2012 The Authors

Session 10: Contaminants: Dioxins and Biomarkers
Tuesday 10-07: 13.30–15.00

10.1.K.
Dioxins and PCBs in the food chain – a never-ending story?

P. FÜRST
Chemical and Veterinary Analytical Institute Münsterland-Emscher-Lippe, Münster, Germany

The major pathway of human exposure to dioxins and PCBs is food. More than 90% of the daily intake is attributed to food and generally almost 80% of this is coming from food of animal origin. Estimations of human exposure performed in Europe and other areas in the 1990s showed that a considerable percentage of the respective populations were already above the WHO-TDI of 1–4 pg TEQ kg^{-1} body weight. Moreover, surveys showed that the intake of an exclusively breast fed baby generally exceeds the dietary intake of adults by two orders of magnitude. The intensification of monitoring programmes for food and feed recently resulted in the identification of a number of dioxin and PCB incidents, either caused by criminal action or by unintentional contamination of the respective commodities. As these incidents do not only have severe financial consequences but most notably spoil the numerous efforts to minimize human exposure, vigilance remains necessary also in the future to identify hitherto unknown sources and consider dioxins and PCBs in food and feed as a continuous matter of concern.

10.2.K.
Interactions of dioxins at gene level: link to toxicity mechanisms and recent advances in food producing species

C. NEBBIA
University of Turin, Grugliasco, Italy

The exposure to polychlorodibenzodioxins, polychlorodibenzofurans, and polychlorobphenyls capable of interacting with the AhR receptor is long known to affect a large array of genes, representing a key event in the toxic mechanism of such compounds. After reviewing the state of the art of the existing knowledge, the keynote will focus on gene alterations recorded in food producing species, especially ruminants, under both *in vitro* and field conditions of exposure.

SUGGESTED READING

1. Yoshioka W, Peterson RE, Tohyama C. (2011) Molecular targets that link dioxin exposure to toxicity phenotypes. *J Steroid Biochem Mol Biol*, **27**, 96–101.
2. Genualdo V, Spalenza V, Perucatti A, Iannuzzi A, Di Meo GP, Caputi-Jambrenghi A, Vonghia G, Rasero R, Nebbia C, Sacchi P, Iannuzzi L. (2011) Fluorescence *in situ* hybridization mapping of six loci containing genes involved in the dioxin metabolism of domestic bovids. *J Appl Genet*, **52**, 229–32.

10.3.
Expression and inducibility of AhR-responsive genes in a bovine mammary epithelial cell line (BME-UV)

V. SPALENZA, F. GIROLAMI, L. MANSISNI & C. NEBBIA
University of Turin, Grugliasco, Italy

INTRODUCTION
Most aryl hydrocarbon receptor (AhR)-agonists, including dioxin-like (DL) compounds (PCDD, PCDF, and PCB) and benzo(a)pyrene (B[a]P), are highly toxic and persistent environmental contaminants of great concern to human and animal health. Their binding to AhR elicits an increase in the expression of several genes encoding for xenobiotic metabolizing enzymes, most notably CYP1A1 and CYP1B1 (Mandal, 2005). Little is known about the expression of such enzymes in the bovine mammary gland. Interestingly, the (CYP-mediated) bioactivation of aflatoxin B1 to aflatoxin M1 coupled to a marked cytotoxicity has been recently demonstrated in a bovine mammary epithelial cell line (BME-UV) (Caruso, 2009). In this report we characterized for the first time the basal expression of AhR-target genes in such cells and their modulation by selected agonists.

MATERIALS AND METHODS
The BME-UV cells were plated in 6-cm dishes and cultured for 24 h until they reached 50% confluence. After replacement with fresh medium, cells were treated with 100 nM of beta-naphthoflavone (β-NAF), B[a]P, PCB126 or PCB77, dissolved in DMSO, and harvested after 2, 4, 8, 24, 48 and 72 h. For dose-response experiments, cells were treated with increasing logarithmic concentrations (from 0.01 to 10 μM) of the different AhR-agonists and lysed after 8 (β-NAF and B[a]P) or 24 (PCB126 and PCB77) hours. The mRNA expression of eight AhR responsive genes (CYP1A1, CYP1B1, AhR, ARNT, AhRR, Bax, GSTA1, and GSTA2) was measured by qPCR. Target gene transcripts were normalized to the GAPDH content, and results were expressed as fold change relative to control cells treated with DMSO alone.

RESULTS
The investigated genes were readily detectable in BME-UV cells under basal conditions, with GSTA2 and AhRR being the most and least expressed, respectively. Moreover, all the AhR-ligands were able to induce to a different degree the expression of CYP1A1, CYP1B1, AhR, ARNT and AhRR in a concentration- and time-dependent manner. Particularly, PCB126 was the most potent inducer and CYP1A1 was the most responsive gene. The treatment with PCBs regulated the expression of target genes up to 72 h, while the effect of β-NAF and B[a]P peaked at 8 h and then rapidly decreased. Unexpectedly, GSTAs expression was reduced by all the ligands, while Bax was repressed only upon PCB126 treatment.

© 2012 The Authors

CONCLUSIONS

Our results demonstrated that the AhR signaling pathway is present and inducible in a bovine mammary epithelial cell line, and that the transcriptional effects of DL-PCBs are more persistent than those of the other tested AhR-agonists. The above findings suggest that BME-UV cells may be employed as a model to study the mammary bioactivation mediated by AhR-dependent CYPs.

REFERENCES

1. Caruso, M., Mariotti, A., Zizzadoro, C., Zaghini, A., Ormas, P., Altafini, A. & Belloli, C. (2009) A clonal cell line (BME-UV1) as a possible model to study bovine mammary epithelial metabolism: metabolism and cytotoxicity of aflatoxin B1. *Toxicon*, **15**, 400–408.
2. Mandal, P.K. (2005) Dioxin: a review of its environmental effects and its arylhydro-carbon receptor biology. *Journal of Comparative Physiology* B, **175**, 221–230.

10.4.

Changes in AhR-Gene battery expression in circulating lymphocytes from cattle accidentally exposed to dioxin and dioxin-like compounds

F. GIROLAMI, M. CARLETTI, P. SACCHI, R. ROSERO & C. NEBBIA
University of Turin, Grugliasco, Italy

INTRODUCTION

Products of animal origin represent one of the major sources of exposure to dioxin-like (DL) compounds (PCDD, PCDF and PCB) for humans. The negative effects of these highly toxic and persistent pollutants are mediated by the activation of the AhR signaling pathway that elicits the transcriptional induction of several genes, including those involved in xenobiotics metabolism. Interestingly, the expression level of these genes (i.e. CYP1A1 and CYP1B1) in human peripheral lymphocytes has been correlated with the exposure to DL-compounds, suggesting their application as non-invasive biomarkers (1). As the AhR signaling pathway is present also in bovine lymphocytes (2), we investigated the changes in target gene expression in these easily accessible cells from dairy cows reared in DL-compounds contaminated areas.

MATERIALS AND METHODS

Lymphocytes were isolated from peripheral venous blood collected from 60 healthy dairy cows, 40 of which came from two farms (A and B) located in a contaminated area (bulk milk TEQ values: A = 18.56 ng g^{-1} fat; B = 8.56 ng g^{-1} fat); the remaining 20 animals, reared in a non-contaminated area, were used as controls (C). Blood sampling was repeated at a 9-months interval, when the contamination level markedly decreased. The mRNA expression of five AhR responsive genes (CYP1A1, CYP1B1, AhR, ARNT and AhRR) was measured by qPCR, normalizing gene transcripts to S24 and PPIA content. Relative expression analysis was performed through qBASE software. To test the influence of exposure level to the *in vitro* stimulation with DL-compounds, lymphocytes collected from 12 cows (six from A, and six from C) were cultured and treated with 100 nM of PCB126 for 2 h (Girolami, 2011). The expression of CYP1A1 and CYP1B1 was measured by qPCR and normalized to GAPDH content. Results were expressed as fold change relative to control cells treated with DMSO.

RESULTS

Bovine lymphocytes expressed to a detectable level only the genes AhR, ARNT and CYP1B1. The expression of CYP1B1 appeared to be correlated to bulk milk TEQ values, being higher ($P < 0.05$, Kruskal–Wallis test) in the most contaminated animals (farm A) upon the first sampling. Concerning the *in vitro* experiments, PCB126 was able to induce both CYP1A1 and CYP1B1 in all the tested samples. However, the extent of CYP1A1 induction in lymphocytes from contaminated animals was lower ($P < 0.05$, Mann–Whitney test) than that of non-contaminated animals, thereby matching what is observed in exposed humans (Landi, 2003).

CONCLUSIONS

In conclusion, significant changes in circulating lymphocyte CYP expression were detected in contaminated cows. Further studies are in progress to ascertain the suitability of such genes as candidate biomarkers of exposure to DL-compounds *in vivo*.

REFERENCES

1. Landi, M., Bertazzi, P., Baccarelli, A., Consonni, D., Masten, S., Lucier, G., Mocarelli, P., Needham, L., Caporaso, N. & Grassman, J. (2003) TCDD-mediated alterations in the AhR-dependent pathway in Seveso, Italy, 20 years after the accident. *Carcinogenesis*, **24**, 673–680.
2. Girolami, F., Spalenza, V., Carletti, M., Perona, G., Sacchi, P., Rasero, R. & Nebbia, C. (2011) Gene expression and inducibility of the aryl hydrocarbon receptor-dependent pathway in cultured bovine blood lymphocytes. *Toxicology Letters*, **206**, 204–209.

© 2012 The Authors

Workshop 4: Traditional Medicine and Monitoring of Drug Usage Tuesday 10-07: 15.30–17.30

Coordinators: A. S. J. P. A. M. van Miert & R. Arowolo

W-4.1.
Veterinary medicine in Nigeria

R. AROWOLO
Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria

W-4.2.
Treatment of animal diarrhoea using medicinal plants: an ethnobotanical survey in Plateau State, Nigeria

N. V. OFFIAH[1], M. S. MAKOSKI[2], I. L. ELSIHA[2], S. MAKAMA[2], J. G. GOTEP[2], C. J. DAWURUNG[2], O. O. OLADIPO[2], A. S. LOHLUM[2] & D. SHAMAKI[2]
[1]*The University of the West Indies, St. Augustine, Trinidad and Tobago;* [2]*Biochemistry Division, National Veterinary Research Institute, Plateau State, Nigeria*

INTRODUCTION
Most tribes of Plateau State, Nigeria practice livestock farming in addition to other trades and like most low skilled farmers in other parts of the world, use medicinal plants for the control of diseases in their animals. There is however scarcity of documented information on the medicinal plants used in the treatment of animal diarrhoea in the State, thus the need for this survey.

MATERIALS AND METHODS
Open-ended questionnaires and guided dialogue techniques were used to gather information on the medicinal plants used by the indigenes for the control of animal diarrhoea in some parts of the State.

RESULTS
Two hundred and forty-eight questionnaires were administered and 132 plants were mentioned as being used for the control of diarrhoea in animals. The survey showed that *Fabaceae* (21%) was the most common family followed by *Combretaceae*, *Moraceae* and *Verbanaceae.* The most common part of the plants used in the preparation of the diarrhoeal remedies was the leaves followed by the stem bark. Administration of plant medicines are mainly by drenching while some are mixed with feed, salt or potash to improve palatability before giving them to the animals.

CONCLUSIONS
Plateau State because of its location has a large reserve of medicinal plants used for the management of diarrhoea in animals. These plants if scientifically evaluated could be potential sources of new drugs for the control of diarrhoea in both humans and animals.

W-4.3.
Silymarin prevents doxorubicin-induced carbonyl stress and regulates the *c-myc* expression at mRNA level in the testis of rats

H. MALEKINEJAD, H. JANBAZ-ACIABAR, M. RAZI & P. TEHRANCHI
Urmia University, Urmia, Iran

INTRODUCTION
Doxorubicin (DOX), an antracycline, is widely used against tumours such as childhood leukemia and testicular cancer. Although DOX has been recognized as a potent and effective anticancer agent, various reports indicate its toxicity against the heart, liver and testis, which hampers its clinical use (1). The current study was carried out to clarify the preventive effects of silymarin (SMN) on DOX-induced testicular damage in rats.

MATERIALS AND METHODS
Wistar male rats were divided into six groups (n = 8), including: controls (C), DOX-treated (DOX, 15 mg kg^{-1}, i.p.), DOX- and SMN-treated and SMN-treated animals (SMN, 50 mg kg^{-1}, orally). Those groups which received either compound, were sub-grouped based on the preventive (PVT), protective (PTT) and/or therapeutic treatment regimens (TPT) of SMN administration. The experimental protocols were approved by the ethical committee of Urmia University in accordance with principles of laboratory animal care. The carbonyl stress assay and histopathological examinations in testis were conducted (2). The expression of c-myc at mRNA level also was analysed.

RESULTS
Pre-treatment and co-treatment with SMN attenuated the DOX-induced carbonyl stress. The DOX-induced histopathological damage including the negative tubular differentiation index (TDI) and repopulation index (RI) were significantly ($P < 0.05$) improved with SMN pre-treatment and concomitant administration of SMN and DOX. SMN in preventive and protective forms prevented also DOX-induced DNA fragmentation in the testis. The c-myc expression at mRNA level was down-regulated in the testis of animals that received SMN as pre-treatment and concomitant administration, respectively.

CONCLUSIONS
Our data suggest that the DOX-induced biochemical and histopathological alterations could be prevented and/or protected by SMN. Moreover, the SMN protective and preventive effects may be attributable to its capacity to reduce DOX-induced carbonyl stress and DNA damage, as indicated by the alterations in c-myc expression levels.

© 2012 The Authors

REFERENCES

1. Damani, M.N., Masters, V., Meng, M.V., Burgess, P., Turek, M. & Oates, R.D. (2002) Postchemotherapy ejaculatory azoospermia: fatherhood with sperm from testis tissue with intracytoplasmic sperm injection. *J Clin Oncol*, **20**, 930–936.
2. Levine, R.L., Williams, J.A., Stadtman, E.R. & Shacter, E. (1994). Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol*, **233**, 346–357.

W-4.4.

Quality and practical use of the list of registered VMPs in Republic of Macedonia

R. VELEV[1], N. KRLESKA VELEVA[2], D. DAMJANOVSKA[2] & S. COKREVSKI[2]

[1]*Faculty of Veterinary Medicine Skopje, Skopje, Macedonia;*
[2]*Replek Farm, Skopje, Macedonia*

INTRODUCTION

According to the WHO guidelines (1), the *Anatomical Therapeutic Chemical System for the Classification of Veterinary Medicines* (ATC-vet) is widely recognized as a classification tool. The aim of this work is to analyse the List of Registered Veterinary Medicinal Products (VMP-s) in the Republic of Macedonia and to evaluate the quality and practical use of this list according to the ATC-vet classification.

MATERIALS AND METHODS

The registered VMPs in Macedonia are classified according to composition of active component/s and main therapeutic indications; further classification is based on target organ systems. An appropriate code from ATC-vet Index was designated to each VMP (1). This work evaluated the list of registered VMPs in alphabetic order with appropriate ATC-vet code and analysed the number of registered VMPs per organ system.

RESULTS

In Macedonia, 348 VMPs were registered or renewed during the period from 1 January 2007 to 31 December 2011 (2). All registered VMPs are imports, from 37 producers from 17 European countries, mainly Serbia 18.6%, The Netherlands 18.3%, Croatia 13.7% and Slovenia 6.8%. The largest number of VMPs are from the QJ group – Antibacterials for systemic use. Tetracyclines, penicillins and sulfonamides are the top three antimicrobial classes, comprising approximately 80% of the total sales, while on the other hand there are no VMPs registered for primary therapeutic effect on cardiovascular system (group QC), for treatment of neoplasms (group QL-Antineoplastic and immunomodulating agents) and for treatment of diseases of eyes and ears (group QS – Sensory organs). Some of the anatomical groups like group QB – Blood and blood forming organs, group QM – Musculo-skeletal system, group QN – Nervous system and group QR – Respiratory system were represented by a significant number of VMPs. A small market for specific VMPs results in lack of interest of the producers to register VMPs with low or no commercial value, creating a serious problem for the veterinary practitioners.

CONCLUSION

The ATC-vet classification gives a detailed view on the real quantitiy of the different classes of registered VMP-s. It is a practical tool for the identification of different groups of VMPs for the veterinary practitioners as well as all others involved in production, trade and distribution of VMPs. Analysed data have a particular use in the assessment of total consumption trends. Currently data indicate a high use of antimicrobials indicating that this class of VMP should be monitored closely to avoid the appearance of antimicrobial resistance and possible consequences for animal and human health.

REFERENCES

1. WHO Collaborating Centre for Drug Statistics Methodology (2012): *Guidelines for ATC-vet classification 2012*, 14nd edn. Oslo, Norway.
2. Velev R. (2012): *Register of veterinary medicinal products in R. Macedonia.* Food and Veterinary Agency of R. Macedonia & Faculty of veterinary medicine – Skopje, 2012.

W-4.5.

Veterinary pharmacovigilance – a pilot study in Tamilnadu, India

G. GHADEVARU

Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

INTRODUCTION

Veterinary pharmacovigilance monitors the safety of veterinary medicines, including vaccines (VAC) used for the prophylaxis, diagnosis or treatment of diseases in animals once they reach the market after authorization (1). The task of veterinary pharmacovigilance is to ensure the protection of the environment as well as the safety of veterinary medicines in animals, animal-derived food and people in contact with veterinary medicines (2). The reporting of adverse drug events (ADE)/reactions is a key part of the process of ensuring the safety of medicines, and plays a part in keeping existing medicines in the market and its availability. In India, there is no pharmacovigilance programme/monitoring of adverse drug events in veterinary medicine. Essential data on the frequency, severity of the treated animal ADE remains unreported in India. Hence, the aim of the study was to assess for the first time the ADEs in treated livestock of Tamil Nadu, India. A 12-month period pilot study was conducted to monitor the ADE for frequently used drugs (labeled/extra labeled drugs). A survey protocol (3) consisting of questionnaire about used drugs in livestock was developed; the questionnaire was distributed to 300 veterinarians of Tamil Nadu state. The veterinarians were instructed to voluntarily report on the various types of drugs used and the ADEs, if any observed.

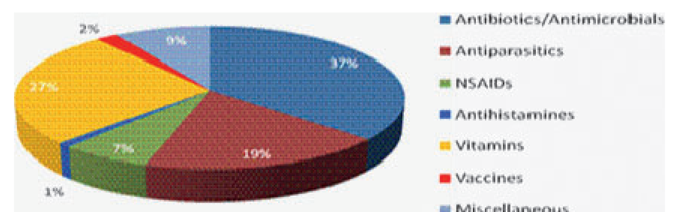


Figure 1. Sources of ADEs recorded from 2011 to 2012 in Tamil Nadu State, India.

© 2012 The Authors

RESULTS

More than 37% ADEs were related to antimicrobials, antiparasitic and anti-inflammatory agents. A further 27% of ADEs were due to vitamins and feed additives. Two cases of ADEs observed in FMD vaccination in cattle and canine Parvo vaccine in dogs. In poultry, tiamulin and salinomycin ADEs induced serious mortality.

CONCLUSIONS

The present study warrants for the need of sustained veterinary pharmacovigilance programmes in livestock for timely ADEs presenting drug detections and drug safety improvement.

REFERENCES

1. Iraguen, D., Urcelay, S. & San Martin, B. (2010) Pharmacovigilance in veterinary medicine in Chile: a pilot study. *Journal of Veterinary Pharmcology and Therapeutics*, **4**, 108–115.
2. EMEA (2009) The European Agency for the Evaluation of Medicinal Products. Veterinary medicines. *Available at*: http://www.emea.europa.eu.
3. VICH (2008) Guidelines on pharmacovigilance of veterinary medicinal products, VICH GL, 24, 7.

© 2012 The Authors

Session 11: Drug Delivery
Wednesday 10-07: 9.00–10.30

11.1.K.

Physicochemical parameters that could influence drug delivery and toxicology of nanomaterials through skin

N. A. MONTEIRO-RIVIERE

Center for Chemical Toxicology Research and Pharmacokinetics, Department of Clinical Sciences, North Carolina State University, Raleigh, NC, USA

Skin is a potential route of exposure to engineered nanoparticles (NP) that may occur in the environment or occupational setting, or after topical dosing with cosmetic or pharmaceutical formulations. It is the one drug delivery portal for nanomaterials that has been extensively studied. There are two phases for assessing hazard and risk after such exposure: penetration and toxicity to cellular elements of the skin. The focus of this presentation is to review some of the physicochemical properties of NP that may enhance or prevent penetration and/or toxicity. There are properties of a chemical/nanoparticle that will determine its propensity to cause dermatotoxicity which is the ability to penetrate skin and subsequently interact with the biological components of skin that could elicit a toxicological response. Due to recent advance in nanotechnology and their use in consumer products such as cosmetics, there is a concern around potential safety issues. The penetration of NP in the skin is a controversial subject, partly because many factors that influence absorption were not studied and the opinions of some investigators were generalized based on limited studies with only a few types of NP. Many of these studies were conducted on very large particles that were not of nanosize. Also, discrepancies may relate to differences in NP composition, surface chemistry, vehicles or solvents, techniques and methods of exposure, and analytical analysis, laboratory conditions and duration of the experiment. There are anatomical differences in species, hair follicle density, thickness, and regional differences in absorption that effect results. The respiratory route of exposure has been studied extensively but skin exposure has been neglected being considered less permeable with a lower perception of risk. However, nanotherapeutic delivery systems are being aggressively developed for transdermal applications and vaccine delivery making definition of realistic physiochemical properties important.

11.2.

Doxycycline loaded poly (lactic-co-glycolic) acid (PLGA) nanoparticles for improved oral bioavailability and pharmacodynamic characters

M. ISMAIL & Y. AL-TAHER

College of Veterinary Medicine and Animal Resources, Alahsa, Saudi Arabia

INTRODUCTION

Doxycycline is a widely used antibiotic which has been effective in the therapy of a large variety of skin, oropharyngeal as well as upper and lower respiratory tract infections for many years. Limited oral bioavailability, poor penetration to deeply seated infections, frequent dosage regimen and emergence of resistant strains have limited its use over the last years. Polymeric particulate carriers have been used for many years to prolong the duration of action and improve stability of numerous drugs (Lemoine and Preat, 1998). Poly (lactic-co-glycolic acid) (PLGA) is one of the most successfully used biodegradable polymers and is approved by the USA FDA and European medicine agency (EMA) in various delivery system in humans. In the present study, doxy PLGA nanoparticles were selected to be evaluated for their oral pharmacokinetic and *in vitro* antimicrobial efficacy.

MATERIALS AND METHODS

Preparation of doxycycline poly lactic-D-glycolic acid (PLGA) nanoparticles by nanoprecipitation method was described earlier. Characterization of nanoparticles: Nanoparticle morphology was obtained by scanning electron microscopy. Nano size and zeta potential were determined by using dynamic light scattering. Pharmacokinetic study: doxycycline PLGA nanoparticles oral disposition kinetics and tissue distribution to skin and lung tissue were compared to free doxycycline after a single dose of 10 mg kg^{-1}. Plasma and tissue samples were collected at different time intervals. The drug was extracted using SPE followed by assay of its concentration by HPLC methods earlier. Compartmental and non-compartmental analysis were done using WinNonlin and different pharmacokinetic parameters were computed accordingly. Antimicrobial activity: minimum inhibitory concentrations (MIC) on standard stocks of Staphylococcus aureus (ATCC 29213) were determined by a microdilution test in a culture broth by comparing samples of doxycycline with and without PLGA.

RESULTS

Doxycycline PLGA nanoparticles exhibited a smooth and spherical shape with diameter of 20 ± 1.4 nm and a zeta potential of −32 ± 2.2 mv. Doxycyline PLGA loaded nanoparticles were absorbed within 2 h compared to the non loaded one (1 h) and a maximum concentration of 4.24 and 3.7 μg ml^{-1} was achieved, respectively. Doxycycline loaded nanoparticles produced sustained release of doxycycline for 48 h in plasma compared to 18 h for the free doxycycline. The encapsulated doxycycline exhibited long elimination half life (7.35 h) and MRT (9.84) against 2.97 and 4.13 h, respectively for the free doxycycline. Relative bioavailability of doxycycline was enhanced fourfold by encapsulation as well as pharmacodynamic parameters such as AUC/MIC, C_{max}/MIC. The MICs of doxycycline against *Staphylococcus aureus* were similar before and after encapsulation. No significant difference in plasma/tissue ratios after encapsulation were observed.

CONCLUSIONS

PLGA polymer encapsulation of doxycycline have a great potential in improving the oral bioavailability and provide a promising method for sustained and controlled delivery of this drug.

© 2012 The Authors

11.3.

Experimental factors affecting *in vitro* absorption of six model compounds across porcine skin

D. KARADZOVSKA, J. D. BROOKS & J. E. RIVIERE
North Carolina State University, Raleigh, NC, USA

INTRODUCTION

Assessing the extent and rate of passive drug absorption through skin is an important requirement for the development of topically administered drugs. Such evaluations are typically carried out *in vitro* using diffusion cell systems incorporating membranes from a variety of animal species. The existing literature highlights a large variation in the combination of experimental variables used for these assessments. Variables such as finite or infinite, saturated or unsaturated doses, vehicle/formulation, and receptor fluid variations complicate the comparison of data. Therefore, the aim of this study was to standardize several experimental variables to identify their impact on the permeability of six model compounds (caffeine, cortisone, diclofenac sodium, mannitol, salicylic acid and testosterone) through porcine skin from three different vehicles.

