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Efficacy of Cefepime and Vancomycin in Preventing of Dacron and ePTEF Vascular Grafts Infection with *Staphylococcus aureus* in Rat

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ABSTRACT

The objective of the present study was to evaluate systemic and local effects of cefepime alone and/or in combination with vancomycin in the prevention of experimentally implanted Dacron and ePTFE vascular grafts infected with *Staphylococcus aureus* in rat. Ninety healthy adult male Wistar rats were divided into two groups of 45 animals each. Then, each group was subdivided into nine subgroups of 5 animals each. Following anesthesia, in each rat one cm^2, sterile Dacron or ePTFE graft was implanted aseptically into dorso-lumbar subcutaneous pocket. The grafts were infected by inoculation of one ml of (5×10^7 CFU/ml) *Staphylococcus aureus* suspension. The rats received vancomycin or cefepime locally, intraperitoneally, or in combinations. The grafts were removed seven days after implantation and evaluated by quantitative culture. Combinations of cefepime with vancomycin resulted in enhanced efficiency of antibiotics in bacterial growth inhibition. Local vancomycin was more effective than local cefepime in the reduction of graft contamination. A combination of intraperitoneal vancomycin, as well as local cefepime, was more effective than a combination of intraperitoneal cefepime as well as intraperitoneal vancomycin in the Dacron group. Local vancomycin-intraperitoneal cefepime combination could be suggested to prevent Dacron and ePTFE vascular graft infected with *Staphylococcus aureus*. Local treatment alone, vancomycin for Dacron graft and cefepime for ePTFE graft, and intraperitoneal treatment alone, vancomycin for ePTFE graft, could also be suggested.

Introduction

The number of patients with vascular implants is constantly on the rise.[1] Prosthetic vascular graft infection is a dreaded, serious complication of vascular surgery. Although, vascular infections are relatively infrequent, they are associated with high rates of significant morbidity, prolonged hospitalization, organ failure, amputation, and mortality.[2-6]

Treatment and prevention of vascular graft infections and those infections caused by biofilm-producing bacteria represent a significant clinical challenge. Antibiotic therapy has decreased the mortality associated with vascular infections; however,

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a successful therapeutic outcome depends on not only the location and extent of the infection, but also the type of organism and its sensitivity to the selected antibiotics.[7]

Asepsis and preoperative administration of systemic antibiotics are effective prophylactic strategies for prevention of prosthetic infection.[8-10] Antimicrobials bound in high concentrations to prosthetic grafts have been proposed as adjunctive prophylaxis for vascular grafts.[9-13] Application of antimicrobial combinations that achieve synergistic activities is one of the important strategies to prevent and control emergence and spread of antimicrobial-resistant microorganisms.[14]

S*taphylococcus aureus (S. aureus)* is one the most common causative organism of the surgical infection site following vascular graft.[2,15-17] Vancomycin is a glycopeptide antibiotic commonly used to treat severe infections caused by methicillin-resistant staphylococci. However, emergence of *S. aureus* strains with reduced susceptibility to vancomycin remains a major concern.[18-21] Cefepime is an injectable cephalosporin with a broad-spectrum activity against many bacterial species, including staphylococci.[21] It is a valid option for single dose prophylaxis in preventing postoperative infections of both biliary tract and surgical wounds.[22] Also, it is valuable in reducing resistant bacilli colonization in pediatric intensive care.[23]

The objective of the present study was to assess systemic and local prophylactic effects of cefepime alone and/or in combination with vancomycin in prevention of experimentally induced meticillin-resistant *Staphylococcus aureus* infection in Dacron and ePTEF vascular prosthetic grafts in rat.

Materials and Methods

The study protocol was approved by Urmia University Research Council and conformed to the Guidelines on the Care and Use of Animals for Scientific Purposes.[24]

Staphylococcus aureus subsp. aureus (ATCC® 6538™) strains (slime producing and methicillin-resistant *S. aureus* [MRSA]) were supplied by Pasture Institute, Karaj, Iran. Vancomycin and cefepime (Sigma-Aldrich; Milan, Italy) were diluted in accordance with manufacturers' recommendations yielding 1 mg/ml stock solution. Solutions of drugs were made fresh on the day of assay. Dacron® (the woven, gelatin-impregnated polyethylene terephthalate, Gelweave Valsalva graft, Sulzer Vascutek, Renfrewshire, Scotland) and ePTFE (expanded polytetrafluoroethylene, Gore-Tex, W.L. Gore & Associates Inc., Flagstaff, Arizona, USA) vascular grafts obtained and used for this study.

