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# PYRUVATE KINASE ACTIVITY IN VARIOUS ORGANS OF RATS EXPOSED TO DINITRO-O-CRESOL AND DICHLORVOS

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*4,6-dinitro-o-cresol (DNOC) and 2,2-dichlorovinyl dimethyl phosphate (DDVP), toxic agents used as pesticides in agriculture were injected intraperitoneally to rats (Rattus norvegicus). Physiological saline was given to the control groups. We investigated the effects on pyruvate kinase (PK) enzyme activities in the liver, kidney, brain and small intestine of rats killed by cervical dislocation 0, 2, 4, 8, 16, 32, 64 and 72 hours after the injection. Statistical analysis was performed using the SPSS statistics program. Generally, DDVP increased PK activity in both male and female rats. DNOC caused inhibitions in PK activity in the liver, kidney and the small intestine, as well as in the brain tissues when compared with control groups.*

*Our study shows that DDVP, more than DNOC, affected the PK activity in various tissues of rats.*

*Key words: DNOC, DDVP, pyruvate kinase, rat*

## INTRODUCTION

The pesticide 4,6 Dinitro-o-cresol (DNOC) is used frequently in agriculture as an herbicide, insecticide, acaricide, larvicide, ovicide, and fungicide. In the plastics industry it is an inhibitor of polymerization of styrene and vinyl aromatic compounds. 2, 2-dichlorovinyl dimethyl phosphate (dichlorvos), (DDVP) is an organophosphate insecticide and fumigant registered for use in controlling flies, mosquitos, gnats, cockroaches, fleas, and other insect pests. DDVP controls insect pests on agricultural sites; commercial, institutional and industrial sites; and for domestic use in and around homes and on pets (U.S EPA, 2006).

DNOC and DDVP, when used in agriculture and in the chemical industry, directly affects workers via respiratory and dermal exposure. Other populations are affected indirectly, as this matter is mixed with soil, water and air (ATSDR, 2006).

Symptoms of acute (short-term) toxicity that appear when one is exposed to DNOC via digestion, respiration and skin are: excessive perspiration, thirst, fatigue, insomnia, rapid breathing, nausea, headache and greenish-yellow pigmentation. Damage to the liver, kidney, and nervous system has been reported in humans following acute exposure. Dermal contact may lead to local necrosis.

Chronic (long-term) exposure also results in the same symptoms in humans. Effects to the cardiovascular, gastrointestinal and central nervous systems, and changes in blood counts of chronically exposed workers, have been reported. Rats chronically exposed to DNOC through digestion, exhibit weight-loss and decreased appetites. Changes in the blood and urine parameters, decreased liver enzyme activity, and changes in organ weights were also reported in rats (U.S EPA, 2006). Bilateral cataracts and blindness have been observed in subjects chronically exposed to DNOC through digestion (OEHHA, 2003).

DDVP is an organophosphate compound reported to have an irreversible neurotoxic effect on acetylcholinesterase (AchE) (Gupta *et al.*, 2005). The enzyme degrades the neurotransmitter acetylcholine in cholinergic synapses. The occurring inhibition provokes an accumulation of acetylcholine in synapses with disruption of the nervous system functions and can result in the death of the organism (Varo *et al*., 2003). DDVP has also been reported to cause methylation of DNA *in vitro*, but there is no evidence of mutagenicity in humans (OSHA, 1997).

With toxic effects of DNOC and DDVP and changes in the energy metabolism having been observed, this study researches and discusses the effects of DNOC and DDVP on PK enzyme activity in the liver, kidney, brain and small intestine of rats.