MATERIALS AND METHODS

Porcine skin was obtained from the dorsal area of euthanized weanling female Landrace/Yorkshire cross pigs and sectioned to provide a dosing area of 0.64 cm^2. The six radiolabeled compounds were applied as varying combinations of finite, infinite, saturated and unsaturated doses in one of three vehicles (propylene glycol, water, and ethanol). The effect of the presence/absence of bovine serum albumin (BSA; 4.5%, w/v) in the receptor phase was also evaluated, using two diffusion cell systems (static and flow-through). Samples of the receptor phase were collected at predetermined intervals post dose application: 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20 and 24 h. Flux of each compound into the receptor phase was monitored over 24 h. Absorption was defined as the total amount (μg) detected in the perfusate for the entire 24 h period. The amount remaining in skin was also calculated. The apparent permeability coefficient (K_p, cm h^{-1}) was determined by dividing the steady state flux calculated using the slope of the cumulative absorption (μg cm^{-2}) versus time regression, by the applied dose concentration (μg ml^{-1}). ANOVA ($P < 0.05$) was carried out on three parameters (amount of dose absorbed, K_p, and amount remaining in skin) to assess the impact of the selected experimental variables on the dermal absorption of the six compounds.

RESULTS

The resultant data was consistent with patterns seen in the published literature, that is, absorption was generally highest from the vehicle where the solubility was greatest. Absorption and K_p values were most often higher from infinite doses rather than from finite doses, and from saturated doses rather than from unsaturated doses. Correlation between the two diffusion cell systems was evident and only a few statistical differences were noted with the presence/absence of BSA in the receptor phase.

CONCLUSIONS

This unique full factorial study design allowed for a comprehensive evaluation of several commonly used experimental variables, permitting direct comparison of results. The data generated from this study design is ideal for the creation of dermal absorption prediction models, which may further assist screening topical formulations.

11.4.

The effects of formulation on the penetration and retention of Budesonide in canine skin

L. A. AHLSTROM[1], S. E. CROSS[2] & P. C. MILLS[3]
[1]*Faculty of Veterinary Science, University of Sydney, Camperdown, NSW, Australia* [2]*Therapeutics Research Centre, Southern Clinical Division, University of Queensland, Princess Alexandra Hospital, Brisbane, Qld, Australia* [3]*School of Veterinary Science, University of Queensland, Gatton, Qld, Australia*

INTRODUCTION

To maximise treatment efficacy and reduce the incidence of systemic side effects in dogs with allergic skin disease, a topically-applied glucocorticoid should penetrate into and be retained within the skin (Wiedersberg *et al.*, 2008). Strategies to enhance the skin penetration and retention of drugs involve modification of the physicochemical properties of either the drug or the formulation (vehicle), to increase the drug's partitioning into and rate of diffusion through the stratum corneum (Wiedersberg *et al.*, 2008). This study was performed to determine if there was a difference in the skin penetration and retention kinetics of a glucocorticoid (budesonide) when applied to normal canine skin *in vitro* at a concentration of 0.025% from a novel, complex formulation (Barazone) and from two simple vehicles.

MATERIALS AND METHODS

Skin was collected from a greyhound (male, entire, approximately 5 years old) euthanized by an intravenous injection of sodium pentobarbital (UQ Animal Ethics Committee approval, SVS/356/06/). Full thickness, lateral thoracic skin was clipped of hair, excised and mounted in Franz-type diffusion cells and a solution containing 4% w/v bovine serum albumin and 0.1% w/v sodium azide in phosphate-buffered saline added to the receptor compartments. One gram of a 0.25 mg ml^{-1} budesonide-containing formulation [Barazone (BZ), isopropyl myristate (IPM) or propylene glycol (PG)] was added to fully hydrated skin ($t = 0$; $n = 12–13$ replicates per treatment group). At regular intervals over 84 h, the amount of budesonide penetrating or retained within the skin was quantified using high performance liquid chromatography. Restricted (or residual) maximum likelihood (REML) mixed model analyses were performed to determine if vehicle had a significant effect on mean flux (J) or skin retention of budesonide. P-values comparing the mean J or skin retention from BZ and IPM with the reference group (PG) were calculated using Chi-square tests.

RESULTS

The statistical model predicted that the mean flux (J) of budesonide from BZ was 9.2-fold ($P < 0.001$) and 105-fold ($P < 0.001$) greater than from IPM and PG, respectively, and the skin retention of budesonide from BZ was more than threefold ($P < 0.0001$) and nearly sixfold ($P < 0.0001$) greater than from IPM and PG, respectively.

© 2012 The Authors

CONCLUSIONS
This study has demonstrated that the excipients of a topical formulation can greatly affect the skin penetration and retention of budesonide in dogs, and consequently could be selected to maximise drug concentration and retention at the site of action. This has the potential to improve the efficacy and safety of, and owner compliance with, topical glucocorticoid therapy in dogs.

REFERENCE
1. Wiedersberg, S., Leopold, C. S. & Guy, R. H. (2008) Bioavailability and bioequivalence of topical glucocorticoids. *European Journal of Pharmaceutics and Biopharmaceutics*, **68**, 453–466.

11.5.
The penetration kinetics of hydrocortisone through the lesional and non-lesional skin of dogs with flea allergy dermatitis
L. A. AHLSTROM[1], S. E. CROSS[2] & P. C. MILLS[3]
[1]*Faculty of Veterinary Science, University of Sydney, Camperdown, NSW, Australia* [2]*Therapeutics Research Centre, Southern Clinical Division, Princess Alexandra Hospital, University of Queensland, Brisbane, Qld, Australia* [3]*School of Veterinary Science, University of Queensland, Gatton, Qld, Australia*

INTRODUCTION
This study was performed to determine if there was a difference in the *in vitro* penetration kinetics of hydrocortisone (HC) through the lesional and non-lesional (normal) skin of dogs with suspected flea allergy dermatitis (FAD).

MATERIALS AND METHODS
Full thickness lesional and non-lesional skin was severity scored, clipped of hair and removed from the dorsal lumbosacral and dorsocaudal thoracic regions, respectively, of five canine cadavers (A–E). The dogs were suspected of having FAD based on their distribution and types of skin lesions. Excised skin (n = 8–10 replicates/region/dog) was mounted in Franz-type diffusion cells and a solution containing 4% w/v bovine serum albumin and 0.1% w/v sodium azide in phosphate-buffered saline (PBS) added to the receptor compartments. A saturated solution of HC (24 μmol ml^{-1}) in equal volumes of PBS and ethanol was prepared, and then 1.2×10^{-4}–2.5×10^{-4} Ci of radiolabelled HC (^{3}H) was added. One millilitre of HC solution was applied to fully hydrated skin (t = 0) and the penetration of HC through the skin was measured by scintillation counting of aliquots taken from the receptor compartment of the diffusion cells at predetermined times over 30 h. Restricted (or residual) maximum likelihood (REML) mixed model analyses were performed to determine if skin lesions had a significant effect on pseudo-steady-state flux (J_{SS}), lag time (t_{lag}) or permeability coefficient (k_P).

RESULTS
Non-lesional skin was confirmed to be histologically normal and the histopathology of lesional skin was consistent with allergic dermatitis. REML mixed model analyses predicted that HC J_{SS} was 10-fold, 4-fold and 1.5-fold greater through the lesional compared to the non-lesional skin of dogs B ($P < 0.0001$), C ($P < 0.0001$) and E ($P > 0.01$), respectively, but 1.5-fold and 1.1-fold greater through the non-lesional compared to the lesional skin of dogs A ($P > 0.08$) and D ($P > 0.5$). When the penetration data for all five dogs was pooled, the model predicted that the k_P and J_{SS} of HC was more than twice as great (95% CI: 1.55–2.71 times as great; $P < 0.0001$) through the lesional compared to the non-lesional skin. There was no significant difference in the t_{lag} for HC penetration through the lesional compared to the non-lesional skin of the dogs.

CONCLUSIONS
This study has confirmed that the transdermal penetration of HC may be increased on average twofold, but could be as high as 10-fold, through the lesional compared to the non-lesional skin of dogs with suspected FAD. However, this trend is not consistent and is likely to be affected by variables such as disease severity, concurrent infections and inter-individual differences in skin characteristics.

11.6.
The biodegradation of metallic magnesium damages nasal epithelial cells and causes an inflammatory response
S. SCHUMACHER, I. ROTH, J. STAHL, W. BÄUMER & M. KIETZMANN
University of Veterinary Medicine Hannover, Hannover, Germany

INTRODUCTION
The development of bioresorbable magnesium-based implants is among the most intriguing fields of research. Degradable implants spare the risks and health care costs of removal surgeries, and magnesium as a material promises some unique advantages compared with other implant materials. It is little thrombogenic and its mechanical characteristics closely resemble those of cortical bone. This is why research so far has focused on cardiovascular and osteosynthesis applications. But a magnesium-based stent also offers a therapeutic option to maintain patency of the frontal sinus aperture in patients with chronic rhinosinusitis. We therefore tested the biocompatibility of degrading magnesium in nasal epithelial cells.

MATERIALS AND METHODS
Primary porcine nasal epithelial cells were isolated from ventral turbinates using a modification of a protocol (1) and grown to confluency before pure magnesium discs or various magnesium ion concentrations were added to the cells. Cell viability, PGE2 and IL-8 production, apoptosis and necrosis were measured as parameters of biocom−patibility. Additionally, amino acid incorporation and activation of the MAP kinases Erk1/2 and p38 were determined to obtain insight into the metabolic effects of degrading magnesium.

RESULTS
Magnesium treated cells showed reduced viability and a high degree of necrosis as well as elevated secretion of IL-8. While the cytotoxic effects seemed to be caused by an extremely high release of magnesium ions from the test materials (final concentration 41–75 mM), we could not yet identify the degradation product responsible for the increase in the proinflammatory cytokine. Amino acid incorporation was not influenced by pure magnesium, but we observed a transient Erk1/2 and a sustained p38 activation which may be involved in the

© 2012 The Authors

inflammatory response because magnesium ions did not mimic this outcome.

CONCLUSION

Magnesium is often considered an uncritical implant constituent because it is an essential element in the body and the surplus of magnesium ions resulting from biodegradation is supposed to be easily excreted via the kidneys. However, our results suggest that the local biocompatibility for the cells in direct contact is not ideal, even though we could recently show that the magnesium concentration at the tissue-implant-interface in an isolated organ model was much lower than in the present cell culture setup (2).

REFERENCES

1. Mao, H., Wang, Y., Yuan, W. & Wong, L.B. (2009) Ciliogenesis in cryopreserved mammalian tracheal epithelial cells at the air-liquid interface. *Cryobiology*, **59**(3), 250–257.
2. Schumacher, S., Stahl, J., Bäumer, W., Seitz, J.M., Bach, F.W., Petersen, L.J. & Kietzmann, M. (2011) Ex vivo examination of the biocompatibility of biodegradable magnesium via microdialysis in the isolated perfused bovine udder model. *International Journal of Artificial Organs*, **34**(1), 34–43.

© 2012 The Authors

Session 12: Antiparasitics
Wednesday 11-07: 9.00–10.30

12.1.K.
Licking behaviour affects the disposition of macrocyclic lactone pour-on formulations in cattle

A. BOUSQUET-MÉLOU
INRA, UMR1331, Toxalim, Research Center in Food Toxicology, and Université de Toulouse, Ecole Nationale Vétérinaire, Toulouse France

Pour-on formulations of macrocyclic lactones (MLs) are considered as transdermal formulations. However, the actual disposition of MLs is considerably influenced by natural licking behaviour, leading to the oral ingestion of a large fraction of the endectocide poured on the backs of cattle. Licking is a component of both self- and allo-grooming, meaning that it also determines exchanges of substances between animals in the same herd. The presentation will discuss the main studies demonstrating this phenomenon and investigate its consequences, in terms of plasma and intestinal ML disposition, intra- and inter-individual variability, as well as issues concerning residues, anthelmintic activity and resistance.

SUGGESTED READING

1. Bousquet-Mélou, A. *et al.* (2004) Endectocide exchanges between grazing cattle after pour-on administration of doramectin, ivermectin and moxidectin. *Int J Parasitol*, **34**, 1299–1307.
2. Bousquet-Mélou, A. *et al.* (2011) Licking behaviour induces partial anthelmintic efficacy of ivermectin pour-on formulation in untreated cattle. *Int J Parasitol*, **41**, 563–569.
3. Laffont, C.M. *et al.* (2001) Licking behaviour and environmental contamination arising from pour-on ivermectin for cattle. *Int J Parasitol*, **31**, 1687–1692.
4. Sallovitz, J.M. *et al.* (2005) Doramectin concentration profiles in the gastrointestinal tract of topically-treated calves: Influence of animal licking restriction. *Vet Parasitol*, **133**, 61–70.
5. Toutain, P.-L. *et al.* (2012) Should licking behavior be considered in the bioavailability evaluation of transdermal products? *Journal of Veterinary Pharmacology and Therapeutics*, **35** (Suppl. 1), 39–43.

12.3.
Differential neurotoxicity of ivermectin and moxidectin in mammals

C. MÉNEZ[1], J. F. SUTRA[1], R. K. PRICHARD[2] & A. LESPIN[1]
[1]*INRA, Toulouse Cedex 3, France;* [2]*McGill University, Montreal, Canada*

INTRODUCTION
Ivermectin (IVM) and moxidectin (MOX), belonging to the very potent antiparasitic macrocyclic lactones family, have similar modes of action through interaction with glutamate receptors in parasitic nematodes and ectoparasites, but display differences in terms of kinetic behaviour and toxicity in several host species. Toxicity of these drugs in mammals is the result of their ability to enter the brain which is essentially controlled by the P-glycoprotein (P-gp) efflux transporter and their lipophilicity. Once in the brain, toxicity occurs through interaction with host GABA(A) receptors. The objective of the study was to compare the toxicity of IVM and MOX *in vivo* and their interaction with GABA(A) receptors *in vitro*.

MATERIALS AND METHODS
Toxicity of IVM and MOX was assessed in P-gp-deficient mice after subcutaneous administration of increasing doses. Survival was evaluated over a 14-days period and the medial lethal dose (LD_{50}) was determined. Drug concentration was measured by HPLC in brain and plasma of P-gp-deficient mice 2 and 24 h after drug administration. In order to investigate the molecular mechanism of their relative toxicity, the activation of rat $\alpha_1\beta_2\gamma_2$ GABAergic Cl^- channel expressed in *Xenopus laevis* oocytes by IVM and MOX was investigated using the two-electrode voltage-clamp technique.

RESULTS
In P-gp-deficient mice, LD_{50} for IVM and MOX were 0.46 and 2.3 μmol kg^{-1}, respectively, demonstrating that MOX was less toxic than IVM. Consistent with this result, MOX had a lower brain-to-plasma concentration ratio (2.4 ± 0.7 versus 5.5 ± 1.6 ml g^{-1} for MOX and IVM, respectively, $P < 0.01$) and entered into the brain more slowly than IVM. *In vitro* electrophysiology measurement showed that IVM and MOX were both able to potentiate the GABA-induced response, demonstrating that IVM and MOX can act as partial agonist on the mammalian GABA(A) receptor when co-applied with the reference agonist GABA. MOX was almost 6-fold more potent in activating chloride currents than IVM with an EC_{50} for potentiation of the GABA(A) receptor of 5.4 ± 1.2 nM, compared with 29.3 ± 3.3 nM for IVM. However, analysis of the Hill coefficient revealed a positive cooperativity binding of IVM on the GABA receptor, suggesting different type of interactions and a higher affinity of IVM compared with MOX for the GABA channel.

CONCLUSIONS
Knowing that the contribution of P-gp in preventing the drug penetration into the brain was previously shown to be considerable for IVM and relatively low for MOX, our results suggest that any impairment of the P-gp transporters in the blood-brain barrier of mammals would have a greater effect on influencing toxicity of IVM than MOX.

12.3.
Contribution of the nuclear receptors PXR and CAR to gene expression modulations induced by fipronil in rodent liver

B. ROQUES[1], M. Z. LACROIX[2], N. MOUSSAOUI[1], J. LEGHAIT[2], S. PUEL[2], F. LASSERRE[2], H. GUILLOU[1], C. VIGIUÉ[2] & P. G. P. MARTIN[1]
[1]*INRA, UMR1331 Toxalim, Toulouse, France;* [2]*INRA, UMR1331 Toxalim, ENVT, Toulouse, France*

INTRODUCTION
Fipronil, an insecticide widely used, is a thyroid disruptor in rat inducing a marked increase in thyroxine (T_4) clearance.

© 2012 The Authors

Fipronil-induced thyroid disruption seems to require a bioactivation of fipronil via its biotransformation into fipronil sulfone by cytochromes P450 (CYP) and an induction of hepatic enzymes involved in thyroid hormone catabolism such as Udp-glucuronosyltransferases (UGT) and sulfotransferases (SULT). The nuclear receptors (NR) Pregnane X Receptor (PXR) and Constitutive Androstane Receptor (CAR) are transcription factors activated by many xenobiotics such as pesticides. Their target genes are involved in the metabolism of xenobiotics and endogenous hormones, including thyroid hormones, and, interestingly, fipronil is a ligand of the human PXR *in vitro*. We thus suspected that CAR and PXR are involved in the regulation of fipronil-induced thyroid disruption in rats. To test this hypothesis, we (i) obtained microarray gene expression profiles in fipronil-treated rats, (ii) checked by RT-qPCR that the fipronil-induced mRNA expression modulations were similar in rats and mice in order to (iii) investigate in wild-type, CAR and PXR-deficient mice the effects of fipronil on both T_4 clearance and hepatic expression of selected genes.

MATERIALS AND METHODS

Fipronil were administered daily through feeding needles for 14 days. In experiment 1, rats were treated with vehicle ($n = 14$) or fipronil 3 mg kg^{-1} per day ($n = 10$). In experiment 2, mice were treated with vehicle or fipronil 5 mg kg^{-1} per day ($n = 8$ each). In experiment 3, mice were treated with vehicle or fipronil 3 mg kg^{-1} per day ($n = 50$ wild-type, 50 CAR-deficient and 50 PXR-deficient mice each). In experiment 4, mice were treated with vehicle or fipronil 10 mg kg per day ($n = 8$ mice/genotype each). Livers were collected for microarray gene expression profiling in rats (experiment 1), to compare the effects of fipronil on hepatic mRNA expression by RT-qPCR in rats and wild-type mice (experiments 1 and 2) and in wild-type and NR-deficient mice at different dosing regimen (experiments 3 and 4). For experiment 3, total blood was collected at 0.25, 2, 4, 8, 12 and 24 h after an intraperitoneal bolus of $^{13}C_6$-LT_4 ($n = 5$–10 mice/genotype/treatment/time) to determine the effect of fipronil on $^{13}C_6$-LT_4 clearance in wild-type and NR-deficient mice.

RESULTS

In rats, fipronil increased the expression of known PXR and/or CAR target genes such as Cyp (Cyp3a1: 11-fold), Ugt and Sult (Sult1b1: 2.5-fold) and transporters involved in thyroid hormone cellular trafficking (Abcc2: 1.6-fold). T_4 clearance was higher in fipronil-treated than in vehicle-treated wild-type mice but not significantly. Furthermore, fipronil-induced mRNA expression modulations were lower in mice than in rats (6-fold, 1.5-fold and 1.2-fold increases for Cyp3a11, Sult1b1 and Abcc2, respectively). Although mice seemed to be less sensitive than rats to fipronil-induced thyroid disruption, fipronil-induced expression of PXR (Cyp3a, Abcb1a), CAR (Cyp2b, Ugt2b) or both (Ugt1a1, Abcc2) target genes were reduced in NR-deficient mice.

CONCLUSIONS

Thus, PXR and CAR contribute to modulate hepatic gene expression following fipronil treatment. Furthermore, our data combined with the known roles of PXR and/or CAR target genes suggest that these receptors could contribute to fipronil-induced thyroid disruption in rodents.

12.4.

Assessment of the inhibitory potency of benzydamine on albendazole hepatic metabolism in sheep

G. VIRKEL, M. L. MATÉ, A. LIFSCHITZ, J. M. SALLOVITZ, C. FARÍAS & C. E. LANUSSE
Facultad Ciencias Veterinarias, UNCPBA, Tandil, Argentina

INTRODUCTION

Albendazole (ABZ), a benzimidazole methylcarbamate anthelmintic compound, is still widely used to control helminth infections in all animal species and man. The hepatic ABZ S-oxidation by both flavin-monooxygenase (FMO) and cytochrome P450 systems (1, 2), accounts for a considerable reduction of its pharmacologic activity. Thus, interference with the oxidative metabolism may enhance the systemic exposure of active drug/metabolites improving its anthelmintic efficacy. Focussed in the search for new potential pharmacologic strategies to inhibit ABZ metabolism, this research assessed the effects of the FMO substrate benzydamine (BZ) on the hepatic S-oxidation of this anthelmintic in sheep.

OBJECTIVE

The effects of BZ were compared to those produced by methimazole (MTZ), a well-known FMO inhibitor of ABZ metabolism.

MATERIALS AND METHODS

Liver microsomes were obtained from adult Corriedale rams ($n = 4$) following the methodology described by Virkel *et al.* (1). MTZ S-oxidation (FMO-dependent specific activity) (3) was assayed in the absence/presence of BZ (750 μM). ABZ S-oxidation was assessed by the amount of ABZ-sulphoxide (ABZSO) formed. The substrate (100 μM) was incubated (15 min at 37°C) either in the absence or in the presence of variable concentrations (12.5–1000 μM) of either BZ or MTZ. Samples were analysed by HPLC. Statistical comparisons were performed using the Student *t*-test.

RESULTS

In pooled sheep liver microsomal fractions the Clint of the specific FMO-dependent enzyme activity (MTZ S-oxidation) was lower in the presence of BZ [53 versus 95 μl (min mg)$^{-1}$]. ABZ was metabolized to its (−) and (+) ABZ-sulphoxide (ABZSO) enantiomers. Both BZ and MTZ inhibited ABZ S-oxidation. The estimated IC50's for both inhibitors are shown in Table 1.

Table 1 Albendazole enantio-selective S-oxidation by sheep liver microsomes. Mean ± SD: IC_{50} values of the metabolic inhibitors benzydamine (BZ) and methimazole (MTZ).

	IC50 (μM)*		
	BZ	MTZ	*P*-value†
(Total) ABZSO	48 ± 5	18 ± 4	0.0004
(+) ABZSO	39 ± 2	16 ± 2	0.0006
(−) ABZSO	290 ± 141[a]	357 ± 86[a]	0.4776

*The concentration of BZ or MTZ causing a 50% inhibition of the production of either (total) ABZ-SO (+) or (−) enantiomer.
†Statistical comparison between metabolic inhibitors.
[a]Statistical different $P < 0.05$ compared to (+) ABZSO.

© 2012 The Authors

CONCLUSIONS

BZ inhibited the hepatic FMO-dependent specific enzyme activity in sheep. Both BZ and MTZ showed a stronger inhibitory potency over the (+)ABZSO production, which is believed to be catalysed by FMO in sheep. Although the inhibitory potency of BZ was lower compared to MTZ, a clinically relevant metabolic interaction after the concomitant administration of two different drugs may occur if adequate concentrations are achieved at the site of biotransformation at the same time. Thus, further studies aimed to evaluate this metabolic interaction *in vivo* are required to determine BZ potential to increase ABZ systemic exposure and efficacy in sheep.

REFERENCES

1. Virkel et al. (2004) Drug Metabolism and Disposition, **32**, 536–544.
2. Souhaili El Amri et al. (1988) The Journal of Pharmacology and Experimental Therapeutics, **246**, 758–764.
3. Dixit & Roche (1984) Archives of Biochemistry and Biophysics, **233**, 50–63.

© 2012 The Authors

Session P1: Prudent Use of Antibiotics
Wednesday 11-07: 9.00–10.30

P-1.1.
What are the public health issues that practitioners have to consider to enforce a sustainable use of antibiotics?

P.-L. TOUTAIN
INRA, UMR1331, Toxalim, Research Center in Food Toxicology, and Université de Toulouse, Ecole Nationale Vétérinaire, Toulouse France

The sustainable use of antibiotics in veterinary medicine necessitates optimizing the dosage regimen not only to minimize the development of resistance among target pathogens (an animal health issue) but also to prevent or minimize the impact of antimicrobial resistance on non-target bacteria including zoonotic pathogens (*Salmonella, Campylobacter, E. coli, etc*), animal commensal flora (mainly gut and skin flora) and also environmental flora (manure, waste, etc). These different public health issues will be presented and discussed to qualify the actual links between animal and human antimicrobial resistance and to explore the possible options that might be enforced to minimize the impact of veterinary antibiotic use on human health.

P-1.2.
Extended spectrum beta-lactamase-producing *Escherichia coli* in animals, humans and the environment

S. GÜNTHER
Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany

Extended-spectrum beta-lactamases producing *E. coli* (ESBL-*E. coli*) represent a major problem in human and veterinary medicine, particular in nosocomial infections. Additionally an onset of community acquired ESBL-*E. coli* infections, an emergence in livestock farming as well as in wildlife and the environment has been observed in recent years. These current developments suggest a successful transmission as well as persistence of ESBL-*E. coli* strains even outside clinical settings as well as a possible zoonotic spread of antimicrobial-resistant bacteria which has been recently discussed controversial. Data presented during this talk on the global molecular epidemiological background of ESBL-*E. coli* as well as the plasmids carrying ESBL-genes in humans, animals and the environment leads us to the conclusion, that the opinion that animal ESBL-producing *E. coli* are the major source of human infections is oversimplified and neglecting highly complex scenarios.

SUGGESTED READING

1. Ewers, C., Bethe, A., Semmler, T., Guenther, S. & Wieler, L.H. (2012) Extended-spectrum β-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective, *Clinical Microbiology and Infection*, DOI: 10.1111/j.1469-0691.2012.03850.x
2. Guenther, S., Ewers, C. & Wieler, L.H. (2011) Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? *Front Microbiol*, **2**, 246
3. Ewers, C., Grobbel, M., Bethe, A., Wieler, L.H. & Guenther, S. (2011) Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted! *Berl Münch Tierärztl Wochenschr*, **124**, 94–101.

© 2012 The Authors

Workshop 5: Emerging and Masked Mycotoxins Wednesday 11-07: 11.00–12.30

Coordinator: R. Krska

W-5.1.

