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Full Length Research Paper

Is sustained release of vancomycin from fibrin glue effective to prevent methicillin-resistant *Staphylococcus aureus* graft infection?

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Prosthetic vascular graft infection remains one of the most serious complications seen after vascular surgery. Recently, fibrin glue has gained attention as a possible means to deliver drug therapies. In this study, the efficacy of vancomycin incorporated fibrin glue for preventing methicillin-resistant *Staphylococcus aureus* (MRSA) infection of prosthetic grafts was investigated. The vascular grafts were implanted into subcutaneous pockets in the backs of 32 rats. Group 1: no graft contamination; Group 2: MRSA contamination; Group 3: vancomycin incorporated fibrin glue graft and MRSA contamination; and Group 4: vancomycin soaked graft and MRSA contamination. The grafts were removed after 7 days and evaluated by a quantitative culture analysis. The quantitative culture values for Groups 2, 3, and 4 were $1.8\times10^{11}\pm 1.4\times10^{11}$, $1.1\times10^{7} \pm 2.4\times10^{7}$, and $2.6\times10^{8} \pm 3.9\times10^{8}$, respectively. The culture values of the Group 2 was significantly higher than those of the Group 3 and Group 4 (p=0.014 and p=0.016, respectively), however, Groups 3 and 4 were comparable (p=0.161). In our study, efficacies of vancomycin-incorporated fibrin and vancomycin alone were comparable. The finding of the current study indicated that a fibrin-based delivery system might not be as effective an option as a vancomycin delivery.

Key words: Vascular graft infection, fibrin glue, methicillin-resistant *Staphylococcus aureus* (MRSA).

INTRODUCTION

Prosthetic vascular graft infection (VGI) remains one of the most serious complications seen after vascular surgery. Although prevention of VGI has improved over time, incidence of VGI is reported to be between 1 and 6% (Kitamura et al., 2005; Stewart et al., 2007). Mortality and extremity amputation rate of vascular graft infection can reach up to 70%, even today (Antonios et al., 2006; Seeger et al., 2000). *Staphylococcus aureus* and *Staphylococcus epidermidis* are common and serious mortality rate, preventing methicillin-resistant *Staphylococcus aureus* (MRSA) infection is crucial causes of prosthetic graft infections. Due to its high (Nasim et al., 2001). Especially in patients with groin incisions, or with other risk factors of infection (diabetic patients, patients with gangrene or ulcers, redo surgery, emergent surgery, chronic use of steroids), prevention from VGI has became more important. It has been known that asepsis and perioperative administration of systemic antibiotics are essential to prevent VGI. However, some Gram-positive pathogens, particularly MRSA, are increasingly resistant to traditional antibiotics that are

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used as prophylaxis. In addition, despite the use of systemic antimicrobial prophylaxis, VGI is still high in the presence of groin incision and skin infection. Therefore, many measures have been advocated to decrease the frequency of VGI occurrence such as silver coated or antibiotics bonded vascular grafts.

Fibrin glue has been studied for decades as a hemostatic and sealant agent. However, more recently, fibrin glue also has gained attention as a possible means to deliver drug therapies (Spicer and Mikos, 2010). Because of its frequency of use in surgical repairs as an adhesive or sealant, surgically relevant drugs have been investigated for release from areas where the material may already be utilized.

In this study, we aimed to investigate the efficacy of vancomycin-incorporated fibrin-glue for preventing MRSA infection of prosthetic grafts using a rat model.

MATERIALS AND METHODS

The commercially available methicillin-resistant S.aureus ATCC 43300 used in this study wereobtained from the Refik Saydam Hifzisihha Institute, Ankara, Turkey.