## MATERIALS AND METHOD

Wistar rats (*Rattus norvegicus*), weighing 250-300 g, and obtained from the Experimental Animals Feeding and Research Centre of Uludag University Medical Science Faculty Bursa-TURKEY were used for this study. For each trial period, 4 rats in the control group, 8 in the DNOC and 8 in the DDVP treated group, were used. Experiments were conducted on 160 rats. Control groups were treated with saline while experimental groups were injected intraperitoneally with 2.8 mg $kg^{-1}$ dose of DNOC (1/10 $LD_{50}$) and 4 $mg.kg^{-1}$ dose of DDVP using 1 mL sterile injectors. DNOC was purchased from Aldrich® and DDVP was purchased from Sigma®. All rats were left without food and water for 24 hours before injection to ensure the same starting metabolisms for animals in the control and experimental groups. Following injection, food and water were regularly given to the animals until the trial periods were completed. Animals were killed via cervical dislocation 0, 2, 4, 8, 16, 32, 64 and 72 hours after the injection. The livers, kidneys, brain and small intestine were quickly removed and perfused in ice-cold 0.15 M KCl. To determine the enzymatic reaction rates, the homogenates were prepared after the addition of ice-cold 0.15 M KCl (1/3 mass/volume) in a glass homogenizer with a teflon pestle, and were homogenized with 4 shots for livers, 3 shots for kidneys and 12 shots for small intestine at 2000 rpm in a T-line laboratory stirrer (model No: 136-2) type homogenizer. Homogenizing was made in ice and each homogenate was centrifuged in a Dupont Instruments Sorval "RC-5 super speed refrigerated centrifuge" at 48000 g for 30 min. Centrifuging and homogenizing at 0-4 °C. PK activity was estimated spectrophotometrically via the Bohringer and Manheim (1973) method. Protein concentration was determined using the Bradford (1976) method. Bovine serum albumin was used as the protein standard.

*Statistical analysis*

Data were analyzed using SPSS 13.0 for Windows. Independent *t*-test was conducted between data of control and experimental periods. The significance was calculated using one-way analysis of variance (ANOVA) and Student's *t*-test. A value of $P<0.05$ was taken as statistically significant. The results were calculated as mean with standard error (±SE) values.

## RESULTS

*Liver:*

PK activity of male rats showed an inhibition at 2 and 4 hours in the livers of animals treated with DDVP, but an increase was observed in other periods. These increases are significant except for the 8$^{th}$ and 72$^{nd}$ hour ($p<0.05$, Table 1). An inhibition was observed in all periods, except for the initial one, when PK activity in the livers of DNOC-treated male rats was examined. These inhibitions are significant, except for 0 period ($p<0.05$). PK enzyme activity in the liver of male rats, while activated by DDVP, is inhibited by DNOC.

From the 4$^{th}$ hour, PK activity in the liver of female rats was activated by DDVP, although DNOC caused an inhibition at the same periods. These results are significant, beginning from 4$^{th}$ hour ($p<0.05$) (Table 2). The PK activities in both male and female rats exposed to DDVP was higher than those exposed to DNOC (Table 1, 2).

*Kidneys:*

When PK activity of the groups treated with saline and DDVP in the kidney was evaluated, activation was found in male rats at all periods (Table 1). These results are significant from 4$^{th}$ hour to 32$^{nd}$ hour ($p<0.05$) (Table 1). PK activity decreased in kidney of male rats in DNOC-treated groups. All experimental periods showed significant values of inhibition in kidney, except for the 0 and 4$^{th}$ hour ($p<0.05$) (Table 1).

From the 0 to 4$^{th}$ hour, a significant activation was observed in PK activity in the kidney of female rats treated with DDVP ($p<0.05$) (Table 2). Except for the 2$^{nd}$ hour, an inhibition in PK activity in kidney of DNOC-treated female rats was observed. Those inhibitions are not significant except for the 72$^{nd}$ hour ($p>0.05$).

Comparisons of the effects of DDVP and DNOC showed that DDVP increased PK activity much more than DNOC. DNOC caused a decrease of PK activity for both male and female rats.

*Brain:*

When PK activity is examined in brains of male rats injected with DDVP, activation was seen at all periods, except for the initial period. These values are considered statistically significant ($p<0.05$) (Table 1). In DNOC-treated groups, the PK activity increased in male rats. Significant increases in the 2$^{nd}$, 4$^{th}$ and 8$^{th}$ hour were noted. DDVP activated PK activity more than DNOC.

Female rats treated with DDVP, showed PK activities at all experimental periods, except the initial period. These results are significant at all periods