Emerging and masked mycotoxins

R. KRSKA, F. BERTHILLER, R. SCHUHMACHER & M. SULYOK
Center for Analytical Chemistry and Christian Doppler Laboratory for Mycotoxin Metabolism, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU), Tulln, Austria

Mycotoxins are secondary metabolites of fungi which are toxic for humans or animals and can be found on a great variety of cereals, food and feed commodities. Food and feed is not necessarily safe, just because the presence of well-known mycotoxins has been ruled out, as they might still be there in disguise. Mycotoxins may also occur in conjugated form, either soluble (masked mycotoxins) or incorporated/associated/attached to macromolecules (bound mycotoxins). These conjugated mycotoxins can emerge after metabolisation by living plants, fungi or mammals and after food processing (Berthiller *et al.*, 2009). Awareness of such altered forms of mycotoxins is increasing, but still reliable analytical methodology, standards, occurrence and toxicity data are lacking. In this paper currently known conjugated mycotoxins, their formation and determination is reviewed. For the latter, especially liquid chromatography – (tandem) mass spectrometry [LC-MS (/MS)] methods are employed. Sample preparation to transfer the bound into soluble forms can involve enzymatic or acidic/alkaline treatment. Especially mycotoxins which are in contact with living plants on the field are prone to be metabolized. This transformation process is not only important regarding food and feed safety but also for the resistance of plants towards fungal induced diseases, like *Fusarium* head blight of wheat. Most of the occurrence studies have emphasized on 'traditional' mycotoxins, such as aflatoxins, ochratoxin A, zearalenone, fumonisins and trichothecenes (Jestoi, 2008). However, in addition to masked mycotoxins, concerns have also been raised recently about so called emerging mycotoxins. These are usually referred to as (unusual) toxins especially produced by *Fusarium* spp. which have become quantifiable due to the availability of high performance mass spectrometric tools, such as LC-MS/MS. These include e.g. enniatins, beauvericin, fusaproliferin, moniliformin and even ergot alkaloids have been referred to this 'new' group of toxins. So far, only limited data is available on these emerging metabolites. In addition, the authors feel that the topic of emerging toxins shall also be considered in view of global warming which has been causing unusal toxin/commodity combinations in several countries. In this context, an LC-MS/MS method for multi-mycotoxin determination was also applied for a semi-quantitative screening of 87 mouldy food samples from private households, including bread, fruits and vegetables (Sulyok *et al.*, 2010). In the 247 investigated sub-samples, 49 different analytes were identified, some of which were never reported before to occur in naturally contaminated food. Enniatins and ergot alkaloids occurred in all samples of (dark) bread/pastries at low $\mu g\ kg^{-1}$-levels. Regulated mycotoxins occurred less often, but the corresponding concentrations exceeded the regulatory limits up to a factor of 1000 in case of patulin.

SUGGESTED READING

1. Berthiller, F., Schuhmacher, R., Adam, G. & R. Krska (2009) Formation, determination and significance of masked and other conjugated mycotoxins. *Analytical and Bioanalytical Chemistry*, **395** (5), 1243–1252.
2. Sulyok, M., Krska, R. & Schuhmacher, R. (2010) Application of an LC-MS/MS based multi-mycotoxin method for the semi-quantitative determination of mycotoxins occurring in different types of food infected by moulds. *Food Chemistry*, **119** (1), 408–416.
3. Jestoi, M. Emerging fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: a review. *Critical Reviews in Food Science and Nutrition*, **48** (1), 21–49.

W-5.2.

The plasma clearance of the *Fusarium* toxin deoxynivalenol is decreased in endotoxemic pigs

S. DÄNICKE[1], B. BROSIG[1], S. KAHLERT[2], P. PANTHER[2], A. K. DIESING[2], J. KLUESS[2], S. KERSTEN[1], H. VALENTA[1] & H. J. ROTHKÖTTER[2]
[1]*Institute of Animal Nutrition, Braunschweig, Germany;* [2]*Institute of Anatomy, University of Magdeburg, Magdeburg, Germany*

INTRODUCTION

Deoxynivalenol (DON) belongs to the *Fusarium* mycotoxins and is frequently detected in cereal grains used as feedstuffs in concentrations high enough to cause adverse effects in pigs. Among others, DON reduces feed intake and acts immunomodulatory in pigs. In laboratory animals an acute low-dose trichothecene exposure influences the immune function by induction of a rapid up-regulation of pro-inflammatory cytokines (Pestka *et al.*, 2004). Lipopolysaccharides (LPS) are cell wall components of gram-negative bacteria and mechanistically, LPS play a critical role in inflammatory responses (Islam and Pestka, 2006). The organism might be exposed to LPS due to gram-negative bacterial infection or via absorption from the gastro-intestinal tract. DON exposure resulted in an increased paracellular permeability to *Escherichia coli* (Pinton *et al.*, 2009). Consequently, one could assume that DON might pave the way for an increased translocation of bacteria and thus LPS from chyme to the liver and eventually to the systemic circulation. At the systemic level it has been demonstrated in mice that LPS priming prior to DON exposure potentiated the expression of pro-inflammatory cytokines compared to DON exposure alone (Islam and Pestka, 2006). Besides the possible interactions between

© 2012 The Authors

DON and LPS at the molecular level, the toxicokinetic behavior of DON in the presence of LPS could be an additional factor influencing the overall interaction between both substances.

MATERIALS AND METHODS

The plasma elimination kinetics of the *Fusarium* toxin deoxynivalenol (DON) was investigated in male castrated pigs (40.4 ± 3.7 kg) when infused intravenously either alone (100 μg kg^{-1} per h, $n = 6$) or together with LPS (7.5 μg kg^{-1} per h, $n = 6$).

RESULTS

The maximum DON concentration at terminating the infusion after 1 h of the DON + LPS group was significantly higher by 61% than that of pigs infused with DON alone. The AUC of the DON + LPS group was approximately twice as high as that of the DON group after 24 h while the initial (0.63 versus 0.6 h) and terminal half-lifes (2.97 versus 2.30 h) remained uninfluenced. The apparent volume of distribution and the plasma clearance were significantly lower for the DON + LPS group compared to the DON group (2.14 versus 1.45 l kg^{-1} and 11.9 versus 5.87 ml kg^{-1} per minutes). Glucuronidated DON seemed to persist longer in the DON + LPS group.

CONCLUSIONS

The clearance of DON was delayed during an LPS induced acute phase reaction in pigs. Whether the higher plasma DON concentrations in endotoxemic pigs are due to a hemodynamically associated longer persistence of the DON glucuronide or associated with an altered glucuronidation activity needs to be examined further.

REFERENCES

1. Islam, Z., & Pestka, J.J. (2006) LPS priming potentiates and prolongs proinflammatory cytokine response to the trichothecene deoxynivalenol in the mouse. *Toxicol Appl Pharmacol*, **211**, 53–63.
2. Pestka, J.J., Zhou, H.R., Moon, Y., & Chung, Y.J. (2004) Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. *Toxicol Lett*, **153**, 61–73.
3. Pinton, P., Nougayrede, J.P., Del Rio, J.C., Moreno, C., Marin, D.E., Ferrier, L., Bracarense, A.P., Kolf-Clauw, M., & Oswald, I.P. (2009) The food contaminant deoxynivalenol, decreases intestinal barrier permeability and reduces claudin expression. *Toxicol Appl Pharmacol*, **237**, 41–48.

W-5.3.

Beauvaricin, alternariol and alternariol monomethyl ester affect maturation of porcine oocytes by different mechanisms

E. J. SCHOEVERS, R. R. SANTOS & B. A. J. ROELEN
Utrecht University, Utrecht, The Netherlands

INTRODUCTION

Beauvaricin (BEA), Alternariol (AOH) and Alternariol monomethyl ester (AME) are metabolites from *Alternaria* and *Fusarium* moulds. These toxins can cause DNA damage and induce apoptosis. By their genotoxic properties oocytes may be particularly vulnerable for these toxins, especially when they resume meiosis. The objective of this study is to determine how BEA, AOH and AME exert a possible toxic effect on the oocyte during maturation.

MATERIALS AND METHODS

Porcine cumulus oocyte complexes were cultured in M199 with 10% porcine follicular fluid for 44 h. For the first 22 h of culture recombinant human FSH was present at a concentration of 0.05 IU ml^{-1}, whereas BEA, AOH and AME were present during the entire culture period at a concentration of 1, 5 10 and 20 μM in 0.05% DMSO. After culture, cumulus cells were removed and the remaining oocytes were fixed and stained for chromation, tubulin and actin, examined by fluorescence microscopy and classified as Germinal Vesicle (GV), Metaphase I (MI), Anaphase I (AI) Telophase I (TI), Metaphase II (MII) or degenerated.

RESULTS

Similar proportions of oocytes were at GV, MI, AI, TI, MII stages or degenerate when exposed to 1 or 5 μM BEA and did not differ from the control group. When exposed to 10 or 20 μM BEA, all oocytes were degenerated. With all concentrations, AOH and AME treated oocytes exhibited higher percentages at the TI stage (14–30%) compared with control oocytes (4%). When exposed to 10 or 20 μM AOH more oocytes exhibited irregular spindles with condensed chromatin or abnormal polar bodies at the MII stage or became degenerated.

CONCLUSION

We conclude that the mechanism of toxicity of BEA is different than that of AOH or AME, but the mechanisms of toxicity need to be further elucidated. In future experiments embryo development will be studied after oocyte exposure to BEA, AOH or AME.

W-5.4

Toxic effects of dietary exposure to T-2 toxin on intestinal and hepatic CYP450 in broilers

A. OSSELAERE, S. J. LI, L. DE BOCK, M. DEVREESE, J. GOOSSENS, V. VANDENBROUCKE, F. P. PASMANS, A. M. MARTEL, P. DE BACKER & S. CROUBELS
Ghent University, Merelbeke, Belgium

INTRODUCTION

Trichothecenes are a group of structurally related secondary metabolites produced by various *Fusarium* species and are widely encountered as contaminants in cereals and agricultural products. Among trichothecenes, T-2 toxin is the most potent. The main uptake route for mycotoxins is feed and food ingestion and thus epithelial cells of the digestive tract can be considered as primary targets. On the other hand, the liver and its cytochrome P450 enzymes are known to play an important role in the biotransformation of T-2 toxin into different derivatives. Until now, little information is available on the effects of T-2 toxin on intestinal and hepatic CYP450 enzymes in broilers.

MATERIALS AND METHODS

Twenty-four, 1-day-old, broiler chickens were obtained from a local hatchery. Animals were allowed to acclimatize for 1 week to the experimental environment with free access to water and a commercial diet free of T-2 toxin. Afterwards, three groups of eight broilers were allocated to different treatment groups. One group received blank feed, the second group received feed contaminated

© 2012 The Authors

with T-2 at a level of 68.0 μg kg^{-1} and the last group also received T-2 contaminated feed, but at a higher level of 752 μg kg^{-1}. During the 21-days experimental period, animals were allowed unrestricted access to fresh water and their respectively designated diets. At the end of the trial, animals were euthanized and liver and intestinal sections were flash-frozen in liquid nitrogen and stored at −80°C until analysis. An RT-PCR method was developed to measure the mRNA expression of CYP1A4, CYP1A5 and CYP3A37. Two different methods were compared for the preparation of intestinal microsomes in broilers. The enzymatic activity of CYP3A37 in liver and small intestines was also measured, applying the substrate midazolam to the microsomal fraction of the respective organs (1).

RESULTS AND CONCLUSIONS

CYP3A37 and CYP1A5 were significantly down-regulated at mRNA level in the liver when comparing the group receiving the highest concentration of T-2 toxin and the control group. Moreover, CYP1A4 was significantly down-regulated in both groups receiving contaminated feed compared to the control. Since the latter already takes place at a common contamination level of 68 μg kg^{-1} T-2 in the feed, this can have consequences for the biotransformation of many substrates such as drugs. In the intestine however, no significant differences could be observed in mRNA expression between groups. Remarkably, enzymatic activity of CYP3A37 was significantly elevated in the ileum and liver in the group receiving the highest level of T-2 compared to the control, which can possibly influence the biotransformation of T-2 itself.

REFERENCES

1. De Bock, L., Boussery, K., Colin, P., De Smet, J., T'Jollyn, H. & Van Bocxlaer, J. (2012) Development and validation of a fast and sensitive UPLC-MS/MS method for the quantification of six probe metabolites for the *in vivo* determination of cytochrome P450 activity. *Talanta*, **89**, 209–216.

W-5.5.

Efficacy of yeast by products added in poultry feed for decreasing ochratoxin A genotoxicity

A. PFOHL-LESZKOWICZ[1], K. HADJEBA-MEDJDOUB[1], J. A. SCHRICKX[2], N. BALLET[3] & J. FINK-GREMMELS[2]
[1]*Institut National Polytechnique Toulouse, Auzeville-Tolosane, France;* [2]*Utrecht University, Utrecht, The Netherlands;* [3]*Lesaffre, Lille, France*

INTRODUCTION

Ochratoxin A (OTA) a mycotoxin contaminating mainly cereals but also other crops, can be accumulated in meat, and is not destroyed by food processing. It is nephrotoxic for pigs and poultry, and is implicated in Balkan endemic nephropathy and associated urothelial cancer in human (Pfohl-Leszkowicz & Manderville, 2012). One of the most promising and economical strategies for reducing animal exposure to mycotoxins is the utilization of adsorbents in feed to reduce gastrointestinal absorption of mycotoxins. The aim of this paper is to evaluate the capacity of several yeasts to decrease genotoxicity of OTA and establish if the decrease is only due to adsorption of OTA on the yeast product.

MATERIALS AND METHODS

Ten chickens per group were fed 2 days with feed including yeast by-products, and then were fed 7 days with feed including yeast by-product and OTA. Genotoxicity was evaluated by detection of DNA-adduct using P^{32} post labelling method. In addition OTA derivatives formed in liver and kidney of poultry were analysed after extraction by HPLC coupled to fluorimetric detection. In parallel, to establish more specifically the contribution of yeast enriched either by glutathione (GSH) or selenomethinonine (SE), human renal cells were exposed to OTA (10 μM) alone or in presence of GSH-Yeast (10 μM) or with SE-Yeast (10 μM). Viability of cells was evaluated using the MTS test. Genotoxicity was analysed by DNA adduct detection, were analyzed under the same conditions as described above for the poultry samples.

RESULTS

Addition of all yeast by-products in feed of poultry reduced the amount of OTA in bile, plasma, liver and kidney except with GSH-yeast. DNA adducts were significantly decreased in the liver. In the kidneys, the number of DNA adducts was increased in the SE-Yeast group, whereas it decreased in all other groups. In cell culture, OTA significantly decreased cell viability (60%; $P < 0.01$) and induced the formation of two OTA-DNA-adducts. Addition of pure GSH or GSH-Yeast partially restores cells viability (70% versus 60%; $P < 0.05$) and avoid DNA adduct formation, explained by conversion of OTA into OTB and 4-OH-OTA. Pure SE does not restore viability whereas SE-yeast has an antagonistic effect (110% versus 60%; $P < 0.01$). SE and SE-yeast increase OTA-DNA adduct formation correlated with the appearance of new OTA metabolites, notably quinone derivatives.

CONCLUSIONS

The decrease of OTA toxicity observed with yeast was not only correlated with adsorption but also with biotransformation of OTA which is modulated by yeast. DNA adduct patterns were correlated with OTA derivatives formed in the kidney. GSH-yeast is better to decrease OTA genotoxicity.

REFERENCES

1. Pfohl-Leszkowicz, A. & Manderville, R.A. (2012) An update on direct genotoxicity as molecular mechanism of ochratoxin A carcinogenicity. *Chem Res Toxicology*, **25**, 252–262.

W-5.6.

Oligosaccharides: a promising new approach for minimizing the pathological effects of deoxynivalenol on the intestinal tract

S. BRABER[1], P. AKBARI[1], A. D. KRANEVELD[2], G. FOLKERTS[2], J. GARSSEN[3] & J. FINK-GREMMELS[1]
[1]*Faculty of Veterinary Medicine, IRAS, Utrecht University, Utrecht, The Netherlands;* [2]*Division of Pharmacy, Faculty of Life Sciences, Utrecht University, Utrecht, The Netherlands;* [3]*Danone Research Center for Specialised Nutrition, Wageningen, The Netherlands*

INTRODUCTION

Non-digestible oligosaccharides (NDO) have demonstrated to not only exert beneficial effects on the composition and activity of the intestinal microflora in the gastrointestinal tract, but these compounds have also been associated with immune-modulating effects in the intestine. One of the most prominent sources of

© 2012 The Authors

oligosaccharides is milk and it is established that particularly collostrial oligosaccharides modulate the enteric immune response. Mycotoxin exposure, a worldwide problem in the livestock industry, causing next to acute intoxication, immunosuppression and gastro-intestinal illness. Specific lesions observed at the intestinal level include induction and promotion of inflammatory reactions and an increased susceptibility to intestinal infections, losses in productivity and reduced weight gain. One of the most prevalent mycotoxins in European cereals and cereal products, important sources of energy and protein for all classes of farmed livestock, is the trichothecene deoxynivalenol (DON). The aim of this study is to investigate the effect of DON on epithelial integrity and to test the hypothesis whether specific oligosaccharides can mitigate the DON related pathological effects at the intestinal epithelial layer.

MATERIALS AND METHODS

Using the human epithelial intestinal cell line Caco2 in a transwell system as an *in vitro* model, we studied the effect of DON on transepithelial electrical resistance (TEER), lucifer yellow (LY) permeability, tight junction protein expression and IL-8 release. In this study the ability of NDO, to inhibit the DON-related effects was investigated.

RESULTS

The TEER of Caco-2 cells was dose-dependently reduced following DON exposure and a significant increase in LY permeability was observed after 24 h. These alterations of the intestinal barrier function were associated with an increase in mRNA expression of occludin, claudin1, claudin3, claudin4, ZO-1 and ZO-2 after 6 h incubation with DON and an increase in IL-8 production after 24 h. Interestingly, after 24 h incubation with NDO the DON-induced impairment of the intestinal barrier was mitigated and the increased IL-8 levels in the apical as well as the basolateral chamber induced by DON were significantly decreased. Furthermore, the claudin4 mRNA expression is further increased after incubation with NDO.

CONCLUSIONS

We conclude that NDO can restore the DON-induced effect on epithelial barrier function and exert an anti-inflammatory effect by reducing IL-8 levels. The results suggested that the intake of galacto-oligosaccharides can reduce the direct effects of DON on the intestinal epithelial integrity. Taken together, the dietary application of specific oligosaccharides to mitigate pathological effects of the fungal toxin DON related to intestinal epithelial cells offers a promising concept for mycotoxin exposure intervention.

W-5.7.

In vitro evaluation of toxin binding efficacy for aflatoxin B1, ochratoxin A, citrinin, zearalenone, T2 toxin, penicillic acid and fumonisin in broiler feeds

G. GHADEVARU

Tamil Nadu Veterinary and Animal Sciences University, Chennai, India

INTRODUCTION

Food safety is concerned about the carry-over of mycotoxins and their metabolites into animal tissues, milk, and eggs after intake of contaminated feed. One of the most practical approaches is the use of non-nutritive adsorbents, which bind mycotoxins, inhibit their absorption from the gastrointestinal tract, and reduce toxicity (1). An *in vitro* model was used to evaluate a commercial adsorbent, by quantifying free mycotoxins using high performance thin layer chromatography method. A multi mycotoxins method was used for extraction and quantification (2).

MATERIALS AND METHODS

In vitro binding ability of a commercial binder (0.2%) on aflatoxin B1 (AF) (500 ppb), ochratoxinA (OA) (1 ppm), citrinin (CT) (100 ppb), zearalenone (ZEA) (1 ppm), Penicillic acid (PA) (500 ppb) Fumonisin (FM) 1 ppm and T-2 toxin (T-2) (1 ppm), when presented alone or in combination, was evaluated at pH4.5 and 6.5 in the diets. Compounded broiler finisher feed weighing 25 g, was added into a 250 ml glass conical flask and the required quantity of mycotoxins standard added to arrive at the desired level of toxin. Binder added at a concentration of 0.2% to these flasks whereas the feed in control flasks of the respective treatment was left untreated. Citric acid-sodium phosphate buffer (100 ml) of the desired pH (4.5/6.5) was added to each flask and the contents incubated at 37°C for 3 h. From each flask, the content was filtered and dried at 37°C for 2 h, while the respective toxin was extracted from the content and quantified. The percentage difference in the toxin content between the beginning and the end of trials in binder treated and control flasks were calculated. The binding of each toxin in different treatments was determined by subtracting the percentage difference in toxin content of the control flasks from that of the treated flasks in the respective treatments (3).

RESULTS

The tested binder showed significantly higher binding ability for AF, whereas those recorded for other toxins were moderate. Binding of each toxin decreased as the number of toxins in feed increased. Higher binding ability for this binder was significantly ($P < 0.05$) noticed at 6.5 pH of the medium.

CONCLUSIONS

In vitro evaluation seems to be a good screening method before actually incorporating binding materials in feed for the control of toxins at feed mills.

REFERENCES

1. Phakamile, T.M., Govinden, R. & Odhav. B. (2008) *African Journal of Biotechnology*, **7**, 2239–2243.
2. Lee, K.Y., Colin, F., Poole, P. & Zlatkis, A. (1980) *Analytical Chemistry*, **52**, 837–842.
3. Manafi, M., Narayanaswamy, H.D. & Pirany, N. (2009) *African Journal of Agricultural Research*, **4**, 141–143.

© 2012 The Authors

Workshop 6: Antiparasitics
Wednesday 11-07: 11.00–12.30

Coordinator: C. Lanusse

W-6.1.
New for Old: anthelmintic mechanisms, resistance, and future

R. J. MARTIN
Iowa State University, Ames, IA, USA

W-6.2.
O-dentatum levamisole receptors: expression illustrates heterogeneity and aspects of resistance

R. J. MARTIN[1], S. K. BUXTON[1], C. NEVEU[2], A. P. ROBERTSON[1] & C. L. CHARVET[2]
[1]*Iowa State University, Ames, IA, USA;* [2]*INRA, UMR1282, Infectiologie et Sante Publique, Nouzilly, France*

INTRODUCTION
The mode of action of three important classes of anthelmintic (cholinergics, macrocyclic lactones, octadepsipeptides) involves increased opening of membrane-ion channels but our knowledge of details of the mode of action of, and mechanisms of resistance to, these compounds is limited. We have used levamisole-sensitive and levamisole-resistant *O. dentatum* as a model to advance our understanding. We have observed in single-channel patch-clamp studies that there are four subtypes of nicotinic acetylcholine receptor (nAChR) present on muscle when activated by high concentrations of levamisole: G25, G35, G40 & G45 (Robertson *et al.*, 1999); and that the G35 subtype is missing in the levamisole-resistant isolate. We have hypothesized that the different nAChR subtypes were due to different combinations of subunits.

MATERIALS AND METHODS
We identified the putative levamisole receptor subunits: Ode-UNC-63, Ode-UNC-38, Ode-UNC-29 and Ode-ACR-8 and expressed them in different combinations in *Xenopus laevis* oocytes along with the ancillary proteins (Hco-UNC-74, Hco-UNC-50 and Hco-RIC-3). Currents activated by acetylcholine, levamisole, pyrantel, tribendimidine, bephenium, nicotine and thenium were recorded by two-microelectrode voltage-clamp at −60 mV. We also cloned a truncated Ode-ACR-8R subunit that was present in the levamisole-resistant isolate.

RESULTS
We observed that four subunit combinations (a, UNC-63: UNC-38:UNC-29: ACR-8; b, UNC-63: UNC-38:UNC-29; c, UNC-63: UNC-29: ACR-8 and; d, UNC-63: UNC-29) produced four functional, pharmacologically diverse receptors. a, was most sensitive to levamisole; b, was most sensitive to pyrantel and tribendimidine; c, was most sensitive to acetylcholine, d, was most sensitive to pyrantel and less sensitive to tribendimidine. When ACR-8R was substituted for the full length ACR-8, the expected a subtype changed to a b subtype (thus the receptor lost its sensitivity to levamisole but retained sensitivity to pyrantel) which suggests a mechanism of resistance to levamisole.

CONCLUSIONS
Here we have recapitulated the pharmacology of the levamisole-sensitive receptors expressed in the *O. dentatum* worms. We confirm that levamisole receptors are a heterogeneous population of channel subtypes and demonstrate that different subunit combinations generate receptor subtypes with distinct pharmacological profiles/patterns. Arrangement of the pentameric receptor ion-channel is plastic; and that truncation of receptor subunits can lead to re-arrangement of the pentameric ion-channel giving rise to a change in the pharmacological sensitivity of the receptor. Our work will help modeling the mode of action of other cholinergic drugs and is paving the way for deciphering the levamisole resistance molecular mechanisms in *O. dentatum*.

REFERENCE
1. Robertson, A.P., Bjorn, H.E. & Martin, R.J. (1999) Levamisole resistance recorded at the single-channel level. *FASEB Journal*, **13**, 749–769.