Cefazolin (Eqizolin, Tum Ekip, Istanbul, Turkey) and Vancomycin (Edicin, Sandoz İlaç, Istanbul, Turkey) were diluted in accordance with manufacturers' recommendations, yielding 1 mg/ml stock solutions. Solutions of drug were fresh on the day of assay. The antimicrobial susceptibilities MRSA strains were determined using the micro-broth dilution method, according to the procedures outlined by the National Committee for Clinical Laboratory Standards (1997)

Rat model

The Animal Ethics Committee of our institution approved this study. Thirty-two adult Wistar rats were used with weight ranging 220–240 g. All rats had free access to standard rat feed and tap water. The rats were randomized into four groups, each of which included 8 rats: Group 1, no graft contamination and no prophylaxis; Group 2, MRSA contamination with cefazolin prophylaxis; Group 3, vancomycin incorporated fibrin glue graft and MRSA contamination with cefazolin prophylaxis; and Group 4, vancomycin soaked graft and MRSA contamination with cefazolin prophylaxis. Prophylaxis was performed with administration of 30 mg/kg intraperitoneal injection of cefazolin 30 min before implantation of the graft, as stated previously in the literature (Atahan et al., 2009).

Preparation of vancomycin fibrin glue grafts

Incorporation of vancomycin in fibrin glue was prepared as follows: In a Teflon cast (1 cm in diameter), 0.12 mL of fibrinogen solution was mixed with 0.12 mL of thrombin solution containing 0.6 mg of vancomycin. A graft sheet (1 cm in diameter) then was placed on the vancomycin fibrin glue.

Surgical technique

Intraperitoneal ketamine hydrochloride (90 mg/kg, Ketalar, Pfizer, Turkey) and xylazine hydrochloride (3 mg/kg, Rompun, Bayer, Turkey) were administered to attain sufficient anaesthesia before the experiment, and additional doses were applied when necessary. The rats' backs were shaved and the skin was cleaned with 10% povidone iodine solution. A subcutaneous pocket was made on the right side of the median line of each rat by means of a 1.5-cm incision. Aseptically, 1 cm^2 sterile, collagen-coated polyester grafts (InterGard, InterVascular, France) were implanted into the pockets. After implantation, 1 ml saline solution containing MRSA strain at a concentration of 2 × 107 CFU/ml was inoculated onto the graft using a tuberculin syringe to create a subcutaneous fluid-filled pocket. The pockets were closed with 5/0 polypropylene sutures (Propilen, Dogsan, Turkey). The animals were returned to individual cages and thoroughly examined daily. All rats were euthanized and grafts were explanted seven days after implantation.

Assessment of infection

The explanted grafts were placed in sterile test tubes, washed in sterile saline solution, placed in test tubes containing 10 ml of phosphate-buffered saline solution and sonicated for 5 min to remove the adherent bacteria from the grafts as described in the previous studies (Atahan et al., 2009; Cook and Farrar, 1978). Quantification of the viable bacteria was performed by preparing serial 10-fold dilutions (0.1 ml) of the bacterial suspensions in 10 mM buffer to minimize the carryover effect and culturing each dilution on blood agar plates. All plates were incubated at 37℃ for 48 h and evaluated for the presence of MRSA. BD Phoenix PMIC/ID automated microbiology system (Becton Dickinson Diagnostic Systems) was used for identification of MRSA. The organisms were quantified by counting the number of colony-forming units per plate. The limit of detection for this method was approximately 50 CFU/cm^2 of graft tissue.

Statistical analysis

Data were presented as mean ± standard deviation (SD). The data obtained from quantitative culture was analyzed with the Kruskal-Wallis test, and multiple comparisons between the groups were performed with the Turkey test. A p-value of 0.05 or less was considered statistically significant.

RESULTS

None of the animals in any group had clinical evidence of drug or fibrin related adverse effects, such as local signs of perigraft inflammation, anorexia, diarrhoea or behavioral alterations, and none of the animals died during the study.

Results of quantitative cultures from groups 2, 3 and 4 are presented in Table 1. In control Group 1, none of the animals showed either anatomical or microbiological evidence of graft infection. However, all animals in Groups 2, 3, and 4 showed different degree evidence of graft infection. The quantitative culture values for Groups 2, 3, and 4 were $1.8 \times 10^{11} \pm 1.4 \times 10^{11}$, $1.1 \times 10^{7} \pm 2.4 \times 10^{7}$, and $2.6 \times 10^{8} \pm 3.9 \times 10^{8}$, respectively. The quantitative graft culture values of the Group 2 was significantly higher than those of Group 3 and Group 4 (p=0.014 and p=0.016, respectively). The quantitative graft culture values of Group

Table 1. Quantitative microbiological results of the study groups.