Table 1. The change in PK activities in the liver, kidney, brain and small intestine in the control, DDVP and DNOC-treated groups of male rats

| Time Hours[r] | | 0<br>Mean±SE | 2<br>Mean±SE | 4<br>Mean±SE | 8<br>Mean±SE | 16<br>Mean±SE | 32<br>Mean±SE | 64<br>Mean±SE | 72<br>Mean±SE |
|---|---|---|---|---|---|---|---|---|---|
| Liver | Control | 8.47±2.69 | 10.49±0.0 | 10.77±0.0 | 10.52±0.0 | 9.08±0.0 | 10.27±0.0 | 11.94±0.0 | 9.76±0.58 |
| | DDVP | 7.35±0.61 | 7.93±0.82 | 9.42±1.12 | 11.74±1.06 | 12.46±0.8* | 13.16±0.16* | 15.21±2.92* | 8.72±0.51 |
| | DNOC | 7.92±1.45 | 1.71±1.06* | 1.76±0.73* | 1.49±0.04* | 2.54±0.19* | 1.68±0.25* | 1.63±0.17* | 1.37±0.0* |
| Kidney | Control | 4.81± 0.0 | 4.11± 0.25 | 6.09±0.0 | 5.73±0.09 | 6.70±0.01 | 6.15±1.32 | 6.90±0.0 | 5.34±0.02 |
| | DDVP | 4.98±0.44 | 5.44±0.05 | 8.40±1.03* | 8.47±0.95* | 10.13±0.24* | 10.77±0.36* | 7.38±0.53 | 7.93±0.68 |
| | DNOC | 4.05±1.03 | 3.14±0.72 | 1.79±0.30* | 3.26±0.28* | 2.70±0.13* | 1.62±0.12* | 1.44±0.04* | 1.35±0.01* |
| Brain | Control | 2.58±0.48 | 2.06±0.54 | 2.30±0.0 | 2.46±1.05 | 1.24±0.0 | 2.57±0.64 | 2.13±0.46 | 1.85±0.0 |
| | DDVP | 2.99±0.39 | 6.81±0.49* | 8.52±0.14* | 7.03±0.66* | 6.64±0.44* | 9.83±1.12* | 9.71±1.33* | 9.0±0.47* |
| | DNOC | 2.94±0.17 | 3.91±0.16* | 4.00±0.43* | 4.42±0.30* | 2.37±0.20 | 3.27±0.02 | 3.45±0.19 | 2.17±0.37 |
| Small intestine | Control | 1.60±0.11 | 2.13±0.29 | 1.79±0.15 | 1.11±0.36 | 2.30±0.89 | 1.26±0.03 | 1.25±0.01 | 1.19±0.01 |
| | DDVP | 1.59±1.17 | 3.95±1.35* | 4.58±0.15* | 3.57±0.01* | 5.06±0.45* | 6.68±0.25* | 2.98±0.33* | 2.67±0.0* |
| | DNOC | 1.20±0.11 | 1.67±0.07 | 0.96±0.01 | 0.35±0.02 | 0.58±0.08* | 0.04±0.001* | 0.04±0.001* | 0.13±0.002* |

* – Data shown in the vertical column are different from control at 0.05 statistical levels
r – All data in the table showed enzyme activities as U.(mg.protein)$^{-1}$
SE – Standard Error

Table 2. The change in PK activities in the liver, kidney, brain and small intestine in the control, DDVP and DNOC-treated groups of female rats