W-6.3.
Comparative pharmacodynamics and pharmacokinetics of moxidectin and ivermectin endectocides: a brief update

R. K. PRICHARD[1], A. LESPINE[2] & C. MÉNEZ[2]
[1]*McGill University, Montreal, QC, Canada;* [2]*INRA, Toulouse, France*

Moxidectin and ivermectin contain a common macrocyclic lactone (ML) ring, but are fermentation products of different organisms. The principal structural difference is that ivermectin, belonging to the avermectin sub-class of MLs, have sugar groups at C13 of the macrocyclic ring, whereas moxidectin, belonging to the milbemycin sub-class of MLs, is protonated at C13. Moxidectin also differs from the avermectins by having a methoxime at C23 and a substituted olefinic side chain at the 25-position. Ivermectin and moxidectin have similar, but not identical broad-spectrum activity against nematodes and arthropods. The longer half-life of moxidectin and its safety profile, allow it to be used in various long-acting formulations. Some important differences between moxidectin and ivermectin in their interactions with various invertebrate ligand-gated ion channels are known and could be the basis of different efficacy and safety profiles. Ivermectin shows profound inhibitory effects on nematode and insect development, and on pharyngeal pumping at nM concentrations. Similar effects are only seen at much higher concentrations of moxidectin. In addition, at nM concentrations ivermectin initially induces an increase in nematode motility while moxidectin is paralytic. Modeling of ivermectin's interaction with glutamate-gated ion channels suggests different interactions will occur with moxidectin because of important structural differences associated with the

© 2012 The Authors

absence of sugar groups and the substitution of the C23 methoxime. Similarly, profound differences between moxidectin and ivermectin are seen in interactions with ABC transporters in mammals and nematodes. While ivermectin is a high affinity inhibitor of ABCB transporters, moxidectin is a much less effective inhibitor of both mammalian and nematode P-glycoproteins. Moxidectin has markedly greater lipophilicity than ivermectin and this leads to a longer elimination half-life and greater volume of distribution. These differences are important for pharmacokinetics, toxicity in animals with defective transporter expression, and probable mechanisms of anthelmintic resistance. Moxidectin has lower mammalian toxicity and ecotoxicity than ivermectin. Resistance to ivermectin has become widespread in parasites of some hosts and moxidectin resistance also exists and is increasing. While there is some degree of cross resistance between ivermectin and moxidectin, ivermectin resistance and moxidectin resistance are not identical. In many cases when resistance to ivermectin is noticed, moxidectin is often fully effective at recommended dose rates. These similarities and differences should be appreciated for optimal decisions about parasite control, delaying, managing or reversing resistances, and also for appropriate anthelmintic combination.

W-6.4.

The new anthelmintic monepantel: pattern of distribution to gastrointestinal contents and mucosal tissues in sheep

A. LIFSCHITZ, G. M. BALLENT, G. VIRKEL, J. M. SALLOVITZ, P. VIVIANI, L. MATÉ & C. E. LANUSSE
Facultad Ciencias Veterinarias, UNCPBA, Tandil, Argentina

INTRODUCTION

The amino-acetonitrile derivatives (AADs) are a new class of anthelmintics (Kaminsky *et al.*, 2008) with activity against a wide range of gastrointestinal (GI) nematodes including those that are resistant to other antiparasitic drugs. The plasma disposition of monepantel (MNP) and its main metabolites monepantel sulphone (MNPSO2) was characterized in sheep (Karadzovska *et al.*, 2009). However information on drug concentrations at target tissues is necessary to understand their pharmacological action. The current work aimed to study the relationship between MNP and MNPSO2 concentrations measured in plasma and in GI tissues of parasite location in sheep.

MATERIALS AND METHODS

Twenty-two (22) healthy Romney Marsh lambs were used following internationally accepted welfare guidelines. Lambs received MNP (Zolvix®; Novartis) orally administered at 2.5 mg kg^{-1}. Blood samples were collected from six animals between 0 and 14 days post-treatment (plasma disposition study). Additionally, 16 lambs were sacrificed at 8, 24, 48 and 96 h post-administration to study the drug concentrations at GI tissues. MNP and MNPSO2 concentrations in plasma and GI tissues (abomasal and intestinal contents and mucosal tissue) were determined by HPLC.

RESULTS

MNP was rapidly oxidized to MNPSO2. MNP plasma concentrations were significantly lower than those observed for MNPSO2. The peak plasma concentrations were 15.1 (MNP) and 61.3 ng ml^{-1} (MNPSO2). The ratio between the systemic availability (AUC values) of MNPSO2 and MNP was 12. Markedly higher concentrations of MNP and MNPSO2 were measured in abomasal and duodenal contents and mucosal tissue compared to those recovered in the bloodstream. The mean MNP and MNPSO2 concentrations in abomasal contents at 24 h post-treatment were 3943 and 204 ng g^{-1} respectively, whereas the mean plasma concentrations were 10.8 (MNP) and 60.3 (MNPSO2) ng ml^{-1}. The biliary secretion of MNPSO2 may contribute to its high concentration recovered in the duodenal content (557 ng g^{-1} at 48 h post-treatment). High concentrations of MNP and MNPSO2 were quantified in all the investigated mucosal tissues. Concentration profiles of both molecules in mucosal tissues reflected their plasma disposition. The concentrations for MNP and MNPSO2 in duodenal mucosa at 24 h post-administration were 293 and 1018 ng g^{-1}, respectively.

CONCLUSION

Although MNP is converted to MNPSO2 in the liver, the large concentrations of both compounds detected over the first 48 h post-treatment in the abomasum and small intestine (duodenum) may greatly contribute to the efficacy against the GI nematodes. Considering that MNPSO2 has shown *in vitro* activity equivalent to MNP, the high levels of MNPSO2 measured in the digestive tract, particularly at the mucosal tissue, may also contribute to the potent nematodicidal activity of the new anthelmintic molecule. The pharmacological information provided here is relevant to achieve a long-standing sustainable use of this novel chemical tool for parasite control.

REFERENCES

1. Kaminsky, R., Ducray, P., Jung, M., Clover, R. *et al.* (2008) *Nature*, **452**, 176–180.
2. Karadzovska, D., Seewald, W., Browning, A., Smal, M., Bouvier, J. & Giraudel, J. (2009) *Journal of Veterinary Pharmacology and Therapeutics*, **32**, 359–67.

© 2012 The Authors

Session P2: Antiparasitics in Companion Animal Practice (Bayer Workshop) Wednesday 11-07: 11.00–12.30

P-2.1.

One Health – a moving target for health professionals

G. VON SAMSON-HIMMELSTJERNA
Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

The concept of 'One Health' supports and requires the interdisciplinary collaboration of all those active in the field of human and animal health. As it is formulated by the 'One Health Initiative' through this collaboration synergism is expected to derive, which will lead to accelerated biomedical discoveries, enhanced public health, expanded scientific knowledge, improved medical education and clinical care. Specifically the field of veterinary parasitology offers manifold opportunities to follow this concept by the interaction of veterinarians, general physicians, pediatrics, public health officers and the pharmaceutical industry, to name just a few of the key stakeholders. In the context of 'One Health' awareness needs to be significantly expanded also with respect to parasites of pet animals, including for example zoonotic gastrointestinal and vector-borne infections.

P-2.2.

Seresto® – an innovative depot formulation for season long protection of dogs and cats from fleas and ticks

D. STANNECK
Bayer Animal Health, GmbH, Leverkusen Germany

P-2.3.

Safety assessment for a novel imidacloprid 10%/ flumethrin 4.5% polymer matrix collar (Seresto®) for season long protection of dogs and cats against fleas and ticks

G. SCHMUCK[1], D. STANNECK[2] & K. J. KRIEGER[2]
[1]*Bayer HealthCare AG, Wuppertal, Germany;* [2]*Bayer Animal Health GmbH, Leverkusen, Germany*

A novel, odour free, water resistant polymer matrix collar, containing 10% imidacloprid and 4.5% flumethrin called 'Seresto', has been approved by European regulatory authorities for use in dogs as of 7 weeks of age and in cats as of 10 weeks of age for the treatment and prevention of flea infestations for 7–8 months, and for the control and repellency (anti-feeding effect) of tick larvae, nymphs and adults in dogs and cats for 8 months.

Imidacloprid and flumethrin were well known substances used in crop protection or livestock. For both, a complete toxicological package according to the guidelines in regard to animal numbers and duration including acute and repeated administration in rodents, repeated administration in dogs, chronic and lifelong administration (carcinogenicity studies) in rodents, genotoxicity studies, immunotoxicity and neurotoxicity studies as well as studies on reproduction and development were performed. These packages are usually performed to assess the safety of the consumer on one side (MRL = maximum residue level), but also for the user (farmer). For Seresto, these data were used to determine the safety for the veterinarian and the user including pet owners and all family members, but also for the target animals cats and dogs.

Safety of the treatment was assessed based on the dermal and oral toxicity of the single compounds (toxicological package) and the combination toxicity in rats. Here, both active ingredients were given together in a sublethal dose (150 mg kg^{-1} bodyweight imidacloprid and 5 mg kg^{-1} bodyweight flumethrin) to investigate a potential synergistic effect. Due to the lack of target, imidacloprid reacts only with the insect nicotinic receptor, and no synergistic effects were observed. This was confirmed in target animal safety studies in kittens, cats, pups and dogs, where 5× overdose (five collars in parallel!) did not induce any toxic side effects.

Safety of the treatment is also underlined by metabolism studies (radioactive), which show that both active substances were quickly metabolized and excreted. Additionally, dermal penetration of both substances is very low. A special focus was directed to the safety of flumethrin in cats. An oral toxicity study in cats showed that 10 mg kg^{-1} bodyweight was tolerated without any clinical symptoms. This is in the same range as in dogs. This demonstrated clearly that no species differences exist with flumethrin in cats and dogs.

P-2.4.

Distribution of flumethrin and imidacloprid in stratum corneum of dogs after application of a Seresto® polymer matrix collar

W. BÄUMER[1], J. STAHL[1], R. KREBBER[2] & K. J. KRIEGER[3]
[1]*University of Veterinary Medicine Hannover, Hannover, Germany;* [2]*Bayer CropScience AG, Monheim am Rhein, Germany;* [3]*Bayer Animal Health GmbH, Leverkusen, Germany*

INTRODUCTION

Fleas and ticks are the most prevalent ectoparasites on dogs and cats, they cause diseases and they are vectors for pathogens like virus, bacteria, and protozoa, including those with zoonotic relevance. A novel, odour free, waterproof polymer matrix collar, containing 10% imidacloprid and 4.5% flumethrin (Seresto®) has recently been approved for dogs and cats. The long duration of efficacy is based on skin compatible neutral oil derivatives which provide a continuous release of the active ingredients. Their bioavailability on the coat of treated dogs has been demonstrated for 8 months (1). Aim of this study was to demonstrate the diffusion of imidacloprid and flumethrin into the horny layers of the dog skin.

MATERIALS AND METHODS

Six laboratory beagles were shaved on the back 5 cm apart from the collar and at a second area on the left hind leg. Ten

© 2012 The Authors

consecutive adhesive tape stripes were collected at each site before application of the collar. Further samples were taken 24 h, 14 and 28 days after applying the collar. The concentration of flumethrin and imidacloprid was determined in the samples by means of HPLC-MS/MS.

RESULTS

No flumethrin or imidacloprid was found in samples before application of collars. Twenty-four hours after application of the collar 20–140 ng flumethrin was found cumulatively in the tape strips. After 14 and 28 days this concentration increased to 1000–1200 ng (back as well as hind leg region). Imidacloprid was already found at high concentration after 24 h (between 4 and 11 μg). These high concentrations were found consistently at later time points (day 14 and 28: 3–7 μg). The distribution of flumethrin in horny layers shows different kinetics compared to permethrin distributed after spot on formulations. By the turnover of the horny layer, permethrin was only measurable in upper horny layers 28 days after application of spot on formulations and the concentration decreases in an exponential order from day 1 to day 28 (Lüssenhop *et al.*, 2012). By contrast due to a continuous release of the active ingredients from the collar there is an initial increase and steady high concentration of flumethrin for 28 days.

CONCLUSIONS

Taken together, these results indicate that flumethrin/imidacloprid is set free continuously and that the horny layers are a second important reservoir apart from the hair coat.

REFERENCES

1. Krieger, K. J., Krebber, R., Delport, P. & Stanneck, D. (2012) Hair kinetics of a novel imidacloprid 10% / flumethrin 4.5% polymer matrix formulation for season long flea and tick prevention in dogs and cats. Proceedings Voorjaarsdagen, Amsterdam.
2. Lüssenhop, J., Stahl, J., Wolken, S., Schnieder, T., Kietzmann, M. & Bäumer, W. (2012) Distribution of permethrin in hair and stratum corneum after topical administration of four different formulations in dogs. *J Vet Pharmacol Ther*, **35**, 206–208.

© 2012 The Authors

Session 13: Pain and inflammation
Wednesday 11-07: 13.30–15.00

13.1.K.
The inflammasome: a new target for anti-inflammatory drugs

C. E. BRYANT
The Department of Veterinary Medicine, The University of Cambridge, Cambridge, UK

The inflammasome is a multi-protein complex that is responsible for processing pro-interleukin 1 beta (IL1B) and pro-IL18 into their active forms and for inducing inflammatory cell death. In humans inhibition of IL1B is emerging as an important therapeutic target with IL1B receptor antagonists such as anakinra showing remarkable efficacy in patients with a number of chronic inflammatory conditions. Activation of the inflammasome is driven by a novel class of cytosolic Pattern Recognition Receptors the Nucleotide Oligomerisation Domain receptors (NLRs). One NLR, NLRP3, is activated in a number of conditions such as gout, silicosis, asbestosis, macular degeneration, Alzheimer's disease and diabetes mellitus and gain of function mutations in this protein are associated with hyper-inflammatory syndromes. The NLRs and the inflammasome may therefore represent a novel target for anti-inflammatory therapy in veterinary medicine.

13.2.K.
The next generation therapeutics for veterinary analgesia

L. PELLIGAND
Veterinary Basic Sciences, Royal Veterinary College, London, UK

Pain is an 'unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in term of such damage'. This definition has not changed since 1994, however great progress has been made in recognition and management of clinical pain in animals. Until now, animal pain management has benefited from the use of human analgesics, however in future years, advances in this veterinary speciality may lead to translation of therapies from animal to human (Flecknell, 2008). Ethical inflammatory or pain models in animals are being continuously refined to allow the improvement in understanding of animal and human pain, particularly in chronic states. However, chemically or surgically induced animal pain models predict less accurately clinical efficacy and adverse effects of candidate molecules than animal models of spontaneously occurring disease. Clinical translational research focusing on the use of privately owned animals with spontaneous pain could bridge the gap between preclinical hopes and clinical efficacy in veterinary and human drug development. Due to the subjective nature of pain assessment in animals, objective markers of pain are lacking to support efficacy studies. The use of force plates, 3-dimension accelerometry and wireless mechanical and thermal testing devices will certainly improve the quality of pharmacodynamic data collected. Expression of the proto-oncogene C-fos within the spinal cord has become an efficient anatomical tool for insight into the organization of nociceptive systems in non-recovery experiments but is still presently too invasive for use in domestic animals (Coggeshall, 2005). Neuroimaging studies such as functional MRI (fMRI) allows a non-invasive study of pain processing in the brain and screening of the analgesic potentials of new analgesic candidates for different types of evoked pain (Arendt-Nielsen and Hoeck, 2011). Although this technology is mainly used in human, neuroimaging biomarkers of pain can be translational and may bridge animal findings in clinical pain conditions, which in turn can provide new possibilities for designing more successful clinical trials. The combination of opioids, alpha-2 agonists, local anaesthetics and Non-Steroidal Anti-Inflammatory Drugs have become the cornerstone of veterinary analgesia. The use of ketamine, an *N*-methyl D-aspartate Receptor antagonist, has been revisited at sub-anaesthetic doses in animals for management of chronic pain. However, identification of an effective NMDA antagonist devoid of hallucinogenic effect for oral use is still lacking. Cannabinoid receptor agonists, ion channels blockers, drugs that target G-protein coupled receptors, norepineprhine re-uptake inhibitors and tricyclic antidepressant are likely to be more highly considered in the future of veterinary analgesia. Due to the limited proof of efficacy of the new analgesics mentioned above, a new trend is emerging for the treatment of pain using a biological approach though the development of monoclonal antibodies (anti-TNF or Interleukin-6 inhibitors) (Hatcher *et al.*, 2011) or a genetic approach using small interfering RNA (siRNA). Thorough exploration of the pharmacokinetic and pharmacodynamic (PK/PD) profile of drugs may help the prediction of the duration of analgesia from experimental models and also the occurrence of side effect. Population PK/PD documents the magnitude and sources of variability in drug concentration-time profile and effect between individuals when standard dosage regimens are administered and identify covariate or cofactors to drive dose adjustment. Dual selectivity behaviour illustrated with lumiracoxib in human or robenacoxib in companion animal (COX-2 enzyme selectivity and tissue targeting) maximise efficacy and reduce toxicity as these compounds are cleared rapidly from plasma and accumulate preferentially at the site of inflammation. Personalised therapy may be achieved for analgesics with narrow therapeutic index by therapeutic drug monitoring (measurement of plasma concentration at peak or trough) to allow dose adjustment in order to bracket plasma concentration within the effective range but remain below the toxic range to limit drug-induced toxicities.

SUGGESTED READING

1. Arendt-Nielsen, L. & Hoeck, H.C. (2011) Optimizing the early phase development of new analgesics by human pain biomarkers. *Expert Rev Neurother*, **11**, 1631–1651.
2. Coggeshall, R.E. (2005) Fos, nociception and the dorsal horn. *Prog Neurobiol*, **77**, 299–352.
3. Flecknell, P. (2008) Analgesia from a veterinary perspective. *Br J Anaesth*, **101**, 121–124.
4. Hatcher, J.P., Chessell, I.P. & Hughes, J.P. (2011) Biologics: the next-generation therapeutics for analgesia? *Expert Rev Neurother*, **11**, 1653–1658.

© 2012 The Authors

Session 14: Pain and Inflammation
Wednesday 11-07: 16.00–18.00

14.1.
Efficacy and safety of mavacoxib in comparison with carprofen in the treatment of pain and inflammation associated with degenerative joint disease in dogs presented as veterinary patients

H. SIX[1], J. MITCHELL[2], C. ANTON[1], E. SIEDEK[1], J. K. TENA[1] & M. R. STEGEMANN[1]
[1]*Pfizer Animal Health, Kalamazoo, MI, USA;* [2]*Royal Veterinary College, London, UK*

INTRODUCTION
An open-label VICH GCP study was conducted post approval to assess the efficacy and safety of Trocoxil™ (mavacoxib) in comparison with Rimadyl™ (carprofen) in the treatment of pain and inflammation associated with degenerative joint disease in dogs.

MATERIALS AND METHODS
Dogs with clinical signs of degenerative joint disease were assigned randomly to treatment with either mavacoxib or carprofen. Mavacoxib was administered orally at the approved label dosage of 2 mg kg^{-1} on days 0 and 14, then every 30 days to day 194 (6.5 months). Carprofen was administered orally according to the approved product label directions (4 mg kg^{-1} once daily in France and Germany, and 2–4 mg kg^{-1} daily in the UK) for up to 194 days. Assessments were made both by the dog's owner and the veterinarian prior to the first treatment and at intervals during the study. Safety was evaluated by capture of solicited reports of any abnormal health from both Owner and the Examining Veterinarian. Cochran Armitage test with site as a stratifying variable was used to compare proportions of improved cases at different time points relative to baseline within a treatment group at 5% level of significance. The non-inferiority of mavacoxib to carprofen was investigated by constructing 90% confidence intervals on difference of proportions of improved cases at each time point using the Newcombe's method.

RESULTS
A total of 2598 dogs (mavacoxib: 1303; carprofen: 1295) were enrolled. Mean age was 9.5 years (range 0.6–18 years). Mean treatment duration was 161 days; 1163 dogs (mavacoxib: 595; carprofen: 568) were treated for 194 days. Sixty percent of mavacoxib-treated dogs and 58% of carprofen-treated dogs received concomitant medications. Forty-three percent of mavacoxib-treated and 50% of carprofen-treated dogs exhibited at least one clinical sign of abnormal health due to any causality; the number of abnormal clinical sign episodes was similar for both treatments. The mean time (days) to onset of abnormal clinical signs was 80 and 76 for mavacoxib and carprofen, respectively. The most commonly observed abnormal clinical signs were digestive tract disorders (21%, 195 mavacoxib; 338 carprofen), systemic disorders (15%, 188 mavacoxib; 213 carprofen), and skin and appendage disorders (11%, 162 mavacoxib; 118 carprofen). Distribution (%, *n*) of adverse event seriousness was as follows: non-serious (75%, 391 mavacoxib, 516 carprofen), lack of efficacy (13%; 72 mavacoxib, 81 carprofen) and serious (12%; 72 mavacoxib, 78 carprofen). Treatment with either mavacoxib or carprofen resulted in a similar pattern of improvement, as assessed by both the dog's owner and the veterinarian. Veterinary assessment of pain showed continuous improvement over the 194 day treatment period in both groups. Owner assessed overall improvement increased from Day 14 (mavacoxib: 68%; carprofen: 70%) to Day 194 (mavacoxib: 87%; carprofen: 84%) in both groups ($P < 0.0001$). The efficacy of mavacoxib was determined to be non-inferior to carprofen at the 5% significance level using a 15% non-inferiority margin.

CONCLUSIONS
Mavacoxib was as effective and had a similar clinical safety profile as carprofen in the treatment of pain and inflammation associated with degenerative joint disease in dogs.

14.2.
Pharmacokinetics and pharmacokinetic/ pharmacodynamic modelling of robenacoxib and ketoprofen in a carrageenan-induced inflammation model in the cat

L. PELLIGAND[1], J. N. KING[2], V. HORMAZÁBAL[3], P.-L. TOUTAIN[4], J. ELLIOTT[5] & P. LEES[5]
[1]*Royal Veterinary College, Hatfield, UK;* [2]*Novartis Animal Health Inc., Clinical Development, Basel, Switzerland;* [3]*The Norwegian School of Veterinary Science, Oslo, Norway;* [4]*UMR 1331 Toxalim INRA/INP/UPS, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France;* [5]*Veterinary Basic Sciences, Royal Veterinary College, Hawkshead Campus, Hatfield, UK*

INTRODUCTION
Robenacoxib and ketoprofen are non-steroidal anti-inflammatory drugs (NSAIDs) licensed for once daily administration in cats, despite their rapid clearance from blood.

OBJECTIVE
We hypothesised that robenacoxib (COX-2 selective) and ketoprofen (COX-1 preferential) would exert anti-inflammatory effects of similar duration and intensity in carrageenan-induced inflammation.

MATERIALS AND METHODS
Eight cats (1–3 yo) weighing 4.0 ± 0.39 kg were enrolled in a randomised, placebo controlled, three period cross-over study (28 days wash-out). In each period, 1.0 ml of a 2% sterile lambda carrageenan solution was injected into a subcutaneously implanted tissue cage. Robenacoxib (2 mg kg^{-1}), racemic ketoprofen (2 mg kg^{-1}) or a placebo formulation was administered subcutaneously. Blood samples were taken from indwelling jugular catheters at pre-determined times for NSAID and serum thromboxane $(Tx)B_2$ (1 h incubation, 37°C) measurements. Tissue cage exudate samples (1 ml) were obtained before and at

pre-determined times (up to 120 h) after carrageenan injection for determination of robenacoxib and prostaglandin (PG)E_2 concentrations. Exudate PGE_2 generation and *ex vivo* serum TxB_2 synthesis inhibition were taken as surrogate markers of COX-2 and COX-1 activity, respectively. Groups were compared using repeated-measurement analysis of variance (SAS PROC MIXED) with main effects for treatment group, time, sex and period. Significance was set at $P < 0.05$. Individual time-concentration curves, pharmacokinetic (PK) variables, effect-concentration curves and pharmacodynamic (PD) parameters for COX-1 and COX-2 inhibition were generated by modelling in WinNonlin 5.2. Average PD parameters were obtained by naïve pooled data analysis.

RESULTS

S(+)ketoprofen was rapidly cleared from plasma, as apparent clearance (CL/F) was 0.114 (95% Confidence Interval: 0.092–0.142) l kg^{-1} per hour and terminal elimination half-life was 1.62 ± 1.14 h. Apparent blood robenacoxib clearance was high [0.684 l kg^{-1} per hour (95%CI: 0.622–0.753)] and terminal half-life was 1.13 ± 0.18 h. Elimination half-lives from tissue cages were 25.9 ± 3.7 h for S(+)ketoprofen (MRT 35.9 h) and 41.5 ± 8.8 h for robenacoxib (MRT 45.7 h). Both drugs significantly reduced exudate PGE_2 between 6 and 36 h. Maximum exudate PGE_2 inhibitions were 92.1% for robenacoxib and 90.9% for S(+)ketoprofen at 9 h. Ketoprofen significantly suppressed (> 97%) serum TxB_2 between 4 min and 24 h, whereas TxB_2 suppression was only transient with robenacoxib (51.2% at 2 h). Geometric mean IC_{50}COX-2 was 48.5 ng ml^{-1} (0.191 μM) for S(+)ketoprofen and 38.2 ng ml^{-1} (0.117 μM) for robenacoxib. Geometric mean IC_{50}COX-1 was 0.17 ng ml^{-1} (0.67×10^{-3} μM) for S(+)ketoprofen and 2950 ng ml^{-1} (9.0 μM) for robenacoxib.

CONCLUSIONS

The inhibition of COX-2 was similar in magnitude and time course for robenacoxib and ketoprofen, whereas COX-1 inhibition was marked and persistent for ketoprofen only.

14.3.
Sphingosine-1-phosphate modulates secretion of IL-12 family cytokines from activated dendritic cells

K. SCHAPER & W. BÄUMER
University of Veterinary Medicine Hannover, Hannover, Germany

INTRODUCTION

Sphingosine-1-phosphate (S1P) is a sphingolipid which mediates its immunomodulatory effect via 5 G protein coupled receptors (S1PR1-5). In vivo, S1P administration reduces inflammatory responses in skin (Reines *et al.*, 2009). We were thus interested, whether S1P modulates the cytokine secretion in activated murine bone marrow derived dendritic cells (DC). Cytokines of the IL-12 family are involved in the initiation and orchestration of Th1 responses. The present study was performed to determine effects of S1P on dendritc cell production of IL-12, IL-23 and IL-27 belonging to the so called IL-12 family due to similarities in their receptor subunit composition.

MATERIALS AND METHODS

Murine bone marrow derived DC were generated from BALB/c mice. After 9 days of cultivation, DC were pre-incubated with S1P or S1P agonists and antagonists and then stimulated with 1 μg ml^{-1} of the TLR4 receptor agonist lipopolysaccharide (LPS). In a second setting, DC were stimulated by keratinocytes activated by 50 μg ml^{-1} LPS and 50 μg ml^{-1} peptidoglycan (transwell system). Cytokines (TNFα, IL-10, IL-12, IL-23 and IL-27) were determined 24 h later in the supernatants by means of ELISAs. Three independent experiments were performed. Different treatment groups were compared by one-way ANOVA followed by Dunnett's *post hoc* test. $P < 0.05$ was considered to be significant.