Group		Quantitative graft culture (CFU/cm^2)
Group 1	No graft contamination	No growth
Group 2	MRSA cont.	$1.8 \times 10^{11} \pm 1.4 \times 10^{11}$ [a, b]
Group 3	MRSA cont.+ VCM incorporated FG graft	$1.1 \times 10^{7} \pm 2.4 \times 10^{7}$
Group 4	MRSA cont.+ VCM soaked graft	$2.6 \times 10^{8} \pm 3.9 \times 10^{8}$ [c]

[a]P = 0.014 vs. Group 3, [b]P = 0.016 vs. Group 4, [c]P = 0.161 vs. Group 3. MRSA cont.; Methicillin-Resistant *Staphylococcus* contamination, VCM; vancomycin, FG; Fibrin glue.

4 were higher than Group 3. However, statistical significant difference could not find (p=0.161).

DISCUSSION

VGIs are most frightening complications seen after vascular surgery. All prosthetic vascular grafts, to varying degrees, are susceptible to infection. Since VGI usually results in graft exclusion with resultant high mortality and morbidity rates, prevention from VGI is an essential target. Most VGIs cause contamination of micro-organisms from skin flora at the time of graft implantation or bacteriemia after the operation. Due to its high mortality rate, preventing MRSA infection is crucial (Nasim et al., 2001). Although, systemic antibiotic prophylaxis reduces the incidence of VGI, especially, in the presence of predisposing factor for VGI such as skin infection, only systemic antibiotic prophylaxis may not enough to prevent it. Therefore, other methods to prevent VGI are needed.

The glycopeptides vancomycin is a bactericidal agent with the ability to inhibit bacterial cell wall synthesis. It is used in controlling infections due to MRSA and for prophylaxis of bacterial endocarditis in patients with prosthetic valves (Cook and Farrar, 1978). In addition, vancomycin is the effective alternative for the treatment of Gram-positive infections in patients allergic to the penicillins (Cook and Farrar, 1978). Fibrin glue has drug carrier capability beyond its hemostatic and sealant property. Therefore, fibrin glue is frequently used as an antibiotic carrier. In this study, we investigated efficacy of vancomycin incorporated fibrin glue for preventing MRSA infection of prosthetic grafts.

In our study, significant inhibitory effect on growth of MRSA was observed in both vancomycin incorporated fibrin graft and vancomycin soaked graft. Similarly, Yasim et al. (2006) concluded that use of vancomycin-soaked graft was effective in preventing primary prosthetic vascular graft infection. Fujimoto et al. (1997) suggested that vancomycin fibrin glue Dacron graft delivery might be useful in preventing graft infection in rats. In addition, Morishima et al. claimed that new vancomycin-containing glue effectively inhibited growth of MRSA was drastically than vancomycin solution group. However, in our study, we could not show any superiority of vancomycin-incorporated fibrin graft than vancomycin-soaked graft. Fibrin glue is frequently used as an antibiotic carrier; however, the drug release periods of fibrin glue is shorter than 24 h (Fujimoto et al., 1997). We think that non-superiority of vancomycin incorporated fibrin graft than vancomycin soaked graft may be caused by drug-release period of fibrin glue.

This study has some limitations. First, our results obtained from experimental rat model and rats might be more resistant to infection than human kind. Second, our subcutaneous graft infection model does not completely simulate the clinical situation of vascular graft infection. Third, the relatively small sample size of our study did not allow us to draw firm conclusions. Further studies using the graft-implanted models in larger sample size will be required to confirm our findings.

In conclusion, our study does not show any statistically significant difference between vancomycin-incorporated fibrin vancomycin alone, in a rat model of graft infection. Although, fibrin is used as a viable option among many drug delivery systems, the finding of the current study indicated that a fibrin-based delivery system might not be an effective option where onset of action of drug is slow such as a vancomycin delivery.

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