| Time Hours[r] | | 0 Mean±SE | 2 Mean±SE | 4 Mean±SE | 8 Mean±SE | 16 Mean±SE | 32 Mean±SE | 64 Mean±SE | 72 Mean±SE |
|---|---|---|---|---|---|---|---|---|---|
| Liver | Control | 5.78±0.05 | 5.16±3.11 | 5.28±2.33 | 5.82±0.46 | 4.75±0.14 | 5.23±0.28 | 5.96±0.90 | 4.30±0..32 |
| | DDVP | 5.47±0.00 | 4.14±0.43 | 9.68±0.27* | 12.37±0.14* | 10.53±0.24* | 12.48±0.69* | 11.30±0.52* | 11.25±0.43* |
| | DNOC | 5.96±0.00 | 6.96±0.67 | 1.73±0.17* | 2.76±0.43* | 2.95±1.13* | 1.94±0.37* | 1.15±0.01* | 1.11±0.01* |
| Kidney | Control | 2.79±0.00 | 2.17±0.03 | 2.08±0.83 | 2.5 ±0.40 | 2.37±0.07 | 1.88±0.00 | 1.90±0.09 | 2.90±0.25 |
| | DDVP | 3.37±0.94 | 4.32±0.40 | 8.35±0.42* | 8.96±0.75* | 9.77±0.63* | 10.41±0.31* | 9.30±0.74* | 9.01±0.11* |
| | DNOC | 2.68±0.83 | 3.33±0.46 | 1.45±0.13 | 2.20±0.07 | 1.36±0.12 | 0.98±0.49 | 0.88±0.03 | 1.42±0.05* |
| Brain | Control | 1.59±0.00 | 1.29±0.01 | 1.52±0.00 | 2.10±0.00 | 1.46±0.26 | 1.23±0.35 | 0.60±0.00 | 1.94±0.43 |
| | DDVP | 1.44±0.09 | 6.67±0.31* | 6.88±0.70* | 8.85±0.72* | 7.88±0.75* | 9.57±2.08* | 10.33±3.18* | 9.99±2.03* |
| | DNOC | 1.68±0.09 | 2.79±0.34 | 1.55±0.14 | 0.65±0.11 | 1.42±0.10 | 1.87±0.15 | 0.44±0.01 | 0.37±0.01 |
| Small Intestine | Control | 1.86±0.01 | 1.79±0.38 | 1.56±0.88 | 1.37±0.01 | 1.51±0.00 | 1.17±0.00 | 1.61±0.22 | 1.56±0.00 |
| | DDVP | 1.75±0.88 | 1.99±0.17 | 2.69±0.00* | 2.40±0.70* | 3.32±0.39* | 3.47±0.00* | 3.33±0.22* | 3.23±0.26* |
| | DNOC | 1.54±0.00 | 0.32±0.04* | 0.43±0.00* | 0.71±0.24* | 0.14±0.01* | 0.08±0.00* | 0.05±0.01* | 0.13±0.00* |

* – Data shown in the vertical column are different from control at 0.05 statistical levels
r – All data in the table showed enzyme activities as U.(mg.protein)$^{-1}$
SE – Standard Error

($p<0.05$) (Table 2). PK activity in female rats in DNOC-treated groups had nearly the same values as the control group. PK activities in the brain of both male and female rats are affected by DDVP, and DNOC showed similar results in the control group.

*Small intestine:*

Although activations were observed in PK activity of DDVP-treated groups in all periods except for the initial period, the inhibition was observed in the intestinal tissue of male rats in the DNOC-treated groups. There was a significant activation in the DDVP-treated groups in all experimental periods, except for the initial period ($p<0.05$). Inhibitions in DNOC-treated groups were significant at the $16^{th}$, $32^{nd}$, $64^{th}$ and $72^{nd}$ hour ($p<0.05$, Table1).

PK activity in the small intestine of DDVP-treated female rats showed activation at all periods. These activations are significant except for the initial and $2^{nd}$ hour ($p<0.05$, Table 2). PK activity of female rats in DNOC-treated groups decreased at all periods. These decreases are significant ($p<0.05$, Table 2). In both genders, DDVP increased PK activity much more than DNOC.

## DISCUSSION

Although somewhat beneficial, uncontrolled pesticide usage causes poisoning and death in non-target animals, as well as humans, and also pollutes the ecosystems and food. DNOC and DDVP, which enter the body through the skin, by respiration and feeding, affect the peripheral nervous system and cause headache, nausea and shivering.

Examining enzyme activities is important for clinical biochemistry, however, in recent years, these tests have been performed in order to explain the effects of pesticides on living organisms. These materials cause tissue defects, resulting in the release of cell enzymes and changes in enzyme concentrations (Mayer *et al.*, 1992). Pyruvate kinase (E.C.2.7.1.40) is an important control enzyme of the glycolytic cycle, which converts phosphoenol pyruvate (PEP) into pyruvic acid. Pyruvate kinase has 4 different isoenzymes (M1, M2, L and R), according to their distribution in the tissues (Muirhead, 1990). It is also used as a marker for some neoplastic and non-neoplastic diseases in laboratory animals and humans (Staib *et al*., 2006).

While PK enzyme activity is inhibited by the effect of DNOC in the liver tissue of male and female rats, it is activated by the effect of DDVP. The reason for these increases and decreases may be the morphological changes occurring in cells. The morphological changes occurring in cells as a result of pesticides, affect the metabolic phenomena within the organisms. In an immunocytochemical study performed with DDVP, a decrease was seen in perforyne, granzyme A and granulysin levels of NK-92CI cells (Li *et al*., 2005). The changes in permeability of the cell membrane, or the destruction of cells, change the enzyme activities. DDVP has caused histopathological changes in the lungs, lymphatic glands and thymus, as well as histopathological and ultrastructural changes in liver, kidneys and heart muscle (Luty *et al*., 1998). It has been suggested that some pesticides

with organophosphates, cause defects in dimensions and surfaces of erythrocytes (Blasiak *et al*., 1991).