RESULTS

There was a significant increase of TNFα, IL-10, IL-12, IL-23 and IL-27 in supernatants of stimulated DC either directly by LPS or indirectly by 'inflamed' keratinocytes. The increase of TNFα and IL-10 was not influenced by S1P treatment, whereas there was a dose dependent inhibition of IL-12 and IL-23 secretion by S1P, which became significant from 0.1 to 10 μM S1P and a tendency of increased IL-27 secretion. The S1P analogue FTY720 (or FTY720P) had only very limited impact on cytokine secretion, whereas the S1P1R agonist SEW2871 also significantly inhibited the IL-12 and IL-23 secretion. As the S1P1R antagonist W146 restored the down-regulation of IL-12 induced by S1P, it is likely that the S1PR1 is the main receptor mediating the modulation of IL-12 cytokine family secretion.

CONCLUSIONS

S1P plays a substantial role in the modulation of IL-12 related cytokines. The interference of keratinocyte-dendritic cell communication might have an impact for the treatment of chronic allergic diseases such as atopic dermatitis or psoriasis with locally acting S1P.

REFERENCES

1. Reines, I., Kietzmann, M., Mischke, R., Tschernig, T., Lüth, A., Kleuser, B. & Bäumer, W. (2009) Topical application of sphingosine-1-phosphate and FTY720 attenuate allergic contact dermatitis reaction through inhibition of dendritic cell migration. *J Invest Dermatol*, **129**, 1954–1962

14.4.
Effects of separate and concomitant TLR2 and TLR4 activation in peripheral blood mononuclear cells of newborn and adult horses

J. C. VENDRIG[1], L. E. COFFENG[2] & J. FINK-GREMMELS[1]
[1]*Faculty of Veterinary Medicine, Veterinary Pharmacology, Pharmacotherapy and Toxicology, Utrecht University, Utrecht, The Netherlands;* [2]*Erasmus Medical Centre, Department of Public Health, Erasmus University Rotterdam, Rotterdam, The Netherlands*

INTRODUCTION

Deficits in both innate and adaptive immune responses, increase the susceptibility of new-born mammals, in comparison to adult individuals, to bacterial infections (Levy, 2007). Toll-like receptors (TLRs) have shown to play a pivotal role in bacterial recognition and subsequent immune responses, accountable for both targeting possible pathogens and tolerating commensal microbiota (Takeuchi and Akira, 2010; Testro and Visvanathan, 2009). Several studies have indicated that activation of certain TLRs, in particular TLR2, can result in suppression of inflam-

© 2012 The Authors

matory pathology (Cario *et al.*, 2007). In this study we investigate effects of separate and concomitant TLR2 and TLR4 activation in Peripheral Blood Mononuclear Cells (PBMCs) of adult horses and foals within 12 h *post partum*. The main objectives of this research were to study the influence of TLR2 activation on the lipopolysaccharide (LPS) induced inflammatory response and to elicit differences in immune responses between new-born and adult equines in this experimental model.

MATERIALS AND METHODS

PBMCs were isolated from the blood of six adult mares and six neonatal foals (age < 12 h). After a 2 h pre-incubation with 0 or 1 μg ml^{-1} Pam3CSK4 (Pam3-Cys-Ser-Lys4), cells were incubated with 0 or 1 μg ml^{-1} LPS (*Escherichia coli* O111:B4), 0 or 1 μg ml^{-1} Pam3CSK4 or a combination of both TLR ligands. After 4 h, samples were collected for ELISA and PCR analysis. In adult horses, additional measurements were made with an incubation period of 2 h. ELISA's were performed for tumour necrosis factor-α (TNF-α) and interleukin-10 (IL-10). mRNA expression of TLR-2, TLR-4, TLR-9, TNF-α, IL-6, IL-10, GAPDH and β-actin was assessed by means of real-time PCR analysis. Acquired data were analysed in a Bayesian three-way analysis of variance (using 95% credible intervals), accounting for possible complex between-horse variation.

RESULTS

In Fig. 1, TNF-α protein levels after 4 h of incubation are shown for both adult horses and foals. Although there were evident differences in amplitude, the pattern of TNF-α production under the different circumstances was comparable in PBMCs from adults and neonates. Incubation with Pam3CSK4 did not have a significant influence on the LPS response in this model. PCR data of TNF-α and IL-6 are generally comparable with the TNF-α ELISA results. In adult horses, IL-10 protein and mRNA levels were influenced more by TLR2 activation than in neonates. Distinct expression patterns of the selected TLRs were observed in foals and adult horses, with striking differences in expression patterns for TLR2 and TLR9. The expression of the selected housekeeping genes was not significantly influenced by TLR2 and/or TLR4 activation in this model.

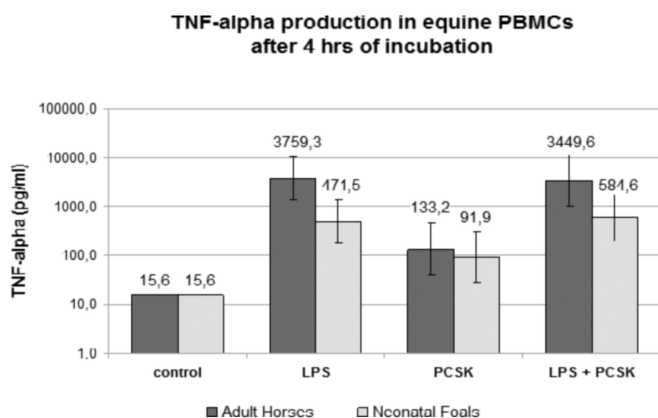


Figure 1. TNF-alpha ELISA results: mean values and 95% credible intervals are given, which were calculated using separate models for adult horses and foals.

CONCLUSIONS

In this model, no significant effect of TLR2 activation on the LPS induced inflammatory response could be demonstrated. Differences in the response of adult and neonatal PBMCs were evident, comprising differences in quantities of protein and mRNA levels as well as expression patterns of cytokines and TLRs.

REFERENCE

1. Cario, E., Gerken, G. & Podolsky, D.K. (2007) Toll-like receptor two controls mucosal inflammation by regulating epithelial barrier function. *Gastroenterology*, **132**, 1359–1374.
2. Levy, O. (2007) Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nature Reviews Immunology*, **7**, 379–390.
3. Takeuchi, O. & Akira, S. (2010) Pattern recognition receptors and inflammation. *Cell*, **140**, 805–820.
4. Testro, A.G. & Visvanathan, K. (2009) Toll-like receptors and their role in gastrointestinal disease. *Journal of Gastroenterology and Hepatology*, **24**, 943–954.

14.5.
The pharmacokinetics of oclacitinib maleate, a novel Janus kinase inhibitor, in the dog

T. COLLARD[1], D. HUMMEL[1], L. KING[1], A. FIELDER[1], F. BOUCHER[1], A. MULLINS[1], S. P. LESMAN[1] & M. R. STEGEMANN[2]
[1]*Pfizer Animal Health, Kalamazoo, MI, USA;* [2]*Pfizer Animal Health, Zaventem, Belgium*

INTRODUCTION

Oclacitinib maleate is a novel selective Janus Kinase (JAK) inhibitor which preferentially inhibits JAK1 over other family members and has been shown to inhibit the activity of pro-inflammatory cytokines such as IL-2, pro-allergic cytokines such as IL-4, and pruritogenic cytokines such as IL-31. The pharmacokinetics of oclacitinib maleate was evaluated in four separate pharmacokinetic dog studies.

MATERIALS AND METHODS

The absolute bioavailability study used a two period, two treatment crossover design with 10 dogs and a 0.282 mg kg^{-1} intravenous and 0.4 mg kg^{-1} oral oclacitinib dose. The effect of food on bioavailability was investigated in a three period, three treatment (IV and oral fasted and fed oral) crossover study with 18 dogs and a dose of 0.5 mg kg^{-1}. The breed effect on pharmacokinetics was assessed in a two treatment, two period crossover study in beagles and mongrels with an IV and oral 0.4 mg kg^{-1} dose. Dose proportionality was evaluated in a parallel design study with eight dogs per group and oral doses of 0.6, 1.8, and 3.0 mg kg^{-1}. In all four studies serial blood samples for plasma were collected up to 48 h post dose. Plasma samples were assayed for oclacitinib concentrations using a LC/MS/MS method, and pharmacokinetic parameters were calculated using a non-compartmental approach using Watson™ (Thermo Fisher Scientific). The statistical analysis was completed with SAS.

RESULTS

In dogs oclacitinib maleate was rapidly and well absorbed following oral administration, with a time to peak plasma concentrations of <1 h and an absolute bioavailability of 89%. Following intravenous administration, the total body plasma

© 2012 The Authors

clearance was found to be low (5.3 ml min^{-1} per kg), and the apparent volume of distribution at steady-state was 942 ml kg^{-1}. The terminal $t_{1/2}$ following IV and PO administration appeared similar with values of 3.5 and 4.1 h, respectively. The prandial state of dogs did not significantly affect the rate or extent of absorption of oclacitinib maleate when dosed orally, as demonstrated by the lack of significant differences in pharmacokinetic parameters between the oral fasted and oral fed treatment groups. The pharmacokinetics of oclacitinib in laboratory populations of beagles and mixed breed dogs also appeared similar. Following oral administration of oclacitinib maleate, $AUC_{0\text{-}t}$ and C_{max} increased dose proportionally from 0.6 to 3.0 mg kg^{-1}.

CONCLUSIONS

Additionally, across all the pharmacokinetic studies, there were no significant differences in oclacitinib pharmacokinetics attributable to sex.

14.6.
Partial purification and characterization of equine calprotectin

C. ZIZZADORO, C. D. CALVANO, A. ARESTA, S. PUNZI & C. BELLOLI
University of Bari, Valenzano, Bari, Italy

INTRODUCTION

Calprotectin (CP) is a small protein which is emerging as an important player in inflammation (Goyette and Geczy, 2011). In humans, CP is abundantly expressed in the cytosol of neutrophils and consists of two calcium-binding proteins assigned to the S100 protein family: S100A8 and S100A9. A possible role of CP in the pathophysiology of inflammatory diseases affecting horses, like laminitis, has been recently proposed (Faleiros *et al.*, 2009). This makes it worth deepening the knowledge of the biochemical and biological properties of this protein in the equine species. As a prelude to such type of studies, the present research has been undertaken to develop a method for purifying equine CP from neutrophils and characterize its key structural components.

MATERIALS AND METHODS

Neutrophils were isolated from blood of healthy horses by density gradient centrifugation. Immunoperoxidase staining of cytospin preparations was performed using the monoclonal antibody (mAb) MAC387 to ascertain CP localization. Then neutrophils were suspended in different buffers (with varying pH values and calcium concentrations), lysed and centrifuged. The so collected crude cytosol fraction was applied to an anion-exchange column, from which proteins were eluted by 0.15–1.0 M NaCl. The starting material and/or its eluted fractions were then subjected to reducing and non-reducing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis using the mAb MAC387 and matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) to assess their protein purity and identify their protein components.

RESULTS

In the cytospin preparations, the mAb MAC387 specifically stained the cytoplasm of the equine neutrophils. Western blot analysis of the cytosol fraction following reducing SDS-PAGE revealed two immunoreactive bands in the expected molecular weight range for S100A8 (10 kDa) and S100A9 (15 kDa). Additional immunoreactive bands of higher molecular weight were observed in cell lysates prepared in some specific lysis buffers and run under non-reducing conditions, including a 25 kDa band compatible with the heterodimer CP. Following anion-exchange chromatography, the fraction eluted at 0.3 M NaCl showed the greatest protein purity and still contained two immunoreactive bands of 10 and 15 kDa when subjected to immunoblotting. MALDI-TOF-MS analysis of this fraction detected four peaks at m/z values of 8701, 10 188, 10 631 and 15 427. The fingerprinting of peptides generated by tryptic digestion suggested that the peaks at 10188 and 15 427 m/z values could be equine S100A8 (100% coverage) and S100A9 (60% coverage) proteins, respectively.

CONCLUSIONS

The present work demonstrates for the first time the presence of the two constitutive proteins of CP, namely S100A8 and S100A9, in the cytosol of equine neutrophils and provides preliminary indications for their successful purification. This will aid future investigations of the biological role(s) of CP and its subunits in the horse.

ACKNOWLEDGEMENTS

Supported by the Italian Ministry of Education, University and Research (PRIN-2008C42LJZ).

REFERENCES

1. Goyette, J. & Geczy, C.L. (2011) Inflammation-associated S100 proteins: new mechanisms that regulate functions. *Amino acids*, **41**, 821–842.
2. Faleiros, R.R., Nuovo, G.J. & Belknap, J.K. (2009) Calprotectin in myeloid and epithelial cells of laminae from horses with black walnut extract-induced laminitis. *Journal of Veterinary Internal Medicine*, **23** (1), 174–181.

14.7.
Expression of calprotectin subunits (S100A8 And S100A9) in equine recurrent airway obstruction

C. ZIZZADORO, M. PIETRA, F. ADDABBO, C. BELLOLI & A. PELI
University of Bari, Valenzano, Bari, Italy

INTRODUCTION

Recurrent airway obstruction (RAO) is a common respiratory disease of horses characterised by neutrophil accumulation in the small bronchi (Léguillette, 2003). In humans, neutrophils have been shown to express high cytosolic levels of a protein complex, calprotectin (CP), consisting of two S100 proteins, S100A8 and S100A9, that are encoded by differently regulated genes and also are produced and act independently as monomers (Nacken *et al.*, 2003). Upon neutrophil activation, these proteins are released into the extracellular environment to probably exert regulatory functions in the local inflammatory response. Moreover, the levels of these proteins in biological fluids proved useful markers of disease activity for various human inflammatory conditions involving neutrophil accumulation. Further to the demonstration that both CP subunits are expressed in the cytosol of equine neutrophils (Zizzadoro *et al.*, 2012), the present study was undertaken to explore the possible release of these proteins in the airways of horses with RAO in relationship with different clinical stages of the disease.

© 2012 The Authors

MATERIALS AND METHODS

To this aim, RAO-susceptible horses (n = 3) were subjected to bronchoalveolar lavage (BAL) at two different sampling times, namely during an asymptomatic condition realized by maintaining the animals on pasture (T0), and during an acute respiratory crisis observed 48 h after housing in a poorly ventilated dusty stable (T1). All procedures were performed according to standard protocols and approved by the local Ethics and Scientific Committee. The recovered BAL fluid (BALF) samples were filtered and centrifuged. The supernatants, after appropriate adjustment of the total protein concentration, were analysed for the presence of S100A8 and S100A9 by western blotting, using the monoclonal antibody MAC387. A lysate of equine neutrophils was run in parallel as positive control.

RESULTS

In the BALF supernatant of all the subjects, irrespective of the sampling time, immune-reactive bands of the expected molecular weight of S100A8 (10 kDa) were not detected. In contrast, a 15 kDa immune-reactive band, likely corresponding to S100A9, was clearly observable in two of the three subjects at T0 and in all the three subjects at T1, with the band signal detected at T1 being, on average, 2.5–3 fold more intense than that detected at T0.

CONCLUSIONS

This study provides the first evidence that a soluble form of the CP subunit S100A9 is released in the airways of asymptomatic RAO horses and undergoes up-regulation during clinical exacerbation. Additional investigations are on-going on a larger cohort of animals to define the potential of this protein as new diagnostic tool and/or drug target for more effective management of equine RAO.

ACKNOWLEDGEMENTS

Supported by the Italian Ministry of Education, University and Research (PRIN-2008C42LJZ).

NB: corrections were made to the author names following initial online publication.

REFERENCES

1. Léguillette, R. (2003) Recurrent airway obstruction—heaves. *The Veterinary Clinics of North America. Equine Practice*, **19** (1), 63–86.
2. Nacken, W., Roth, J., Sorg, C. & Kerkhoff, C. (2003) Myeloid representatives of the S100 protein family as prominent players in innate immunity. *Microscopy: Research and Technique*, **60** (6), 569–80.
3. Zizzadoro, C., Calvano, C.D., Aresta, A., Punzi, S. & Belloli, C. Partial Purification and Characterization of Equine Calprotectin. *Proceedings of the 12th EAVPT International Congress* (8–12 July 2012, Noordwijkerhout, the Netherlands).

14.8.
Development of a cytometric bead array screening tool for the simultaneous detection of pro-inflammatory cytokines and acute phase proteins in porcine plasma

H. WYNS, S. CROUBELS, K. DEMEYERE, A. WATTEYN, P. DE BACKER & E. MEYER

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

INTRODUCTION

Multiplex assays currently are a very popular tool for the simultaneous detection of biomarkers of infection and inflammation. Whereas specific and sensitive *Enzyme-Linked* Immuno Sorbent *Assays* (ELISAs) are well-suited to perform single factor analysis, for multi-parameter analyses, this approach is time-consuming and expensive. Cytometric bead array (CBA) is a flexible, bead-based flow cytometric application for the simultaneous detection of various soluble proteins of interest. Therefore, the aim of the present study was to develop and validate a CBA 3-plex assay for the major pro-inflammatory cytokines TNF-α, IL-1β and IL-6, and an additional CBA 2-plex assay for the major acute phase proteins (APPs) CRP and pig-MAP in plasma of lipopolysaccharide (LPS)-challenged pigs. Furthermore, results were compared to commercial ELISA kits.

MATERIALS AND METHODS

Four pigs with a mean body weight (BW) of 24.9 kg were intravenously challenged with 15 μg ultrapure LPS kg BW^{-1} (*Escherichia coli* serotype O111:B4). Plasma was isolated and stored at −70°C until analysis. Capture antibodies were covalently coupled to the surface of beads with unique fluorescence intensities (Becton Dickinson Biosciences). Detection antibodies were conjugated with R-Phycoerythrin (R-PE). A mixture of beads was firstly incubated with an appropriate standard mixture. Subsequently, a mixture of detection antibodies, either directly or indirectly conjugated to R-PE, was added to accomplish the desired sandwich format. The samples were finally analyzed on a BD FACSArray™ Bioanalyzer. ELISAs were purchased from R&D Systems, PigCHAMP Pro Europe S.A. and ALPCO Diagnostics.

RESULTS AND CONCLUSIONS

Table 1 shows validation parameters of the developed CBA 3-plex assay and the commercial ELISAs as mentioned by the manufacturer. Similar concentration-time profiles were observed for TNF-α, IL-1β, IL-6 and pig-MAP with CBA and ELISA in plasma from LPS-challenged pigs. Up till now, the CBA 2-plex assay could not be validated. On the other hand, the optimized and validated CBA 3-plex protocol provides a fast, flexible and cost-effective screening tool for simultaneous determination of pro-inflammatory cytokine profiles in porcine plasma. This technique will be applied in future research to study the immunomodulatory properties of drugs in a porcine LPS inflammation model.

Table 1. Limits of detection (LOD), mean intra- and inter-assay coefficients of variations (CV%) of the CBA 3-plex assay and commercial ELISAs

Cytokine	LOD (ng/mL)		Intra-assay (CV%)		Inter-assay (CV%)	
	CBA	ELISA	CBA	ELISA	CBA	ELISA
TNF-α	0.363	0.004	8.45	4.86	15.71	8.90
IL-1β	0.109	0.007	2.26	5.77	15.48	6.27
IL-6	0.005	0.002	2.33	4.00	11.41	5.97

Session 15: Toxicology
Wednesday 11-07: 11.30–15.30

15.1.
Can clenbuterol meat residues cause athletes to fail doping tests?

T. MARTIN JIMENEZ
University of Tennessee, Maryville, TN, USA

INTRODUCTION
Over the last few years, many elite athletes have tested positive for clenbuterol. In some cases, anti-doping agencies have accepted the explanation of food contamination.

OBJECTIVE
The objective of this study was to evaluate whether consuming contaminated meat could result in positive doping tests.

MATERIALS AND METHODS
A compartmental model (1) was developed (WinNonlin 5.2. Pharsight, Mountain View, CA) that simultaneously fitted the published plasma and urine PK data (2). This model was used to predict clenbuterol urine concentrations. Given its extensive documentation, our reference was the case of the cyclist Alberto Contador during the 2010 Tour de France. In this case, two steaks were consumed approximately 24 and 6 h before the doping control. The most commonly reported dose for illegal use in cattle is 10 $\mu g\ kg^{-1}$ q12 h for 21 days, which results in average residue concentrations in muscle of 12.5 and 1.25 ng g^{-1} at 0- and 4-day withdrawal times (WDTs), respectively (3). A 200 gr steak would contain approximately 2.5 and 0.25 μg of clenbuterol at 0- and 4-day WDTs, respectively. A daily urine volume of 1.5 l divided into eight equally-spaced voidings was used in our simulations.

RESULTS
Plasma and urine data were best fit with a compartmental model with first order absorption and mono-exponential decay. The absorption rate constant (ka) was 1.73 1/h, the elimination rate constant (kel) was 0.025 1/h, the extravascular volume of distribution (V/F) was 3.8 l kg^{-1}, and the fraction excreted in urine (fe) was 23%. The urine concentration in the athlete was 50 pg ml^{-1} 24 h after the last negative control. For the amounts of clenbuterol in a 200 gr steak used in this study, the predicted urine concentration 24 h after initial exposure was 50–500 pg ml^{-1}. Larger doses in cattle have been reported that would result in similar urine concentrations even with larger WTDs.

CONCLUSIONS
The ingestion of meat from cattle illegally treated with clenbuterol can result in urine concentrations above the limit of quantitation of the most sophisticated assays available. Our data indicates that, under current international regulations, sport competitions in countries with widespread clenbuterol use or insufficient monitoring, may result in accidental cases of positive doping. This suggests the need to open a discussion as to whether some form of concentration threshold should be established.

REFERENCES
1. Gibaldi, M. & Perrier, D. (1982) *Pharmacokinetics*, 2nd edN. Revised and Expanded. Marcel Dekker Inc., New York.
2. Yamamoto, I., Iwata, K. & Nakashima, M. (1985) Pharmacokinetics of plasma and urine clenbuterol in man, rat, and rabbit. *Journal Pharmacobiodynamic*, **8**, 385.
3. Sauer, M.J., Pickett, R.J.H., Limer, S. & Dixon, S.N. (1995) Distribution and elimination of clenbuterol in tissues and fluids of calves following prolonged oral administration at a growth-promoting dose. *Journal Veterinary, Pharmacology & Therapeutics*, **18**, 81.

15.2.
Transcriptomic markers meet the real world: diagnostic genes of anabolic treatment in beef cattle

S. PEGOLO, C. MONTESISSA, S. FERRARESSO, C. PELLIZZARI, L POPPI, M. CASTAGNARO & L. BARGELLONI
University of Padua, Legnaro, Padua, Italy

INTRODUCTION
The use of growth-promoters in beef cattle, despite the EU ban, remains a frequent practice motivated by the positive effects of these substances mainly on body weight gain and increased feed conversion. To elude official controls, new anabolic compounds are developed and/or growth promoters are administered at low doses or combining different substances in hormone cocktails. To keep pace with such a moving target, constant innovation in screening and validation methods is necessary. The goal of the present work was to identify gene expression patterns that could classify unknown commercial samples as negative or putative positive (suspect) by comparison with untreated controls.

MATERIALS AND METHODS
Bovine-specific DNA-microarrays were used to obtain global gene expression profiles of skeletal muscle samples from beef cattle collected at the slaughterhouse and of negative control animals. Statistical analyses (Principal Component Analysis, Significance Analysis of Microarray and Prediction Analysis of Microarray) of the transcriptomic profiles were performed and followed by functional annotation of the differentially expressed genes through the DAVID knowledgebase. Further qRT-PCR validation of a small set of genes identified as potential biomarkers for anabolic treatment was carried out.

RESULTS
Principal Component Analysis on the gene expression data clearly identified four clusters of samples. Two clusters included the negative controls as well as a few commercial samples, while the remaining two groups clustered just specimens collected at the slaughterhouse. Based on functional analysis of the differentially expressed genes and independent evidence from direct and indirect methods, animal clustering in the latter two groups could be considered as suspects. To tentatively classify such suspect cases with a small panel of diagnostic markers, a discriminant analysis was carried out, using the software

© 2012 The Authors

Prediction Analysis of Microarray on a training set of putative positive and negative samples, and validated on all available animals. This led to the identification of the 10 most discriminant genes, which were then validated by qRT-PCR. The overall correlation of average fold-change values calculated for the two methods (qRT-PCR and DNA-microarray) showed a very high correlation coefficient (Spearman's rho = 0.988; $P < 0.001$) and the slope of linear regression line was 0.853 suggesting a correlation close to one-to-one between microarray and qRT-PCR data.

CONCLUSIONS
A small panel of diagnostic genes has been developed, which might allow routine analysis for illicit treatment detection, without substantial loss of discriminatory power. On the other hand, global transcriptomic tools (DNA-microarrays, RNA-seq) are becoming increasingly affordable and rapid, and soon a whole-transcriptome analysis will be feasible in routine practice. Such an approach avoids *a priori* selection of candidate markers and allows the identification of complex transcriptomic signatures as well as the comparison with other transcriptomic, proteomic, and metabolomic studies.

15.3.
Use of hepatic and testicular molecular biomarkers to detect growth promoters misuse in cattle: a preliminary application Under field conditions

R. M. LOPPARELLI, E. ZORZAN, V. ZANCANELLA,
A. SADOCCO, M. GIANTIN, C. MONTESISSA & M. DACASTO
Dip Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, Legnaro, Padova, Italy

INTRODUCTION
Growth promoters (GPs) are banned at the European Community level. Nevertheless, GP misuse in cattle remains of concern. In the past decade an increasing interest toward the set up and validation of molecular biomarkers to be used side by side with official analytical methods has been recorded (1). In preceding pilot studies, a number of tissue-specific responsive genes have been identified (2,3). In this study, these biomarkers were preliminarily tested under field conditions.

