Original communication

In-vivo evaluation of the effect of cyanoacrylate on prosthetic vascular graft infection – does cyanoacrylate increase the severity of infection?

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Summary: *Background:* Prosthetic vascular graft infection (PVGI) is a complication with high mortality. Cyanoacrylate (CA) is an adhesive which has been used in a number of surgical procedures. In this in-vivo study, we aimed to evaluate the relationship between PVGI and CA. *Materials and methods:* Thirty-two rats were equally divided into four groups. Pouch was formed on back of rats until deep fascia. In group 1, vascular graft with polyethyleneterephthalate (PET) was placed into pouch. In group 2, MRSA strain with a density of 1 ml 0.5 MacFarland was injected into pouch. In group 3, 1 cm 2 vascular graft with PET piece was placed into pouch and MRSA strain with a density of 1 ml 0.5 MacFarland was injected. In group 4, 1 cm 2 vascular graft with PET piece impregnated with N-butyl cyanoacrylate-based adhesive was placed and MRSA strain with a density of 1 ml 0.5 MacFarland was injected. All rats were scarified in 96th hour, culture samples were taken where intervention was performed and were evaluated microbiologically. Bacteria reproducing in each group were numerically evaluated based on colony-forming unit (CFU/ml) and compared by taking their average. *Results:* MRSA reproduction of 0 CFU/ml in group 1, of 1410 CFU/ml in group 2, of 180 200 CFU/ml in group 3 and of 625 300 CFU/ml in group 4 was present. A statistically significant difference was present between group 1 and group 4 ($p < 0.01$), between group 2 and group 4 ($p < 0.01$), between group 3 and group 4 ($p < 0.05$). In terms of reproduction, no statistically significant difference was found in group 1, group 2, group 3 in themselves. *Conclusions:* We observed that the rate of infection increased in the cyanoacyrylate group where cyanoacrylate was used. We think that surgeon should be more careful in using CA in vascular surgery.

Keywords: Vascular graft infection, cyanoacrylate, in-vivo

Introduction

Prosthetic vascular graft infection (PVGI) is a rarely seen complication in 1–5 % of patients [1]. Although rarely seen, it is one of the most serious complications leading to high rates of mortality and morbidity. Despite various protective methods against infection, it has been reported that mortality risk ranges from 10 % to 50 % and amputation risk ranges from 4 % to 14 % [2]. The most common cause of infection is known to be bacterial colonization occurring due to wound site in perioperative period [3]. Though Staphylococcus aureus is the most common pathogen, the rate of methicillin-resistant Staphylococcus aureus (MRSA) has been increasing and MRSA is related to the increased amputation rate in PVGI [4–6]. Removing the graft and using antibiotics over a long period is the main treatment in graft infection [6]. Owing to this damaging table, various approaches have been studied in order to prevent PVGI and to control the infection.

Cyanoacrylate (CA) is a tissue adhesive which has been used for a long time in a number of surgical and interventional radiology procedures including vascular surgery in order to provide tissue integrity and to control bleeding that cannot be stopped by standard methods [7]. There have been a lot of studies that have investigated the antibacterial characteristic of CA and its effect on wound site contamination as well as its adhesive character [8–10]. However, a number of those are in vitro studies and there has been no consensus yet.

In the literature there have been studies reporting that CA when used in living tissue in vivo may have a tendency to bacteremia and infection due to contamination. Therefore, in this in vivo experimental study, we aimed to show the effect of CA on PVGI.

Materials and methods

This study was carried out in Uludağ University Faculty of Medicine Experimental Animal Breeding Application and Research Center. It was done at room temperature (21–23 C). 32 adult male Sprague-Dawley rats weighing 230–280 gr were used.

Approval was obtained from Uludağ University Animal Experiments Local Ethics Committee for the study (approval number: 2017-07/01). Thirty-two rats were equally divided into 4 groups. Inhaled anesthesia was performed with sevoflurane. The dorsal area of the rats was chosen as the surgical site. Under sterile conditions, the surgical site was cleaned with polyvinylpyrrolidone iodine (Batticon®; Adeka). A pouch was formed by descending until muscular fascia near the vertebrae by vertical incision. No same surgical instrument was used in another rat. 150 million/ml methicillin-resistant Staphylococcus aureus (MRSA) bacterial suspension was prepared in order to be used in the study.