A similar phenomenon was seen in brain and small intestine tissues. Unlike DNOC, PK activity of both male and female rats was activated by DDVP. In a study made by Sarin and Gill (1999), it was suggested that DDVP prevented oxygen from being transferred to tissues, causing glycogen levels in the brain to decrease. In another study, it was shown that DDVP decreased the glycogen synthase activity, while increasing the glycogen phosphorylase activity and causing hyperglycemia (Kuliszewska and Szymczyk, 1979). Increasing glucose quantities may cause PK activity to increase, too. Another study showed that DDVP causes hyperglycemia (Seifert, 2001). Romero *et al*. (2006) suggested that a 20 mg $kg^{-1}$ dose of DDVP decreases the glycokinase activity of the liver. In other study, Dere *et al.* (2007) have shown that generally, while DNOC sometimes caused significant activations of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), and increased High Density Lipoproteins (HDL), triglycerides and urea in male and female rats. Cholesterol levels did not display any significant variations.

DNOC inhibits PK activity. It may indicate a toxic effect by binding to cell macromolecules. Luk'ianchuk (1983) demonstrated that DNOC causes toxicity by binding to human serum albumin. Hrelia *et al*. (1994) recorded that DNOC increases structural chromosome damages in the bone marrow cells of rats. Moreover, DNOC forms chain breakages in DNA, thus DNA damages (Grilli *et al*., 1991). Ken *et al*. (2003) stated that DNOC causes damages in the germ cells of rats. The reason PK activity decreases with DNOC, may be due to a decrease in the insulin level. Decreasing insulin levels may cause PK amounts to decrease in the liver. It has been suggested that a lack of insulin, or insulin synthesis anomalies, may change PK activity (Saxena *et al.*, 1992).

In the kidney tissue of male rats, the effects of DNOC inhibited PK activity. In female rats, similar results were seen when compared to the control groups, only increasing in the 72$^{nd}$ hour. The effects of DNOC on PK activity may be the result of decreasing PK synthesis. As stated, DNOC damages DNA and RNA (Hrelia *et al*., 1994). Changes in enzyme amounts may also be connected to decreases in primary gene transcription levels. Low enzyme activity decreases the tendency of glucose to convert into pyruvate (Valera *et al.*, 1993). A lack of PK may cause defects in the ATP levels of the tissues. Thus, lack of glycolysis causes defects in cells, resulting in anemia and hepatitis (Sedano *et al*., 2006).

In all tissues observed, increasing PK activity caused pyruvate to increase. Increasing pyruvate may also increase the toxic effect of DDVP. One study showed that the toxic effect of DDVP in hepatocytes increases when glycolytic substrates like pyruvate, lactate and fructose are added to the environment (Yamano and Morita, 1992).

Our study demonstrated that the effect of DDVP on the PK activity in all tissues observed is much more than the effects of DNOC. Accordingly, we consider that DDVP possesses a greater disease risk than DNOC. Some studies state that PK activity is higher in hepatoma, tyroid and breast cancers, than in normal tissues (Verhagen *et al*., 1985; Ignacak and Guminska, 1993).

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## AKTIVNOST PIRUVAT KINAZE U RAZLIČITIM ORGANIMA PACOVA IZLOŽENIM DINITRO-O-KREZOLU I DIHLORVOSU

DERE E, ARI FERDA i TOSUNOGLU H

SADRŽAJ

U ovom radu su izneti rezultati ispitivanja uticaja 4,6-dinitro-o-krezola (DNOC) i 2,2 – dihlorvinil dimetil fosfata (DDVP-dihlorvos) na aktivnost enzima piruvat kinaze (PK) u jetri, bubrezima, mozgu i tankim crevima pacova posle intraperitonealne aplikacije. Ovi agensi se koriste kao pesticidi u poljoprivredi. Životinje su bile žrtvovane 0, 2, 4, 8, 16, 32, 64 i 72 sata posle aplikacije toksina, a kontrolna grupa je na isti način bila tretirana fiziološkim rastvorom. Rezultati su ukazali da DDVP povećava aktivnost PK u svim ispitivanim tkivima dok DNOC smanjuje aktivnost ovog enzima.