MATERIALS AND METHODS
Ninety-five cattle testis and liver aliquots were collected by chance at slaughterhouses, placed in microtubes with RNAlater® and stored at −80°C until use. A robust set of negative controls (44 animals), from earlier pilot studies, were included in the study, too. Total RNA was extracted with TRIzol® Reagent and gene expression profiles measured by using a quantitative Real Time RT-PCR approach (qPCR). Seven and eight target genes were chosen for liver and testis, respectively. Results were elaborated (Hierarchical Clustering, HCL, and Principal Component Analysis, PCA) by using the GenEx software (4).

RESULTS
In liver, HCL clustered samples into three main groups, supported by PCA: negative controls and most of random samples were clustered together ('negatives'), while three animals were distinctly grouped in another cluster ('suspects'). Further nine samples, assigned to negatives by GenEx, generated a different cluster; therefore, they were classified as 'doubtful'. In testis, three 'suspects'and three 'doubtful' were identified besides 'negatives'. Considering both tissues as a whole, the software identified three 'suspects'and two 'doubtful'.

CONCLUSIONS
This study aimed to test a set of candidate genes and a popular *software* for qPCR data processing and analysis upon a large number of random samples. The approach allocated samples into three different clusters, representing different expression profiles. Presented data suggest that transcriptome analysis and bioinformatic tools, coupled with a robust database of negative controls, might be helpful for tracking GP abuse in cattle. Further studies are needed to confirm these promising results.

ACKNOWLEDGEMENTS
Project supported by a grant from Regione del Veneto (Dgr 2888 07/10/2008) to M.D.

REFERENCES
1. Nebbia, C., Urbani, A., Carletti, M., Gardini, G., Balbo, A., Bertarelli, D. & Girolami, F. (2010) Novel strategies for tracing the exposure of meat cattle to illegal growth-promoters. *The Veterinary Journal*, **189**, 34–42.
2. Giantin, M., Lopparelli, R.M., Zancanella, V., Martin, P.G., Polizzi, A., Gallina, G., Gottardo, F., Montesissa, C., Ravarotto, L., Pineau, T. & Dacasto, M. (2010) Effects of illicit dexamethasone upon hepatic drug metabolizing enzymes and related transcription factors mRNAs and their potential use as biomarkers in cattle. *Journal of Agricultural and Food Chemistry*, **58**, 1342–1349.
3. Lopparelli, R.M., Zancanella, V., Giantin, M., Ravarotto, L., Pozza, G., Montesissa, C. & Dacasto, M. (2011) Steroidogenic enzyme gene expression profiles in the testis of cattle treated with illicit growth promoters. *Steroids*, **76**, 508–516.
4. Berkvist, A., Rusnakova, V., Sindelka, R., Garda, J.M.A., Sjogreen, B., Lindh, D., Forootan, A. & Kubista, M. (2010) Gene expression profiling – Clusters of possibilities. Methods, **50**, 323–335.

15.4.
Investigations about the natural occurrence of prednisolone and prednisone in urine samples from cows reared in different housing systems

C. NEBBIA[1], M. LEPORATI[2], M. CARELETTI[3], P. CAPRA[4], A. ATTUCCI[5], G. BARBARINO[6] & M. VINCENTI[7]
[1]*University of Turin, Grugliasco, Italy;* [2]*Centro Regionale Antidoping, Orbassano, Italy;* [3]*Department of Animal Pathology, University of Turin, Grugliasco, Italy;* [4]*Istituto Zooprofilattico Sperimentale PLVA c/o Centro Regionale Anti-doping, Orbassano, Italy;* [5]*ASL CN1, Servizio Veterinario, Cuneo, Italy;* [6]*Regione Piemonte – Direzione Sanità Pubblica, Torino, Italy;* [7]*Department of Analytical Chemistry, University of Turin, Torino, Italy*

INTRODUCTION
In an attempt to partly explain the increase in non-compliances for prednisolone or prednisone recently occurring in the EU, and particularly in Italy, stressing conditions were tested to clarify the origin of glucucorticoids in cattle urine as these could result from a 'natural' synthesis starting from cortisol or cortisone,

© 2012 The Authors

respectively (1). To verify this hypothesis a survey was conducted under field conditions in cows of different breeds reared in different housing systems.

MATERIALS AND METHODS

The survey was conducted in 131 untreated cows (age range 2.5–8 years) of Piemontese or Friesian breeds, reared in six beef cow-calf enterprises and six dairy farms, respectively, adopting tie-stall barn or littered louse-house systems. Urine sampling was performed under standardized conditions and as recommended by the National Residue Plan. Prednisolone, prednisone, and cortisol urinary levels were measured with a HPLC-MS/MS method (2), with a CCa of 0.66 ng ml^{-1} for prednisone and of 0.67 ng ml^{-1} for prednisolone. LOD values were estimated around 0.05 ng ml^{-1} for both analytes.

RESULTS

According to the EU legislation in force, none of the 131 examined urine samples could have been officially declared as noncompliant for either analyte. Interestingly, the only cows in which very low prednisolone urinary levels (0.1–0.3 ng ml^{-1}) could be determined were reared under loose housing systems and showed cortisol levels higher than those recorded in negative counterparts bred in tie-stall barns; this was probably due to differences in the response to stressing events like routinely sampling procedures. In our study, measured urinary prednisolone concentrations were remarkably lower than those obtained by Pompa *et al.* (1) in ACTH-challenged cows and of the same order of magnitude of those detected in finishing bulls administered with prednisolone at a growth-promoting schedule (2).

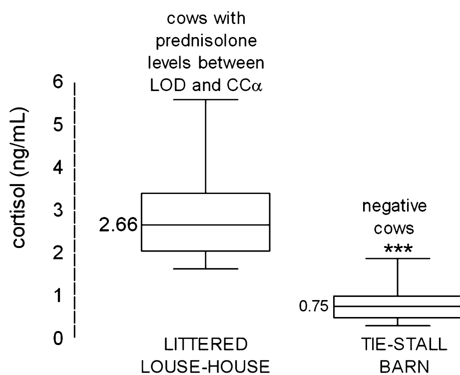


Figure 1. Box plots depicting urinary cortisol levels, prednisolone traces and housing systems in untreated cows ($n = 7$ for both groups). ***$P = 0.006$ Mann–Whitney.

CONCLUSIONS

Further research is warranted to ascertain whether the prednisolone/cortisol urinary ratio could be used to discriminate between the pharmacological use or abuse of the drug and the possible non exogenous origin linked to stressing conditions.

REFERENCES

1. Pompa, G., Arioli, F., Casati, A., Fidani, M., Bertocchi, L. & Dusi, G. (2011) Investigation of the origin of prednisolone in cow urine. *Steroids*, **76**, 104–110.
2. Cannizzo, F.T., Capra, P., Divari, S., Ciccotelli, V., Biolatti, B. & Vincenti, M. (2011) Effects of low-dose dexamethasone and prednisolone long term administration in beef calf: chemical and morphological investigation. *Analytica Chimica Acta*, **700**, 95–104.

15.5.

Effects of truck transportation and slaughtering the presence of cortisol, cortisone, prednisolone, and prednisone in urine, liver and adrenals from untreated cows

C. NEBBIA[1], M. LEPORATI[2], P. CAPRA[3], S. GATTO[4], A. ATTUCCI[5], G BARBARINO[6], F. GIROLAMI[7] & M. VINCENTI[8]
[1]*University of Turin, Grugliasco, Italy;* [2]*Centro Regionale Antidoping, Orbassano, Italy;* [3]*Istituto Zooprofilattico Sperimentale PLVA c/o Centro Regionale Anti-doping, Orbassano, Italy;* [4]*ASL TO3 – Servizio Veterinario, Pinerolo (TO), Italy;* [5]*ASL CN1, Servizio Veterinario, Cuneo, Italy;* [6]*Regione Piemonte – Direzione Sanità Pubblica, Torino, Italy;* [7]*Department of Animal Pathology, University of Turin, Grugliasco, Italy;* [8]*Department of Analytical Chemistry, University of Turin, Torino, Italy*

INTRODUCTION

To get more insight into the relationship between stress and the possible 'endogenous' synthesis of prednisolone claimed by some authors (1), the effects of truck transportation and the subsequent slaughtering was assessed in untreated cows by measuring the concentrations of cortisol and cortisone in urines as well as in liver and adrenals from the slaughtered animals. All samples were also analyzed for the presence of prednisone and prednisolone.

MATERIALS AND METHODS

The study was conducted in 15 untreated cows at the end of their productive cycles reared in different farms. Urines were first collected in living animals at the farm; after some days, animals were transported by truck to the abattoir, slaughtered after a short wait, and further subjected to a second urine sampling directly from the bladder. Specimens of liver and adrenals were also collected. Prednisolone and prednisone were measured essentially as described (2), while cortisol and cortisone were determined with a validated HPLC-MS/MS method.

RESULTS

Cortisol and cortisone concentrations ranging from non-detectable to less than 3.5 ng ml^{-1} were found in all but one urine sample collected at farm and none of the specimens contained measurable amounts of prednisone or prednisolone. Truck transportation and slaughtering induced a sharp rise in urinary cortisol and cortisone; only in samples displaying the highest concentrations of both compounds (above 48 ng ml^{-1}) the presence of low prednisolone concentrations (0.60 and 0.57 ng ml^{-1}, respectively) could be detected. One liver sample was found to contain measurable amounts of cortisol. Adrenal specimens exhibited variable levels of either cortisone and cortisol or, in one cow only, prednisone and prednisolone (3.4 and 4.2 ng ml^{-1}, respectively), in that case, the corresponding urines proved negative for both compounds. In line with the results of a previous study on three artificially stressed cows (1), our findings confirm that stressful events such as truck transportation followed by slaughtering cause the urinary levels of cortisol and cortisone to rise up to several fold as compared to those

© 2012 The Authors

recorded in samples collected at farm. However, only in two out of 15 tested cows such an increase was paralleled by the appearance of trace amounts of prednisolone in the same matrix. The adrenal origin of this glucocorticoid is supported by the recovery of both prednisone and prednisolone in the adrenal gland of a cow.

CONCLUSION

Data from this study confirm the overall very limited incidence of false urinary non compliances for prednisolone in the cow and support the use of the urinary prednisolone to cortisol or cortisone ratio to discriminate between the pharmacological use or abuse of the drug and its possible link to stressful events.

REFERENCES

1. Pompa, G., Arioli, F., Casati, A., Fidani, M., Bertocchi, L. & Dusi, G. (2011) Investigation of the origin of prednisolone in cow urine. *Steroids*, **76**, 104–110
2. Cannizzo, F.T., Capra, P., Divari, S., Ciccotelli, V., Biolatti, B. & Vincenti, M. (2011) Effects of low-dose dexamethasone and prednisolone long term administration in beef calf: chemical and morphological investigation. *Analytica Chimica Acta*, **700**, 95–104

15.6.
Characterization of maternal and fetal bisphenol A disposition in a physiologically-based sheep model

T. CORBEL[1], V. GAYRARD[2], C. VIGUIÉ[2], S. PUEL[2], M. Z. LACROIX[2], P.-L. TOUTAIN[2] & N. HAGEN-PICARD[2]
[1]*INRA, UMR1331, Toxalim, ENVT, Toulouse, France;* [2]*UMR1331 Toxalim INRA/ENVT, Toulouse, France*

INTRODUCTION

Bisphenol A (BPA) is one of the most highly produced chemicals worldwide and the human exposure to BPA is thought to be ubiquitous. As the foetal period represents a critical BPA exposure window, an integrative model of pregnant ewe based on physiological considerations was developed to determine if the pregnancy-associated physiological changes and the metabolic specificities of the feto-placental unit can influence BPA toxicokinetic. The aims of this study were to determine the toxicokinetic parameters of BPA and its inactive metabolite BPA-Glucuronide (BPA-G) in the feto-maternal compartments and to characterize the placental transfer of BPA and BPA-G.

MATERIALS AND METHODS

In a first longitudinal study, four ewes were infused IV for 24 h with BPA [2 mg $(kg\ d)^{-1}$] at four physiological stages: 1 month before breeding, 1 and 4 month of pregnancy, and 1 month after lambing. Blood samples were collected at each period during the three last hours of the infusion and during 48 h after the end of infusion. Percentage of unbound BPA was evaluated for each period by equilibrium dialysis. In a second study, BPA [2 mg $(kg\ d)^{-1}$ and 5 mg $(kg\ d)^{-1}$ for ewes and fetus respectively] and BPA-G [3.54 mg $(kg\ d)^{-1}$] were infused IV for 24 h to seven pregnant ewes or to five fetus at 4 month of gestation. Maternal and fetal plasma and amniotic fluid was collected during the three last hours of infusion thereafter for several days until lambing. BPA, BPA-G and BPA-Sulfate (BPA-S) concentrations were assayed by UPLC/MS/MS.

RESULTS

The pregnancy stage modified neither maternal internal exposure to BPA and its metabolites nor plasma clearance of BPA. The BPA clearance was fivefold higher in fetus than in adults [150 ml $(kg\ min)^{-1}$ versus 30 ml $(kg\ min)^{-1}$], with values in adult very closed to the human. Three percent of the BPA dose infused to the pregnant ewe was transferred across the placenta to the fetus. The feto-placental unit appeared to be highly efficient to conjugate BPA. The conjugated metabolites (BPA-G and BPA-S) remained trapped in the feto-placental compartment. The BPA fraction not bound to plasma protein i.e. the bioactive BPA form, was 2-fold higher in the fetus than in the adults (11% versus 6%), suggesting that for the same dose (total BPA) the foetus is twofold more exposed than ewes.

CONCLUSIONS

For a BPA maternal exposure of 2 mg $(kg\ d)^{-1}$, the fetus received in late pregnancy a BPA dose of about 1 mg $(kg\ d)^{-1}$ when adjusted to its bodyweight. The resulting fetal plasma concentrations of BPA-G and BPA-S were 5–10-fold higher than the maternal ones and these metabolites remained trapped in amniotic fluid until lambing. This high internal exposure to BPA metabolites raises the question of a possible local deconjugation and reactivation of these conjugated forms in target organs (gonads, brain, 3DOTS) and provides new insights for human risk assessment.

15.7.
Molecular and biochemical biomarkers in environmental monitoring: a study with a benthic fish living in the Venice Lagoon

R. M. LOPPARELLI[1], S. PEGOLO[1], M. ARMANI[2], D. BERTOTTO[1], M. GIANTIN[1], M. PAULETTO[1], V. ZANCANELLA[1], E. ZORZAN[1], F. CAPOLONGO[1], G. BINATO[2], F. MUTINELLI[2] & M. DACASTO[1]
[1]*Dip Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, Legnaro, Padova, Italy;* [2]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy*

INTRODUCTION

The Venice Lagoon (VEL) is a coastal ecosystem known to be heavily contaminated (Locatello, 2009). In the present study, the effects of persistent organic pollutants on the aryl hydrocarbon receptor (AhR) and cytochrome P450 1A (CYP1A) expression and catalytic activity (ethoxyresorufin O-deethylase, EROD) were measured *in situ* by using *Zosterisessor ophiocephalus*, a benthic species living in the VEL and showing a resident behaviour.

MATERIALS AND METHODS

Fishes were sampled during spring and autumn seasons from three VEL areas (Porto Marghera, Val di Brenta and Porto Canale) with a high, intermediate or low level of contamination, respectively. A total of 189 pools, each consisting of the liver of three animals, were prepared. Total RNA was extracted and liver microsomes obtained by using common procedures. Species-specific AhR, CYP1A and β-actin (reference gene) coding sequences were identified and sequenced. Aryl hydrocarbon receptor and CYP1A mRNA levels as well as CYP1A apoprotein and EROD were measured by using a quantitative Real Time RT-PCR approach, immunoblotting and a HPLC method,

respectively. Confirmatory residue analyses (non-dioxin-like and dioxin-like polychlorinated biphenyls) were executed on the lipid component of pooled muscle by gas-chromatography.

RESULTS

When compared to Porto Canale, significant increases of CYP1A expression and EROD were noticed in samples from Porto Marghera and Val di Brenta. Data from residues analysis mirrored this trend to induction. Furthermore, season-differences were observed for CYP1A expression (higher in the spring, which represents the reproductive season) and EROD activity (higher in the autumn season). Contrasting results were obtained for AhR gene expression.

CONCLUSIONS

This integrated biomarker approach confirmed Porto Marghera as the most polluted area of VEL (Zonta, 2007). Collectively, CYP1A expression was proved as a suitable biomarker of effect in Zoosterisessor ophiocephalus; therefore, this species may, in turn, be considered as a good sentinel species for VEL environmental monitoring *in situ*. Less clear-cut results were obtained for EROD. Present AhR data need further molecular investigations, also in light of its role in other physiological mechanisms, including reproduction (Calò, 2010).

REFERENCES

1. Locatello, L., Matozzo, V. & Marin, M.G. (2009) Biomarker responses in the crab Carcinus aestuarii to assess environmental pollution in the Lagoon of Venice (Italy). *Ecotoxicology*, **18**, 869–877.
2. Zonta, R., Botter, M., Cassin, D., Pini, R., Scattolin, M. & Zaggia, L. (2007) Sediment chemical contamination of a shallow water close to the industrial zone of Porto Marghera (Venice Lagoon, Italy). *Marine Pollution Bulletin*, **55**, 529–542.
3. Calò, M., Alberghina, D., Bitto, A., Lauriano, E.R. & Lo Cascio, P. (2010) Estrogenic followed by anti-estrogenic effects of PCBs exposure in juvenil fish (*Sparus aurata*). *Food and Chemical Toxicology*, **48**, 2458–2463.

ACKNOWLEDGEMENTS

Project supported by grants from Regione del Veneto (Dgr. 309403/10/2006) to M.D. and F.M. and Universita degli Studi di Padova (60A08-4049/11) to M.G.

15.8.

Honeybees, honey and pollen as sentinels to monitor pesticides presence in environment

O. LAMBERT[1], S. PUYO[1], C. THORIN[2], L. WIEST[3], A. BULETE[3], F. DELBAC[4] & H. POULIQUEN[1]

[1]*LUNAM Université, ONIRIS, Plateforme Environnementale Vétérinaire, CVFSE, Nantes, France;* [2]*LUNAM Université, ONIRIS, Unité de Statistiques, Nantes, France;* [3]*Service Central d'Analyse (SCA), CNRS, ISA, UMR5280, Solaize, France;* [4]*Clermnt Université, CNRS, UMR6023, LMGE, Aubière, France*

INTRODUCTION

Pesticides are widely present in the environment and are suspected occurring adverse effects on wildlife health. Honeybees and apicultural matrices are commonly presented as appropriate sentinels to monitor the occurrence of agricultural and veterinary treatments residues in the environment. The aim of this study was to investigate the contamination of 18 apiaries to pesticides through analyses of bees, honey and pollen over 2 years regarding the landscape and seasonal context. The risk assessment for bees and human health will be discussed.

MATERIALS AND METHODS

Eighteen apiaries from Pays de la Loire and Bretagne (France) were selected: 5, 6, 5 and two apiaries were located in cultivated landscapes, hedgerow landscapes, urban landscapes and small islands respectively. Three apicultural matrices were collected from eight colonies from each apiary. Samples were collected at four times per year during 2008 and 2009. In total, 141, 141 and 128 samples were collected for honey, honeybees and pollen respectively. Samples were analysed for 80 pesticides and veterinary drugs, by a multi residue analysis. For the analysis, a modified QuEChERS method was used followed by GC-ToF and LC-MS/MS.

RESULTS

Thirty-six substances residues were found in all the matrices (Table 1). Carbendazim, coumaphos, amitraz II, triphenylphosphate, phosmet, carbaryl and tau-fluvalinate were the most frequently detected substances. The honeybee samples collected in spring were the most contaminated, while those collected at the end of summer were the less contaminated. The contamination levels according to landscapes decreased in the following order: cultivated > hedgerow > urban > island, but the difference was not statistically significant.

Table 1 Descriptive parameters concerning the pesticides residues found for each apicultural matrix

Matrix	Number of residues	Maximum in a single sample	Mean per sample analyzed	% Of samples contaminated by		
				At least 1 pesticide	2 or more pesticides	3 or more pesticides
Honey	28	8	2.9	95.7	80.8	57.4
Honeybees	20	6	1.4	72.3	36.2	18.4
Pollen	23	7	1.1	58.6	30.5	11.7

© 2012 The Authors

CONCLUSIONS

Our results showed a wide contamination of the apicultural matrices by pesticides and veterinary drugs and were consistent with those previously published in the USA (2) and in Europe (1). Substances used for the control of *Varroa destructor* (ectoparasite of honeybees), coumaphos, amitraz and tau-fluvalinate, were found in all matrices and were the most frequent detected substances. Such results were similar to previous results (1, 2). Concentrations measured in bees were generally low but nine samples showed pesticides at concentrations close to the LD_{50} in bees indicating that sub-lethal effects were highly likely. In view of the contamination levels and taking into account the food consumption of the population, the contamination by pesticides of honey and pollen is of no public health concern for the apiaries studied.

REFERENCES

1. Chauzat et al. (2011) Environmental Toxicology & Chemistry, **30**, 1–9.2.
2. Mullin *et al.* (2010) *PLoS ONE*, **5**: 1–19.
3. Wiest *et al.* (2011) *J Chromatography A*, **1218**, 5743–5756.

15.9.

Toxicovigilance of pesticides: the use of time-series analyses to investigate the effects of mitigation decisions

J. BERNY[1], J. VEIN[1], D. VEY[1] & A. DECORS[2]

[1]*Vetagro Sup, Marcy l'étoile, France;* [2]*ONCFS, St Benoist, France*

INTRODUCTION

The EU has re-evaluated all the existing pesticides and, as a result, only 300–400 active substances are now available instead of over 900 in the late 90s'. Among the reasons for banning pesticides, ecological effects, including non-target poisoning have been considered. The objective of this work was to demonstrate that passive toxicovigilance, as organized under the SAGIR network, could provide valuable information with respect to non-target effects of pesticides and of management decisions (banning, restrictions of use).

MATERIALS AND METHODS

The SAGIR network has been established in 1986 in order to monitor wildlife diseases in France. All toxicological analyses are conducted at the same laboratory. Cases were retrieved from the SAGIR database. Several pesticides were selected for investigation: strychnine, some cholinesterase inhibitors, neo-nicotinoid insecticides and anticoagulant rodenticides. Information regarding species, circumstances of exposure were taken into consideration. All case-series were analyzed statistically for long-term trend (12-month moving average), seasonal effect and unexpected individual events using the R statistical software-epidemiology tools package.

RESULTS

Time-series analyses from 1995 until 2010 indicate that banning may be effective if the products are actively eliminated and not available from other sources or countries. Strychnine, for instance, is still detected, although it was partially restricted in 1984 and completely banned in 2000. A surprising result of our analyses is the detection of shifts in pesticide poisoning cases with obvious 're-emerging' toxicants in the recent years, such as lindane and alpha-chloralose. Analysis of pesticide poisoning incidents involving compounds such as furathiocarb or neonicotinoids showed very clear long-term and seasonal effects. Comparing these effects with management decisions could point out some effective management decisions (coating-color change, restrictions of use) as well as ineffective decisions (restrictions of use on corn or sunflower). Figure 1 describes the actual chronological series, the annual (long-term) trend, the seasonal effect and the individual (random) effect of furathiocarb poisoning incidents over 15 years.

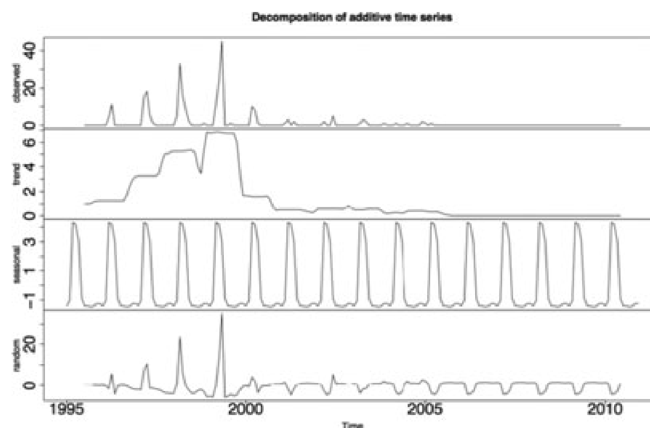


Figure 1. Decomposition of additive time series of Furathiocarb poisoning in birds.

CONCLUSIONS

Spontaneous case reports of wildlife poisoning cases can be analyzed chronologically to identify long term trends and seasonal patterns of poisoning, as well as unexpected events. Comparing these data with management decision can outline effective/ineffective management decisions. As a consequence, despite obvious biases and limitations, passive toxicovigilance monitoring may be a very helpful tool to monitor non-target effects of pesticides and the impact of management decisions.

© 2012 The Authors

Session P3. Innovations in Antimicrobial Therapy Wednesday 11-07: 13.30–15.30

P-3.1.K.
The Mutant Prevention Concentration strategy for minimizing the risk of antimicrobial resistance

J. M. BLONDEAU
Royal University Hospital, University of Saskatchewan, Saskatoon Health Region

The Mutant Prevention Concentration (MPC) defines the antimicrobial drug concentration threshold necessary to block the growth of the least susceptible cell present in high density bacterial populations-such as those seen during acute infection. MPC is determined by measuring the amount of drug required to inhibit the growth of ~1–10 billion bacterial cells. MPC values are then compared with drug pharmacology data and the likelihood for resistance selection/prevention interpreted from this data. To date, MPC measurements have been applied to various classes of antimicrobial agents against key companion animal pathogens. Most recently, a newly approved fluoroquinolone pradofloxacin has been evaluated in our laboratory by MPC measurements. Based on drug pharmacology and low MPC values, pradofloxacin shows a low propensity to select for resistance with key companion animal pathogens. This presentations will provide an overview of the MPC approach and show comparative data for numerous antimicrobial agents representing various drug classes.