- *In group 1 (n = 8)*: A 1 cm^2 sterile vascular graft with polyethylene terephthalate (PET) piece was placed into the pouch in the rats and the incision was sutured.
- *In group 2 (n = 8)*: An MRSA strain having a density of 1 ml 0.5 McFarland was injected into the pouch in the rats and the incision was sutured.
- *In group 3 (n = 8)*: A 1 cm^2 sterile vascular graft with PET was placed into the pouch, an MRSA strain having a density of 1 ml 0.5 McFarland was injected, and the incision was sutured.
- *In group 4 (n = 8)*: A 1 cm^2 sterile vascular graft with PET piece impregnated with N-Butyl Cyanoacrylate-based adhesive was placed into the pouch after the adhesive had dried and 1 ml of 0.5 McFarland density MRSA strain was injected.

All the rats in the 4 groups were sacrificed in the 96th hour and the culture samples and graft pieces were taken from the surgical sites under sterile conditions. The culture materials were placed into 1 ml sterile serum and transferred to the laboratory at 4° C. Samples were both taken directly and diluted 1/10 and 1/100 after tubes containing vortexed graft by maintaining its temperature for one minute were submitted to sonication. 100 µl was taken from each of them and placed on 5 % Columbia sheep blood agar (Germany; Becton Dickinson). The culture media were incubated at 35° C under atmospheric conditions. The incubated culture media were examined in the 48th hour in terms of reproduction. Bacteria colonies were counted and were confirmed to be MRSA. For each group, bacteria count in direct planting and dilutions were evaluated on colony-forming unit/ml (CFU/ml) and were compared by taking their averages.

Statistical analysis

The conformity of the variables to normal distribution was examined by Shapiro-Wilk test. Permanent variables were expressed with their mean values. ANOVA test was used in comparisons conducted among groups. A multicomparison procedure was performed in order to determine the different groups after the ANOVA test by using the Tukey-Kramer approach. The GraphPad InStat program was used for statistical analysis and $p < 0.05$ was accepted as statistically significant.

Results

After rats were sacrificed and when the incisions were opened, the macroscopic findings in tissues were as follows:

- In group 1: With mild edema.
- In group 2: With edema, with erythema, with purulent appearance.
- In group 3: With edema, with erythema, with purulent appearance.
- In group 4: With edema, pale, with heavy purulent appearance.

The culture results of all groups were evaluated in the 48th hour of incubation. No reproduction was seen in any graft in group 1 and those grafts were accepted to be sterile. Reproduction in different numbers was seen in samples in the other groups. Although the lowest reproduction occurred in group 2, in which only MRSA was injected, the highest reproduction occurred in group 4 in which MRSA was injected on the graft with cyanoacrylate. When reproduction was numerically evaluated in all groups, MRSA reproduction was present in 0 CFU/ml in group 1, 1410 CFU/ml in group 2, 180200 CFU/ml in group 3 and 625300 CFU/ml in group 4, respectively (Figure 1).

In terms of reproduction, a statistically significant difference was present between group 1 and group 4 ($p < 0.01$), group 2 and group 4 ($p < 0.01$), and group 3 and group 4 ($p < 0.05$). There was no statistically significant difference between groups 1, 2 and 3 in terms of reproduction.

Discussion

PVGI is a devastating condition that may lead to limb loss, sepsis, multiorgan failure and death [3]. Diabetes mellitus, obesity, renal failure, and immunodeficiency, as well as various identified risk factors such as groin incision, prolonged operative time, emergency surgery, and interventions carried out to the surgical site in the preoperative period, increase susceptibility to infection [11, 12, 25].

Antisepsis, antibiotic prophylaxis effective on skin flora and administered at the right time, blood sugar regulation, maintaining body temperature during the perioperative period, sufficient oxygenation, careful wound care and preventing the graft from contact with the skin during operation are the generally accepted principles in order to minimize the risk of PVGI [5]. Moreover, there have been

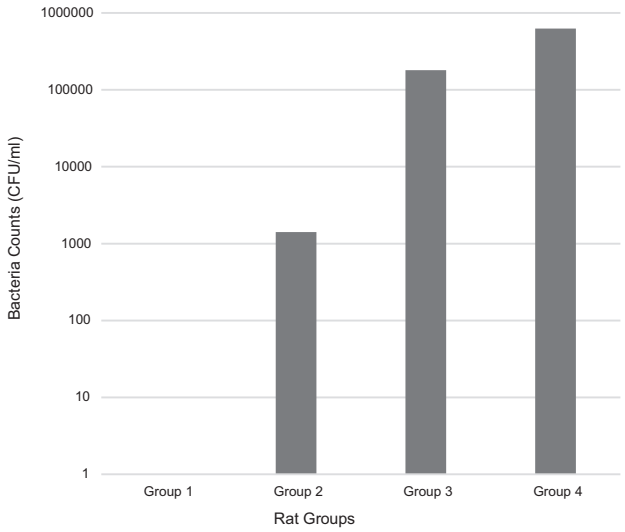

Figure 1. Reproduced bacteria counts in each group measured by CFU/ml, represented by logarithmic graph.

various studies on the use of antibiotic coated graft and antibiotic prophylaxis over a long period, but no certain opinion exists about their efficiency [6, 13].