P3.2.K.
Animal-specific immunomodulatory antimicrobials

H. P. HAAGSMAN[1], E. J. A. VELDHUIZEN[1], F. J. BIKKER[1,2] & A. VAN DIJK[1]
[1]*Division of Molecular Host Defence, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands;* [2]*Department of Oral Biochemistry, Academic Centre for Dentistry Amsterdam (ACTA), Free University and University of Amsterdam, Amsterdam, The Netherlands*

The innate immune system produces a wide array of small cationic peptides to combat pathogenic microorganisms. These so-called Host Defence Peptides (HDPs) are multifunctional peptides whose classic functionality is associated with their broad-spectrum antimicrobial activity against a variety of Gram-negative and Gram-positive bacteria, fungi, protozoa and some enveloped viruses. HDPs use multiple mechanisms to kill bacteria, e.g. cell wall permeabilisation, inhibition of DNA replication and protein synthesis. Besides direct killing of pathogens, HDPs also regulate immune functions of the host. The multiple actions of HDPs have precluded the development of resistance despite the fact that these peptides are part of the natural host defence of animals for millions of years. Based on HDPs of chicken and pig, small peptides were designed that exhibit antibacterial and immunomodulatory activities. Structure-function relationships of the chicken peptide CATH-2 will be presented. The potency of HDP-derived antimicrobials will be discussed.

SUGGESTED READING

1. van Dijk, A., Veldhuizen, E.J.A. & Haagsman, H.P. (2008) Avian defensins *Vet Immunol Immunopathol*, **124**, 1–18
2. van Dijk, A., Molhoek, E.M., Bikker, F.J., Yu, P.-L., Veldhuizen, E.J.A. & Haagsman, H.P. (2011) Avian cathelicidins: paradims for the development of anti-infectives. *Vet Microbiol*, **153**, 27–36

P3.3.K.
Bacteriophage applications: where are we now?

S. HAGENS
Micreos, Wageningen, The Netherlands

Bacteriophages are bacterial viruses and have been used for almost a century as antimicrobial agents. In the West, their use diminished when chemical antibiotics were introduced, but they remain a common therapeutic approach in parts of Eastern Europe. Increasing antibiotic resistance in bacteria has driven the demand for novel therapies to control infections and led to the replacement of antibiotics in animal husbandry.

SUGGESTED READING

1. Monk, A.B., Rees, C.D., Barrow, P., Hagens, S. & Harper, D.R. (2010) Bacteriophage applications: where are we now? *Lett Appl Microbiol*, **51** (4), 363–369.

© 2012 The Authors

Session 16: New Veterinary Products and Technologies Wednesday 11-07: 16.00–18.00

16.1.

Pharmacokinetics of the novel atypical opioid tapentadol in dogs

M. GIORGI[1], A. MEIZLER[2] & P. C. MILLS[2]
[1]*University of Pisa, San Piero a Grado, Pisa, Italy;* [2]*School of Veterinary Science, The University of Queensland, Brisbane, Qld, Australia*

INTRODUCTION

The novel opioid tapentadol (TAP) belongs to a new pharmacological class with a dual action as an agonist of the mu opioid receptor and a noradrenaline reuptake inhibitor (Kress, 2010). TAP may provide a useful alternative for pain management in veterinary medicine, particularly species where opioids have limited efficacy or unsuitable pharmacokinetic (PK) features (Giorgi, 2012).

MATERIALS AND METHODS

Six normal dogs (four male and two female), aged 4–10 years, and weighing 13–35 kg, were randomly assigned to two treatment groups, using an open, single-dose, two-treatment, two-period, and randomized cross-over design, with a 1-week washout period. Each dog in group I received a single oral dose of 200 mg dog^{-1} of TAP [sustained release (SR) tablet; Palexia, Grunenthal, Germany] after a 12 h overnight fast. The SR formulation could not be divided since this would affect drug release. In group II dogs were intravenously administered TAP (50 mg dog^{-1}). Blood samples were collected at 0, 5, 15, 30, and 45 min, and 1, 1.5, 2, 4, 6, 8, 10 and 24 h after drug administration. Plasma was analyzed by an HPLC-FL method (Giorgi *et al.*, submitted). The PK calculations were performed using WinNonLin v 5.3 using standard non-compartmental equations.

RESULTS

Some weight-related adverse effects (sedation and panting) were detected in IV group, while light sedation was noticed in the oral group. After IV administration, the TAP concentrations were detectable up to 6 h. The half-life was in the range 38–61 min, in line with what was previously reported in rodents (Tzschentke, 2006). After oral administration, variability in PK parameters among the subjects was measured. Generally, drug absorption was rapid (T_{max} 45–60 min), but the oral bioavailability was low (4.4 ± 2.3), which was similar following administration of other opioids in dogs (KuKanich, 2009).

CONCLUSIONS

TAP would appear to have a short duration of effect in dogs if the concentration necessary for analgesia (5–300 ng ml^{-1}) was extrapolated from humans. In conclusion, this is the first report describing the PK of TAP in dogs and the findings suggest that it may be a useful addition to the analgesics available in this species.

16.2.

The prevention of urinary tract infection in dogs by using sustained release varnish of chlorhexidine coated urinary catheters

E. LAVY, T. BRINKER, M. FRIEDMAN, S. STEINBERG & G. SEGEV
Hebrew University of Jerusalem, Rehovot, Israel

INTRODUCTION

Canine urinary tract infection (UTI) is common (1) and is associated with urinary catheters (2). Attempts to prevent catheter associated UTI are usually unsuccessful due to biofilm formation. The use of antibiotics may even predispose to resistance (3). The aim of this prospective study was to assess the safety and efficacy of a sustained release varnish of chlorhexidine coated urinary catheters in preventing biofilm formation and UTI in dogs.

MATERIALS AND METHODS

Thirteen dogs had a coated urinary catheter placed for a median of 72 h (study group) and 13 dogs received an untreated urinary catheter for a median of 36 h (control group). Presence and intensity of biofilm formation on the urinary catheter was assessed and compared between the groups by evaluating colony forming unit (CFU) counts of biofilm bacteria (*t*-test), and by semi-quantitatively using confocal and electron microscopy (Fisher Exact test).

RESULTS

Median CFU count was significantly lower in the study compared to the control group [median 125 CFU ml^{-1} (range, 0–7.5×10^3) versus 10×10^5 CFU ml^{-1} (range, 0.75–7.5×10^7), $P < 0.001$] The degree of intensity and the presence of bacteria on the urinary catheter as evaluated by confocal and electron microscopy, respectively, were significantly lower in the study group. The proportion of dogs with positive urine culture prior to catheter removal tended to be lower in the study compared to the control group (1/8, 11% versus 6/12, 50%, $P = 0.06$).

CONCLUSION

Sustained release varnish of chlorhexidine coated urinary catheters is safe and effective in the prevention of biofilm formation on urinary catheters, and potentially of catheter associated UTI.

REFERENCES

1. Ling, G.V. (1984) Therapeutic strategies involving antimicrobial treatment of the canine urinary tract. *J Am Vet Med Assoc*, **185**, 1162–1164.
2. Trautner, B.W. & Darouiche, R.O. (2004) Catheter-associated infections: pathogenesis affects prevention. *Arch Intern Med*, **164**, 842–850.
3. Koseoglu, H., Aslan, G. & Esen, N. (2006) Ultrastructural stages of biofilm development of *Escherichia coli* on urethral

© 2012 The Authors

catheters and effects of antibiotics on biofilm formation. *Urology*, **68**, 942–946.

16.3.

Preliminary evaluation of the efficacy of finrozole for the inhibition of oestrus in bitches

K. HEINONEN[1], A. BANTING[2], S. BEASLEY[1] & L. REČEK[3]
[1]*Vetcare Oy Mäntsalä, Finland;* [2]*La Bergerie, St Etienne de Chigny, France;* [3]*BioTest s.r.o. Konárovice, Czech Republic*

INTRODUCTION

Finrozole is a novel non-steroidal inhibitor of aromatase that has been shown to decrease 17β oestradiol to increase progesterone and testosterone blood concentrations in humans (1) and that is being developed for the inhibition of oestrus in dogs.

A study has been conducted in arctic fox vixens (*Vulpes lagopus*) that have a close hormonal pattern to bitches (2,3). The pharmacokinetic profile of Finrozole is similar to that observed in dogs (unpublished data). When Finrozole is administered before peak 17β oestradiol levels are reached, oestrus is suppressed. The decrease in oestradiol and the increase in progesterone blood levels were confirmed (4). The purpose of the study was to confirm that Finrozole inhibits oestrus in bitches. The study was approved by the Institutional Animal Care and Use Committee and the Committee for Animal Protection of the Czech Republic.

MATERIALS AND METHODS

The study was conducted on six Beagle bitches aged 1.7–7 years. Each animal received Finrozole daily, as capsules, prior to feeding, for 21 consecutive days at a dose rate ranging from 25.57 to 31.96 mg kg^{-1} per day. Treatment started 3 days after the confirmation of the clinical signs of heat (day 1). Clinical signs, Adverse Drug Reactions (ADR's), were recorded in all the animals daily. Vaginal smears were taken from day −2 to day 4, 6–8 and days 10, 12, 20, 22, 28. The bitches were put in contact with a male dog on days 8 and 10.

RESULTS

The animals remained in good health throughout the trial; no ADR's were observed. The bitches showed clinical signs of heat that disappeared during treatment. No mating occurred. Vaginal cytology showed the bitches to be in the pro-oestrus phase. After the start of treatment, rapid changes occurred and vaginal cytology revealed metoestrus at the scheduled time of mating. No evidence of oestrus was seen.

CONCLUSIONS

Finrozole effectively inhibits oestrus in bitches when given during early pro – oestrus, hence confirming that this is a novel and promising approach for the control of heat in bitches.

REFERENCES

1. Ahokoski, O., Irjala, K., Huupponen, R., Halonene, K., Salminen, E. & Scheinin, H. (1998) Hormonal effects of MPV-2213ad, a new selective aromatase inhibitor in healthy male subjects. *Br J Clin Pharmacol*, **45**, 141–146.
2. Farstad, W.K. (1993) Periovulatory endocrinology, oocyte maturation, fertization and fertility in female blue fox *Alopex lagopus.* PhD Thesis, Norwegian College of Veterinary Medicine, Oslo, Norway.
3. Valtonen, M. & Jalkanen, L. (1993) Species specific features of oestrus development and blastogenesis in domestic canine species. *J Reprod Fertil*, **47**, 133–1374.
4. Lindeberg, H. *et al.* (2012) The effect of a new aromatase inhibitor, Finrozole, on reproductive functions of arctic foxes, 12th EAVPT Congress.

16.4.

Pharmacokinetics of fluorescein for gastrointestinal confocal endomicroscopy in dogs

M. J. SHARMAN, C. S. MANSFIELD & T. WHITTEM
University of Melbourne, Werribee, Australia

INTRODUCTION

Fluorescein is a low molecular weight, water soluble, fluorophore routinely used intravenously for diagnostic retinal angiography in people and dogs. Recently fluorescein has been utilised for clinical confocal endomicroscopy (CEM) of the gastrointestinal tract in people. This novel technology allows acquisition and evaluation of *in vivo* histologic-equivalent images of the gastrointestinal mucosal architecture, but relies upon the use of fluorophores to provide sufficient fluorescent contrast. Although fluorescein is commonly used clinically, evaluation of its pharmacokinetics in both people and animals is limited. The aim of the current study was to evaluate the efficacy, pharmacokinetics and relative safety of fluorescein in dogs for gastrointestinal CEM.

MATERIALS AND METHODS

Six healthy adult mixed-breed dogs (1F, 5M) with a mean weight of 18.5 kg (Range 12.2–21.6 kg) were included in the study. Dogs were anaesthetised for standard upper gastrointestinal endoscopy and CEM. Fluorescein (15 mg kg^{-1}, 10% aqueous solution; Sigma Aldrich) was administered intravenously to provide fluorescent contrast for CEM and blood samples were collected at 5, 10, 20, 30, 45, 60, 90 and 120 min. Cardiorespiratory variables were monitored throughout anaesthesia and dogs were monitored for a minimum of 6 h post-procedure for obvious adverse effects. Plasma sample analysis was by C18 reverse phase HPLC with UV/Vis detection with hydrocortisone as the internal standard. Calibration curves were produced by preparing pooled canine plasma at known concentrations. The curve was linear within the range of 0.1–50 mg l^{-1} using a log-log transformation. The lower limit of quantification for the assay was set at 0.1 mg l^{-1}, being the lowest concentration on the standard curve. The concentration data were evaluated by compartmental analysis. The best model was chosen using visual inspection of weighted (1/y) residuals and Akaike's Information Criterion.

RESULTS

Evaluation of canine mucosal architecture *in vivo* via CEM was achievable using fluorescein and imaging was optimal during at least the first 30 min following intravenous administration. Fluorescein plasma concentrations were adequately described by a two compartment model in 5/6 dogs and by a one compartmental model in one dog. The latter dog was excluded from overall analysis. Mean plasma concentration 5 min after a

single injection of fluorescein was 57.6 ± 18.2 mg/L and plasma concentrations decreased bi-exponentially thereafter with a mean concentration of 2.5 ± 1.3 mg/L at 120 min. Mean terminal plasma elimination half-life ($t½\beta$) was 34.8 min ± 8.9. No unexpected adverse effects were observed, although discolouration of the sclera and urine were noted following the procedure. Based upon a calculated clearance of 9.1 ml kg^{-1} per minutes a constant rate infusion (CRI) at a rate of 0.18 mg kg^{-1} per minutes was estimated to be adequate to maintain plasma concentration at 20 mg l^{-1} for optimal CEM imaging following initial bolus administration.

CONCLUSIONS

Fluorescein administered IV at the selected dose (15 mg kg^{-1}) provided sufficient fluorescent contrast to allow histologic-equivalent evaluation of mucosal morphology and vasculature, via CEM, for 30 min adverse effects were minor and transient. Use of a CRI following initial fluorescein bolus may be useful to maintain plasma concentrations and allow for prolonged imaging procedures.

16.5. Pharmacokinetics of ketorolac in horses undergoing orchiectomy

P. CAGNARDI, C. FERRARESI, G. RAVASIO, A. PECILE, S. CARLI, R. VILLA & A. ZONCA
Università degli Studi di Milano, Milano, Italy

INTRODUCTION

Ketorolac (KET) is a potent non-steroidal anti-inflammatory drug, approved for use in humans, that possesses a strong analgesic activity (Gills, 1997). It seems to be as effective as morphine, but without the troublesome side effects of opioids and this may allow its use in the control of short-term pain in surgical patients (Brocks, 1992). Due to the favourable anti-inflammatory and analgesic activities, KET could represent an useful tool to control pain in animals as well. The aim of the study was to determine the pharmacokinetics of KET after a single IV dose of 0.5 mg kg^{-1} administered in the pre-operative period in horses undergoing gonadectomy.

MATERIALS AND METHODS

Animals: Five male horses undergoing orchiectomy, 294 ± 51 kg b.w., 2 years old and clinically healthy. *Drug:* ketorolac tromethamine IV at the dose of 0.5 mg kg^{-1} b.w. after the induction of anaesthesia i.e. about 10 min before the beginning of the surgery. Blood samples were collected before administration and during the following 36 h. KET was quantified by an intra-laboratory validated HPLC method with UV-visible detection (Limit of quantification, LOQ 0.01 μg ml^{-1}) (Pasloske *et al.*, 1999). A two-compartmental analysis was performed on KET serum concentrations (WinNonLin 6.1). Intra-operative cardio-respiratory variables were monitored and post-operative pain was assessed up to 12 h by a visual analogue scale.

RESULTS

The dose selected (0.5 mg kg^{-1} b.w. IV) did not produce adverse effects. Drug concentrations were quite low (2.63 ± 0.72 μg ml^{-1} at 0.08 h) and decreased rapidly, so that KET was close to the LOQ at 3 and 4 h and then was never detected. Mean pharmacokinetic parameters (±SD) are listed in Table 1. Cardio-respiratory variables were among physiological ranges, pain scores were low and the rescue analgesia was never administered.

Table 1 Pharmacokinetic parameters after IV ketolac tromethamine administration in five horses at the dose of 0.5 mg kg^{-1} b.w.

Parameter (units)	Mean ± SD
AUC $_{0-\infty}$	1.75 ± 1.03
$t_{1/2\ K1}$ (h)	0.06 ± 0.02*
$t_{1/2\ K2}$ (h)	0.59 ± 0.2*
V_t (ml kg^{-1})	107.55 ± 78.24
Cl_β (ml h^{-1} kg^{-1})	339.99 ± 120.19
$AUMIC_{0-\infty}$ (h μg ml^{-1})	0.87 ± 0.28
MRT $_{0-\infty}$ (h)	0.59 ± 0.29
V_{ss} (ml kg^{-1})	218.83 ± 134.26

$AUC_{0-\infty}$ = mean area under serum concentration curve from 0 extrapolated to infinity. $t_{1/2\ K1}$ (h) = distribution half-life; $t_{1/2\ K2}$ (h) = elimination half-life; V_t (ml kg^{-1}) = volume of distribution in the central compartment; Cl_β (ml h^{-1} kg^{-1}) = body clearance; $AUMIC_{0-\infty}$ (h μg ml^{-1}) = area under moment curve; $MRT_{0-\infty}$ (h) = mean residence time; V_{ss} (ml kg^{-1}) = volume of distribution at steady state.
*Harmonic mean ± pseudo SD.

CONCLUSIONS

KET administered IV at the dose of 0.5 mg kg^{-1} b.w. was characterized by a very rapid distribution and short elimination half-life, low body clearance and volume of distribution, characteristics that could make ketorolac valuable especially for racing horses. The use of ketorolac in horse should be further investigated and studies at higher doses would be advocated to clarify the therapeutic effects of KET in moderate to painful surgery.

REFERENCES

1. Gillis, J.C. & Brogden, R.N. (1997) Ketorolac. *Drugs*, **53** (1), 139–188.
2. Brocks, D.R. & Jamali, F. (1992) *Clinical pharmacokinetics*, **23** (6), 415–427.
3. Pasloske, K., Renaud, R., Burger, J. & Conlon, P. (1999) *Journal of Veterinary Pharmacology and Therapeutics*, **22**, 314–319.

16.6. Pharmacokinetics of dexmedetomidine in calves

P. CAGNARDI[1], L. LUCATELLO[2], G. RAVASIO[1], C. FERRARESI[1], A. BOCCARDO[1], V. LOCATELLI[1], C. MONTESISSA[2] & D. PRAVETTONI[1]
[1]*Università degli Studi di Milano, Milano, Italy;* [2]*Università degli Studi di Padova, Padova, Italy*

INTRODUCTION

Dexmedetomidine (DEX) is the dextro-rotary and active enantiomer of the racemic mixture medetomidine, a selective alpha-2-agonist with centrally mediated sympatholytic, sedative, and analgesic effects. Due to its property to preserve respiratory

function, the use of DEX is also increasing in pediatrics (Lee *et al.*, 2007) It has marketing authorisation in small animal practice and is one of the most potent alpha-2-agonist available commercially (Marcilla *et al.*, 2012). The positive DEX characteristics make its use advisable also in other species, such as food producing animals in which the use of drugs is restricted by the necessity to respect MRLs and withdrawal times. Most sedatives, such as alpha-2-agonists, do not require MRLs (Annex II), nevertheless only few are approved in cattle. The aim of this study was to evaluate the kinetic profile of DEX in clinically healthy calves and to suggest a possible use for the drug in this species.

MATERIALS AND METHODS

Animals: Six calves (Holstein Friesian), 49 ± 7 kg body weight, 41 ± 13 days old and clinically healthy. *Drug:* dexmedetomidine IV at the dose of 5 mg kg^{-1} b.w. Blood samples: collected in EDTA tubes from jugular catheter before treatment and during the following 720 min. DEX was quantified by a validated HPLC/MS/MS method (limits of quantification and detection 0.023 and 0.006 ng ml^{-1}, respectively) (Lee *et al.*, 2007; Li *et al.*, 2009). A two-compartmental analysis was performed (WinNonLin 6.1) on DEX plasma concentrations.

RESULTS

The table shows mean pharmacokinetic parameters (±SD). The selected dose of 0.5 mg kg^{-1} b.w. IV did not produce adverse effects. The induction of anaesthesia was rapid (3 min) and a good sedation with a smooth recovery in 74 ± 18 min was obtained. Cardio-respiratory variables were within physiological ranges for the whole period.

Dexmedetomidine pharmacokinetic parameters after IV administration at the dose of 0.5 µg/kg in calves

Parameters (units)	Mean ± S.D.
C_0 (µg/ml)	0.011±0.01
$t_{1/2\lambda 1}$ (min)	7.19±4.61[a]
$t_{1/2\lambda 2}$ (min)	65.65±53.30[a]
$AUC_{0\to\infty}$ (min.µg/ml)	0.18±0.07
$AUMC_{0\to\infty}$ (µg.min/ml)	16.57±20.1
$MRT_{0\to\infty}$ (min)	89.07±82.75
Cl_B (ml/min/kg)	31.4±10.65
V_c (ml/kg)	752.83±509.19
V_{dss} (ml/kg)	2620.82±1911.87

C_0 = serum concentration at time 0; $t_{1/2\lambda 1}$ = distribution half-time; $t_{1/2\lambda 2}$ = elimination half-time; $AUC_{0\to\infty}$ = area under serum concentration-time curve; $AUMC_{0\to\infty}$ = area under moment curve; $MRT_{0\to\infty}$ = mean residence time; Cl_B = body clearance; V_c = volume of distribution in central compartment; V_{dss} = volume of distribution at steady state; [a] harmonic mean±pseudo SE

CONCLUSIONS

To the authors' knowledge, this is the first pharmacokinetic study on DEX in calves. DEX was characterised by rapid distribution and short elimination half-life, high body clearance and volume of distribution. At the dose adopted, DEX would be helpful for short term clinical procedures in young calves although further studies would be advocated to clarify the therapeutic role of the drug in calves.

REFERENCES

1. Lee, J.I., Su, F., Shi, H. & Zuppa, A.F. (2007) *Journal of Chromatography B*, **852**, 195–201.
2. Li, W., Zhang, Z., Wu, L., Tian, Y., Feng, S. & Chen, Y. (2009) *Journal of Pharmaceutical and Biomedical Analysis*, **50**, 897–904.
3. Marcilla, M.G., Schauvliege, S., Segaert, S., Duchateau, L. & Gasthuys, F. (2012) *Veterinary Anaesthesia and Analgesia*, **39**, 49–58.

16.7.

Biotransformation in rats of R,S-2-hydroxy-4-methyl selenobutanoic acid (HMSeBA), a new source of organic selenium

P. RICHEZ[1], E. BALTUSSEN[2], Y. MERCIER[3], M. BRIENS[3] & P. REY[3]

[1]*TransPharm, St Genies des Mourgues, France* [2]*Notox, 's Hertogenbosch, The Netherlands* [3]*Adisseo, Antony, France*

INTRODUCTION

Selenium exerts its effects via selenoproteins, hydrogen selenide and methylated selenium compounds. Deficiency may delay the development of immunocompetence and increase animal susceptibility to infectious diseases. Selenium is available in the form of inorganic salts (e.g. sodium selenate or selenite) or organic salts (e.g. selenomethionine obtained from yeasts). Ingested selenomethionine is either metabolised directly to reactive forms of selenium or is stored instead of methionine in body proteins as its metabolism is closely linked to protein turnover (1). The aim of this study was to investigate whether 2-Hydroxy-4-methyl-selenobutanoic acid (HMSeBA), a new purified organic form of selenium obtained by chemical synthesis, is a precursor of selenomethionine *in vivo*, and hence may be considered as a chemically-defined source of organic selenium that can be used to prevent selenium deficiencies.

MATERIALS AND METHODS

Eight female Crl:WI (Han) (outbred, SPF-Quality) rats, 7–10 weeks old, were dosed with HMSeBA 1 mg kg^{-1} body weight (equivalent to Se 0.4 mg kg^{-1}) for four consecutive days; blood and muscle samples were taken from two rats killed 0, 1, 3 and 6 h post last dosing. Specific analytical methods were developed to analyse the plasma and muscle samples: HPLC-ICP-MS for HMSeBA, Seleno-methionine (Se-Met) and other possible Seleno metabolites (e.g. Seleno-cystine and Seleno-cysteine) and ICP-MS for total Selenium.

RESULTS

Total selenium concentrations rapidly increased after dosing (from 600 to 700 $\mu g\ l^{-1}$) and steady-state concentrations of 1400–2000 $\mu g\ l^{-1}$ were obtained 1–6 h post-dosing. HMSeBA concentrations were low at $T + 1$ h and below the Limit of Quantification at all subsequent time points whereas selenomethionine and selenocysteine were detected up to 6 h post dosing.

CONCLUSIONS

This showed that oral administration of HMSeBA led to selenium incorporation in tissues, at least partly through the formation of selenomethionine. HMSeBA was thus rapidly and extensively absorbed and metabolised to form selenium and selenium derivatives in blood and tissues. Hence, its use in food animals will not result in any tissue residues of unchanged HMSeBA. Organic selenium in the form of yeast is less toxic than inorganic selenium in the form of sodium selenite (2). One explanation for this is that the selenium yeast may be more slowly converted than selenite to a toxic form. A similar pattern can be expected

© 2012 The Authors

for HMSeBA, a purified (100%) form of organic selenium for food animals.

REFERENCES

1. Schrauzer, G. (2000) Selenomethionine: A Review of Its Nutritional Significance, Metabolism and Toxicity. *J Nutr*, **130**, 1653–16562.
2. Vinson, J.A. & Bose, B. (1987) In: *Selenium in Biology and Medicine*. Eds Combs, G.F., Levander, O.A., Spallholz, J.E. & Oldfield, J.E., Van Nostrand, NY, USA.

16.8.

Pharmacokinetics of chloramphenicol in koalas (*Phascolarctos cinereus*) following administration of chloramphenicol sodium succinate.