CA is a liquid adhesive having a common use in both peripheral artery surgery and venous insufficiency treatment as well as many other areas of medicine. In various in vitro studies carried out, it has been reported that CA has an antibacterial effect against gram-positive and gram-negative bacteria [7, 9]. Therefore, we believe that examining whether it has an antibacterial effect in in vivo conditions and examining its relationship with infection are important. In our study, it was seen that the infection rate was high in the group in which CA was included. We consider the fact that no reproduction occurred in group 1 suggests that our study is reliable in terms of sterilization. The fact that reproduction appeared in group 2 and group 3 was an expected result. However, the fact that the highest reproduction rate occurred in group 4 where CA was included may seem to contradict with the literature. This may be associated with the behavior of CA in biologic tissue.

In various studies, it has been shown that CA has a cytotoxic effect in biologic tissue. In a study conducted on human neuroblastoma by Landegren et al., they showed that ethyl cyanoacrylate and butyl cyanoacrylate had a transient toxic effect [14]. Montanaro et al. revealed the cytotoxic effect of two different CA types [15]. Although these studies argue that cytotoxicity is temporary and may be acceptable when CA is diluted, necrotic tissue may form a medium for infection. Furthermore, there have been histopathological studies reporting that CA caused serious inflammation and foreign body reaction in tissue to which it was applied [16, 17]. These changes in tissue may increase the infection when contaminated.

The adhesive character of CA is associated with polymerization reaction. It forms a hard layer by polymerizing rapidly after contact with air and living tissue. Wang et al. analyzed factors affecting polymerization of N-butyl cyanoacrylate and revealed that contact with tissue and blood increased the rate and speed of polymerization of CA [18]. Romero et al. also indicated that polymerization reaction made a significant contribution to the antibacterial character of CA [19]. Having dried the grafts in group 4 at room temperature, we placed them into the rats' bodies. As a result, our study may not be able to explain the relationship between the polymerization reaction of CA in living tissue and its effect on infection. When taking into consideration that CA used in the graft during vascular surgery is polymerized with room air prior to tissue contact, tissue and blood may not contribute to the rate and speed of polymerization reaction in such surgeries.

In different in vivo studies carried out on patients with cirrhosis who have had gastrointestinal system variceal bleeding, it could not be shown that CA had antibacterial efficiency [20, 21]. Randi et al. reported that recurrent bacteremia developing after intervention on a 43-year-old cirrhosis patient with gastric variceal bleeding was associated with CA [22]. Some authors have even suggested long-term antibiotic prophylaxis, pointing out that CA injection may develop susceptibility to infection in patients with gastric variceal bleeding [23]. In these studies, it has been reported that bacteremia results from contamination of gastrointestinal system flora. The most common cause of PVGI is also graft contamination occurring during perioperative period [24, 25]. Hence, the use of CA in procedures with high risk of infection due to contamination may increase susceptibility to infection.

In recent years, the use of CA in the treatment of venous insufficiency has been increasing [26]. Taking into consideration that CA resulted in a foreign body reaction in in vivo conditions and that it may affect infection negatively, it may lead to destructive results on patients with venous ulcer who are already prone to infection [27].

For whatever reason it is used, we believe that CA may aggravate infection in in vivo conditions and therefore, we should be more careful about sterilization when it is used compulsorily.

Limitations

Our study has some limitations. In this study, histopathological evaluation of changes in living tissue was not performed. In addition, only the relationship between N-butyl cyanoacrylate and infection was examined. The relationship between other types of CA used in medicine and infection should be evaluated. There is a relationship between the polymerization reaction and the effect of CA on infection. We did not perform a chemical analysis of this relationship.

Conclusions

In this in vivo study, we observed that the rate of infection increased in the group in which CA was used.

This result shows that CA, which is said to have an antibacterial effect, could not produce that effect in in vivo conditions and even increased the severity of infections. As a result, different in vivo studies should be carried out in which detailed histopathological and microbiological analyses are made. Until these studies are completed, we are of the opinion that vascular surgeons and interventional radiologists should not behave by trusting the antibacterial effect of CA, and that conversely, when used compulsorily, they should be more careful about protection from infection owing to contamination.

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Conflicts of interests
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