L. A. BLACK, A. J. MAC LACHLAN & M. GOVENDIR
The University of Sydney, Sydney, NSW, Australia

INTRODUCTION

Chloramphenicol is one of the few antibacterials advocated for treating the most common infectious disease of koalas, chlamydiosis, as the macrolides and tetracyclines used as first line treatment for chlamydiosis in humans induce emaciation and death in koalas (Brown, 1984; Osawa, 1990). The pharmacokinetic (PK) profile of chloramphenicol has not been reported in healthy koalas. The two PK studies published for koalas have shown subtherapeutic plasma concentrations following conventional antibiotic dose administration to diseased koalas (Govendir, 2012; Griffith, 2010). The inactive prodrug, chloramphenicol sodium succinate (CSS), is the only formulation available for intravenous veterinary use; it is hydrolysed to chloramphenicol after administration. This study aimed to characterise the PK profile of chloramphenicol in clinically normal koalas following CSS administration, to inform the design of koala-specific chloramphenicol dosing regimens.

MATERIALS AND METHODS

Clinically normal koalas (n = 13) were administered a single dose of CSS intravenously (IV) (equivalent to 25 mg kg^{-1} chloramphenicol; n = 6) or subcutaneously (SC) (equivalent to 60 mg kg^{-1} chloramphenicol; n = 7). Serial plasma samples were collected over 24 h and chloramphenicol concentrations were determined using a validated high performance liquid chromatography (HPLC) assay. In vitro plasma protein binding was determined by ultrafiltration. Data was subjected to non-compartmental PK analysis.

RESULTS

The median systemic clearance (CL) and half-life (t1/2) of chloramphenicol after IV CSS administration were 0.52 (range 0.35–0.99) l h^{-1} per kg, and 1.13 (range 0.76–1.40) h respectively. SC CSS resulted in a high median maximal concentration (C_{max}) (20.4; range 13.9–25.2 μg ml^{-1}) and short median t_{max} (1.25 h; range 1.0–2.0 h). The bioavailability (F) of CSS after SC administration ranged from 0.49 to 0.95 (median, 0.68). Protein binding was 58.4 ± 4.4% (mean ± SD) across the assay range.

CONCLUSIONS

As an antibacterial with time-dependent effects, the rapid t_{max} and relatively high CL of chloramphenicol following SC CSS administration render this formulation inadequate for once-daily dosing in koalas. However, the data from this study will be useful for comparisons to the poorly soluble chloramphenicol base formulation, which cannot be administered intravenously.

REFERENCES

1. Brown, A.S., Wood, A.D. & Dickens, R.K. (1984) *Dirty Tail and Other Chlamydial Diseases in Koalas. Control and therapy series*, no 1792. The University of Sydney, NSW.
2. Govendir, M., Hanger, J., Loader, J.J., Kimble, B., Griffith, J.E., Black, L.A., Krockenberger, M.B. & Higgins, D.P. (2012) Plasma concentrations of chloramphenicol after subcutaneous administration to koalas (*Phascolarctos cinereus*) with chlamydiosis. *Journal of Veterinary Pharmacology and Therapeutics*, **35** (2), 147–154.
3. Griffith, J., Higgins, D., Li, K., Krockenberger, M. & Govendir, M. (2010) Absorption of enrofloxacin and marbofloxacin after oral and subcutaneous administration in diseased koalas (*Phascolarctos cinereus*). *Journal of Veterinary Pharmacology and Therapeutics*, **33** (6), 595–604.
4. Osawa, R. & Carrick, F. (1990) Use of a dietary supplement in koalas during systemic antibiotic treatment of chlamydial infection. *Australian Veterinary Journal*, **67** (8), 305–307.

16.9.

Plasma pharmacokinetics of meloxicam after oral, subcutaneous and intravenous administration in the koala

B. KIMBLE[1], L. BLACK[1], S. GILCHRIST[2], A. GILLETT[3], S. JOBBINS[1], K. LI[1] & M. GOVENDIR[1]
[1]*The University of Sydney, Sydney, Australia;* [2]*Sydney Wildlife World, Sydney, Australia;* [3]*The Australia Zoo Wildlife Hospital, Beerwah, Australia*

INTRODUCTION

The koala, an iconic Australian animal can be traumatized by feral animals or vehicle strikes especially during the breeding season when they are most active, therefore analgesics and anti-inflammatory drugs are used for this species. Meloxicam, a non-steroidal anti-inflammatory drug, is approved for the treatment of pain and inflammation in people and some veterinary species. The currently recommended dosage regimen for koalas has been extrapolated from dogs (0.1–0.2 mg kg^{-1} oral s.i.d) (Blanshard, 2008). As koalas are known to have highly efficient hepatic metabolism (Jones, 2008), the aim of this study was to investigate the pharmacokinetic profile of meloxicam in koalas.

MATERIALS AND METHODS

Fifteen clinically normal koalas were recruited. Single doses of meloxicam were administered intravenously (n = 5, 0.4 mg kg^{-1}), subcutaneously (n = 1, 0.2 mg kg^{-1}) or orally (n = 3, 0.2 mg kg^{-1}), and multiple doses were given to two groups of koalas via the oral or subcutaneous routes (n = 3 for both routes) with a loading dose of 0.2 mg kg^{-1} for day 1 followed by 0.1 mg kg^{-1} s.i.d for a further 3 days. After drug administration, serial blood samples were collected to determine plasma drug concentrations at time points over 4–5 elimination half-lives ($t_{1/2}$). Plasma drug concentrations were quantified with vali-

© 2012 The Authors

dated liquid chromatography. Plasma protein binding was determined via ultrafiltration method.

RESULTS

Meloxicam had a rapid clearance (0.44 ± 0.2 l h^{-1} per kg), a volume of distribution$_{(area)}$ of 0.72 ± 0.22 l kg^{-1} and a volume of distribution at steady state of 0.22 ± 0.12 l kg^{-1}. The median $t_{1/2}$ was 1.19 h (range 0.71–1.62 h). Plasma protein binding was 98%. Oral bioavailability, either from single or multiple doses, appeared to be low. Subcutaneous bioavailability was 56% after a single dose and 70% after multiple doses.

CONCLUSIONS

Meloxicam administration at comparable dose rates and dosage intervals produces different pharmacokinetic profiles in dogs versus koalas. Compared to dogs, the koala has almost incomplete oral absorption and a much shorter $t_{1/2}$ (1.19 versus 24 h). The pharmacokinetic profile seen in koalas is consistent with what is known about their efficient metabolic pathways and also suggests barriers to oral and subcutaneous absorption. This study reinforces that PK/PD studies are necessary when formulating dosage regimens for specific species.

REFERENCES

1. Blanshard, W. & Bodley, K. (2008) Koalas. In: *Medicine of Australian mammals*. Eds Vogelnest, L. & Woods, R., pp. 312–315. CSIRO, Melbourne, Vic.
2. Jones, B., El-Merhibi, A., Ngo, S., Stupans, I. & Mckinnon, R. (2008) Hepatic cytochrome P450 enzymes belonging to the CYP2C subfamily from an Australian marsupial, the koala (*Phascolarctos cinereus*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* **148** (3), 230–237.

© 2012 The Authors

Session P4: Evidence-Based Dosing Regimens for Antibiotics: PK/PD Approaches
Wednesday 11-07: 16.00–18.00

P-4.1.
Differential activity of veterinary antibiotics on target pathogens versus digestive flora: development of a pharmacodynamic selectivity index for public health perspective

M. VASSEUR, A. FERRAN, P.-L. TOUTAIN &
A. BOUSQUET-MÉLOU
INRA Toxalim UMR 1331, Toulouse, France

INTRODUCTION
The main challenge of antimicrobial chemotherapy in animals consists of eradicating pathogenic bacteria while minimizing the transmission of resistances from animal to man. In this respect, a strategy would be to select antibiotics specifically targeting pathogenic bacteria while sparing gut flora, the critical reservoir of resistance genes in animals. We propose a pharmacodynamic index to quantify the selectivity of antibiotics on pathogenic versus commensal bacteria. For illustrating our proposal, we took the example of two beta-lactams – amoxicillin (AMOX) and cefquinome (CEFQ), a fourth-generation cephalosporin, against *Pasteurella multocida*, a pathogen responsible for lung infections, and *Escherichia coli*, an indicator of commensal Enterobacteriaceae harboured in gut flora.

MATERIALS AND METHODS
We performed *in vitro* time-killing experiments with different concentrations of AMOX and CEFQ on *E. coli* (10^7 CFU ml^{-1}) and *P. multocida* (10^5 or 10^7 CFU ml^{-1}). Results were modelled with an equation describing the antibacterial activity (bacterial inoculum change) versus antibiotic concentration $\Delta I(a) = \Delta I_{MAX} - (((\Delta I_{MAX} - \Delta I_{MIN})*(a/EC_{STATIC})\gamma)/((a/EC_{STATIC})\gamma\text{-}(\Delta I_{MAX} - \Delta I_{MIN})))$ where ΔI(a) is the Mean Inoculum Change (LogCFU ml^{-1}) over 24 h for an antibiotic concentration ($\mu g\ ml^{-1}$), ΔI_{MAX} and ΔI_{MIN} (LogCFU ml^{-1}) the Mean Inoculum Changes obtained without antibiotic or with increasing antibiotic concentrations respectively, EC_{STATIC} ($\mu g\ ml^{-1}$) the antimicrobial concentration producing bacteriostatic effect ($\Delta I(EC_{STATIC}) = 0$), and γ the sigmoid coefficient of the curve. Based on the concentration-effect relations, we defined the selectivity index (SI) as the ratio of ECSTATIC for *E. coli* over EC90, the concentration producing 90% of maximal antibacterial effect against *P. multocida*.

RESULTS
Concentration-effect curves indicated antibacterial activities of both antibiotics correlating to their MIC for bacterial species, CEFQ being more potent than AMOX (ECSTATIC 8-to-10-fold lower). With high 10^7-inocula of *P. multocida*, SI values were 5.78 and 0.74 for AMOX and CEFQ respectively, meaning that AMOX was more potent against *P. multocida* than *E. coli* whereas CEFQ was equally potent against both bacteria. EC90 against 10^5-inocula of *P. multocida* were lower compared to 10^7-inocula for both AMOX (eightfold) and CEFQ (threefold), leading to SI values of 47.69 (AMOX) and 2.36 (CEFQ) for 10^5-inocula. Acting against a low pathogenic inoculum increased selectivity of both antibiotics.

CONCLUSIONS
We propose a pharmacodynamic index for assessing antibiotic selectivity in the context of public health protection. Despite the fact that CEFQ is much more potent than AMOX against both *P. multocida* and *E. coli*, selectivity towards the lung pathogen was higher for AMOX, indicating that concentrations required for efficacy are less likely to impact intestinal Enterobacteriaceae. Moreover, selectivity of both antibiotics can be improved when acting against lower pathogenic inocula, corresponding to early stage of infection. Further investigations in animals will be required to determine the impact on selectivity of pharmacokinetic behaviour of the antibiotics, which controls their distribution in the anatomic locations of both the targeted pathogenic flora and the commensal intestinal flora.

P-4.2.
Comparative mutant prevention concentration and minimum biofilm eradication concentration to selected veterinary fluoroquinolones in *Actinobacillus pleuropneumoniae*

D. DAMTE, S.-J. LEE & S. C. PARK
Kyungpook National University, Daegu, South-Korea

INTRODUCTION
The 'mutant selection window (MSW)' hypothesis has been developed to describe how drug exposures below the mutant prevention concentration (MPC) may create the selection of resistant bacterial strains. Hence, several recent studies have emphasized the importance of MPC-based dosing strategies to improve therapeutic outcome and restrict the selection of resistant mutants. The aim of this study was to evaluate the comparative activity, in terms of both MIC and MPC, of three fluoroquinolones used in pig industry against recent *A. pleuropneumoniae* isolates from pigs with clinical signs of respiratory disease. Additionally, the impact of biofilm on susceptibility of antimicrobials was evaluated.

MATERIALS AND METHODS
A total of 12 clinical isolates of *A. pleuropneumoniae* were collected from pigs. The MIC for all clinical isolates and a quality control strain (ATCC 27090) was determined in triplicate using the Clinical and Laboratory Standards Institute (CLSI) broth micro-dilution method. The MPC of all the three fluoroquinolones was determined as described in Gebru *et al.* (2012). The minimum biofilm eradication concentration (MBEC) was determined using the Calgary Biofilm Device (CBD) following the manufacture's instruction. The interpretative criteria of clinical breakpoints were taken from CLSI M31-A3 as available. To obtain further insight into the clinical utility of our *in vitro* data, we integrated the pharmacodynamic (PD) data obtained in this

© 2012 The Authors

study with published pharmacokinetics (PK) data at the recommended clinical doses of fluoroquinolones in pigs, and computed various PK-PD indices (Ding *et al.*, 2010).

RESULTS

The *in vitro* activities, in terms of MIC, MPC, MBEC and MPC/MIC ratios, and the proportion of resistant isolates are presented in Table 1. Integrating our data with reported PK data at the recommended dose ranges revealed that with current doses all three fluoroquinolones could not achieve maximum plasma concentration (C_{max}) greater than the MPC of 90% isolates (C_{max}/MPC_{90}). The overall rank of potency against *A. pleuropneumoniae*, based on C_{max}/MIC, C_{max}/MPC, the area under concentration-time curve (AUC)/MIC, and AUC/MPC values, was in decreasing order: difloxacin > marbofloxacin > enrofloxacin.

Table 1. Comparative activity of three fluoroquinolones against clinical isolates of *A. pleuropneumoniae* from pigs

Parameters	Antibiotics (μg/ml)		
	Enrofloxacin	Difloxacin	Marbofloxacin
$Rnage_{MIC}$	0.125-4	0.125-1	0.125-1
MIC_{50}	0.25	0.125	0.5
MIC_{90}	0.5	0.25	0.5
R (%)*	8.33	0	0
$Range_{MPC}$	0.5-8	0.5-4.0	0.5-8
MPC_{50}	2	1	0.5
MPC_{90}	8	2	4
MBCE	>512	512	>512
$(MPC/MIC)_{50}$	8	8	1
$(MPC/MIC)_{90}$	16	8	8

*Resistantrates (R)

CONCLUSIONS

High doses of difloxacin and marbofloxacin could minimize the selection of resistant mutants, whereas the possibilities of selecting mutants with the conventional doses of enrofloxacin seem high.

REFERENCES

1. Gebru, E., Damte, D., Choi, M.J., Lee, S.J., Kim, Y.H. & Park, S.C. (2012) *Veterinary Microbiology*, **154**, 384–394.
2. Ding, H., Li, Y., Chen, Z., Rizwan-UL-Haq, M. & Zeng, Z. (2010) *Journal of Veterinary Pharmacology and Therapeutics*, **33**, 507–510.

P-4.3.

Dose regimen determination based on PK/PD and clinical evaluations for an amoxicillin form (Cofamox 15 L.A.) given intramuscularly in pigs to control *Streptococcus suis* infections

P. RICHEZ[1], M. W. N. DE BRUIN[2], X. BURREN[3] & A. VAN NES[2]
[1]*TransPharm, St Genies des Mourgues, France;* [2]*University of Utrecht, Fac. Vet. Med., Utrecht, The Netherlands;* [3]*Coophavet, St. Herblon, France*

INTRODUCTION

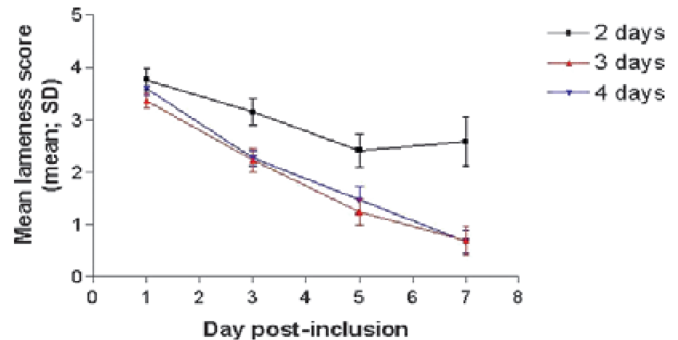
Amoxicillin is of significant interest as a substitute for third generation cephalosporins against *Streptococcus suis* infections in swine. It is a time-dependent bactericidal antibiotic with particularly low MICs (MIC_{90} = 0.014 $\mu g\ ml^{-1}$) and no observed resistance.[1] PK/PD analyses revealed that a daily dose of 15 mg kg^{-1} bw IM was required for a new formulation (Cofamox 15 L.A., Coophavet, France) to fulfill PK/PD criteria, i.e. maintain inhibitory concentrations for > 50% of the dosing interval.[2] The aim of this study was to confirm the efficacy of this dosage and determine optimum treatment duration in clinical cases of *S. suis* infections.

MATERIALS AND METHODS

Sixty-five pigs aged 5–14 days with evidence of *S. suis* infection confirmed by bacteriological investigations were randomly assigned to three groups treated with Cofamox 15 L.A. 15 mg kg^{-1} bw IM either two, three or four times at 24-h intervals. All animals were examined on Day 1 (inclusion), 3, 5 and 7. The animals were examined for general signs (rectal temperature, general condition, behaviour), arthritis (number of joints affected, lameness based on a scoring system derived from Main *et al.* (2000)[3], circumference of enlarged joints, skin lesions), and other (particularly neurological) signs. Lameness score was used as the main efficacy endpoint and as a basis for inter-group statistical analyses (ANOVA).

RESULTS

Lameness scores over time are shown in Figure 1. Figure 1: Lameness score (mean ± SD) after a 2-, 3- or 4-day treatment course in *S. suis* infections in pigs. These results confirm that the test product is effective at the daily dose of 15 mg kg^{-1} bw per day when given for 3 or 4 days (no significant difference) but not for 2 days.



CONCLUSIONS

This therefore, on PK/PD and clinical grounds, justifies use of Cofamox 15 L.A. at a daily IM dose of 15 mg kg^{-1} bw for 3 days to control *S. suis* infections.

REFERENCES

1. Richez, P., Keck, N. & Burren, X. (2012) *Streptococcus suis*: bactericidal profile of amoxicillin, susceptibility and resistance in porcine clinical strains. *12th Int. Cong. EAVPT, Noordwijkerhout, the Netherlands.*
2. Richez, P., Chick, B. & Burren, X. (2012) Pharmacokinetics of amoxicillin given intramuscularly in pigs in the context of a PK/PD assessment against streptococcus suis infections in pigs. *12th Int. Cong. EAVPT, Noordwijkerhout, the Netherlands.*
3. Main, D.C.J., Clegg, J., Spatz, A. & Green, L.E. (2000) Repeatability of a lameness scoring for finishing pigs. *Veterinary Record*, **147**, 574–576.

© 2012 The Authors

P-4.4.

Integration of pharmacokinetic and pharmacodynamic data for tulathromycin in healthy and pneumonic calves

T. J. POTTER[1], J. ILLAMBAS[2], A. RYCROFT[2], P.-L. TOUTAIN[3], M. Z. LACROIX[3] & P. LEES[2]
[1]*Westpoint Veterinary Group, Warnham, UK;* [2]*The Royal Veterinary College, Hatfield, UK;* [3]*MR 1331 Toxalim INRA/INP/UPS., Toulouse, France*

INTRODUCTION

The aim of this project was to undertake pharmacokinetic-pharmacodynamic (PK-PD) integration of data for tulathromycin in healthy and pneumonic calves.

MATERIALS AND METHODS

PK data for tulathromycin were established in separate groups of healthy and pneumonic calves. Tulathromycin (Draxxin, Pfizer Animal Health) was administered subcutaneously at 2.5 mg kg^{-1} in both studies. The first study used 10 healthy female Aberdeen Angus Holstein Friesian Cross calves aged 3–5 months. The second study established serum PK, clinical response and lung pathology in a *Mannheimia haemolytica* model of calf pneumonia in eight Holstein Friesian bull calves, aged 12 weeks. Clinical findings were compared with a group of eight untreated control calves with induced pneumonia. Serial blood samples were collected to establish serum PK in both studies and clinical parameters were monitored over a 48 h period in the second study. For the strain of *M. haemolytica* used to induce pneumonia, minimum inhibitory concentration (MIC) was determined in Mueller Hinton Broth (MHB).

RESULTS

PK variables and PK-PD surrogates for tulathromycin in healthy and pneumonic calves are presented in Table 1. Although different calves were used in the two studies, PK and PK-PD variables were similar, except for $AUC_{0-\infty}$, which was lower in pneumonic calves. In the pneumonia model study, significant differences were observed in respiratory rate and rectal temperature between the treated and untreated animals and at *post mortem* there was significantly less lung consolidation (12% versus 24%) in the tulathromycin treated group compared to control animals.

Table 1 PK/PD Integration for tulathromycin in healthy and pneumonic calves

	Healthy calves	Calves with induced disease
Variable (units)	MHB MIC (2.07 μg/mL)	MHB MIC (2.07 μg/mL)
C_{max}/MIC	0.36	0.45
AUC_{0-24}/MIC (h)	4.59	4.07
$AUC_{0-\infty}$/MIC (h)	23.15	12.73
C_{av}/MIC from 0 to 24 h	0.19	0.17
C_{av}/MIC from 24 to 48 h	0.15	0.12

CONCLUSIONS

At the currently licensed dose rate tulathromycin resulted in significantly lower respiratory rates, rectal temperatures and less lung consolidation when compared to untreated control animals in a *M. haemolytica* model of calf pneumonia. The AUC_{0-24}/MIC ratio means that the average tulathromycin plasma concentration over the first 24 h was approximately fivefold lower than the MIC (2.07 μg ml^{-1}). It is clear that a clinical effect was obtained with plasma concentrations much lower than the *in-vitro* MIC.

ACKNOWLEDGEMENTS

This study was supported by DEFRA, United Kingdom.

P-4.5.

Integration of pharmacokinetic and pharmacodynamic data for amoxicillin in healthy and pneumonic calves

T. J. POTTER[1], J. ILLAMBAS[2], A. RYCROFT[2], P.-L. TOUTAIN[3], M. Z. LACROIX[3] & P. LEES[2]
[1]*Westpoint Veterinary Group, Warnham, UK;* [2]*The Royal Veterinary College, Hatfield, UK;* [3]*UMR 1331 Toxalim INRA/INP/UPS., Toulouse, France*

INTRODUCTION

The aim of this project was to undertake pharmacokinetic-pharmacodynamic (PK-PD) integration of data for amoxicillin in healthy and pneumonic calves.

MATERIALS AND METHODS

PK data for amoxicillin were established in separate groups of healthy and pneumonic calves. Amoxicillin (Betamox LA, Norbrook Laboratories) was administered intramuscularly at 15 mg kg^{-1} in both studies. The first study used 10 healthy female Aberdeen Angus Holstein Friesian Cross calves aged 3–5 months. The second study established serum PK, clinical response and lung pathology in a *Mannheimia haemolytica* model of calf pneumonia in eight Holstein Friesian bull calves, aged 12 weeks. Clinical findings were compared with a group of eight untreated control calves with induced pneumonia. Serial blood samples were collected to establish serum PK in both studies and clinical parameters were monitored over a 48 h period in the second study. An LCMS method was used to determine amoxicillin concentrations in serum. For the strain of *M. haemolytica* used to induce pneumonia, minimum inhibitory concentration (MIC) was determined in Mueller Hinton Broth (MHB).

Table 1 PK/PD Integration for amoxicillin in healthy and pneumonic calves

	Healthy calves	Calves with induced disease
Variable (units)	MHB MIC (0.15 μg/mL)	MHB MIC (0.15 μg/mL)
C_{max}/MIC	18.53	9.27
AUC_{0-24}/MIC (h)	171.9	119.6
$AUC_{0-\infty}$/MIC (h)	238.4	187.2
C_{av}/MIC from 0 to 24 h	7.16	5.97
C_{av}/MIC from 24 to 48 h	1.71	1.79
T>MIC (h)	53.11	>48

RESULTS

PK variables and PK-PD surrogates for amoxicillin in healthy and pneumonic calves are presented in Table1. The PK variables C_{max} and AUC were significantly lower in pneumonic calves. The T > MIC for calves with induced disease was > 48 h as the sampling schedule stopped at 48 h. In the pneumonia model study, significant differences were observed in rectal temperature between the treated and untreated animals and at *post mortem* there was significantly less lung consolidation (14% versus 24%) in the amoxicillin treated group compared to control animals.

CONCLUSIONS

Disease may have a significant impact on amoxicillin PK and this should be taken into account in the selection of dose schedules for clinical use.

ACKNOWLEDGEMENTS

This study was supported by DEFRA, United Kingdom.

© 2012 The Authors

Plenary Session
Thursday 12-07: 9.00–12.30

PL-1.
Veterinary pharmacology: personal reflections on past achievements, current status and future prospects

P. LEES[1], J. FINK-GREMMELS[2] & P.-L. TOUTAIN[3]
[1]*The Royal Veterinary College, Hawkshead Campus, Hatfield, Herts., UK;* [2]*Faculty of Veterinary Medicine, IRAS, Veterinary Pharmacology, Pharmacotherapy and Toxicology, Utrecht University, Utrecht, The Netherlands;* [3]*Ecole Nationale Vétérinaire de Toulouse, Toulouse, France*

Manuscript submitted to the Journal of Veterinary Pharmacoloy and Therapeutics.

PL-2.
Ensuring safe food and medical products through stronger regulatory systems abroad: a new study by the Institute of Medicine of the National Academies

J. E. RIVIERE
College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

Globalization has fundamentally changed the way that drug and food products are both manufactured and distributed in the world today. Most existing mature regulatory agencies were established when both food and drug production and consumption were local. This no longer exists. The US Food and Drug Administration commissioned the Institute of Medicine of the National Academies to study this issue and provide advice on how medical product and food safety could be insured as more developing countries join the global production and consumption network. The presentation will be summary of this committee's findings.

SUGGESTED READING

1. Riviere, J.E. & Buckley, G.J., Eds (2012) *Ensuring Safe Foods and Medical Products through Stronger Regulatory Systems Abroad*. Institute of Medicine, The National Academies Press, Washington, DC.

© 2012 The Authors