Ninety adults healthy Wistar male rats weighing 200-250 g were used in the present study. They divided randomly into two Dacron and ePTFE groups of 45 rats each. Then, each group was subdivided into 9 subgroups ($Dacron_1$-$Dacron_9$ and $ePTFE_1$- $ePTFE_9$) of 5 animals each (Table 1). In uncontaminated control subgroups ($Dacron_1$ and $ePTFE_1$), the grafts were remained uncontaminated and no prophylactic antibiotic was administered. In untreated control subgroups ($Dacron_2$ and $ePTFE_2$), the grafts were inoculated with *S. aureus* and prophylactic antibiotic was not administered. In experimental subgroups ($Dacron_3$–$Dacron_9$ and $ePTFE_3$–$ePTFE_9$) the grafts were contaminated with inoculation of *S. aureus* and vancomycin or cefepime were used locally, systematically or in combinations. Accordingly, in local administration route, the grafts were impregnated with vancomycin (1 mg/ml, $Dacron_3$ and $ePTFE_3$) or cefepime (3 mg/ml, $Dacron_5$ and $ePTFE_5$) prior to implantation of the grafts. In the systemic route, vancomycin (10 mg/kg, $Dacron_4$ and $ePTFE_4$) and cefepime (60 mg/kg, $Dacron_6$ and $ePTFE_6$) were administered intraperitoneally 30 min before implantation of the grafts. The rats of $Dacron_7$ and $ePTFE_7$ subgroups received vancomycin impregnated grafts with intraperitoneal cefepime. The rats of $Dacron_8$ and $ePTFE_8$ subgroups received cefepime impregnated grafts with intraperitoneal vancomycin. The rats of $Dacron_9$ and $ePTFE_9$ subgroups received both vancomycin and cefepime intraperitoneally.

Each rat was anesthetized with ketamine 70 mg/kg (Alfasan, Netherland) and xylazine 5 mg/kg (Bayer, Germany) intraperitoneally. The skin of dorso-lumbar region was shaved, cleaned and prepared for surgery using scrub povidone iodine. One cm^2 sterile Dacron or ePTFE graft was implanted aseptically into a subcutaneous skin pocket (Figure 1). Antibiotic bonding was obtained immediately before implantation by impregnation of the grafts for 20 min in a sterile solution of the antibiotics. Skin incision was closed using 4-0 nylon sutures in cruciate pattern and sterile saline solution (1 ml) containing methicillin-resistant strain of *S. aureus* at a concentration of 5×10^7 CFU/ml was inoculated on the graft surface using a tuberculin syringe to create a subcutaneous fluid-filled pocket. The animals were returned to individual cages and thoroughly examined daily.

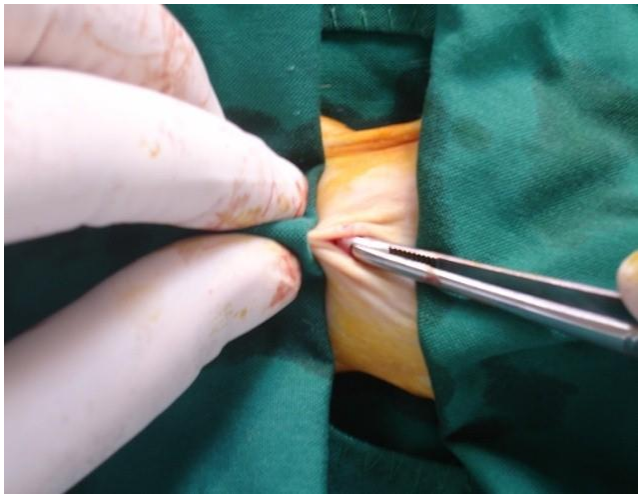

Figure 1. Aseptically implantation of one cm^2 sterile Dacron or ePTFE graft into subcutaneous skin pocket of dorso-lumbar region.

All grafts were removed seven days following the implantation. The explanted grafts were placed in sterile tubes, washed in sterile saline solution, placed in tubes containing 10 ml of phosphate-buffered saline solution and sonicated for 5 minutes to remove the adherent bacteria from the grafts. Quantitation of viable bacteria was performed by culturing serial dilutions (0.1 ml) of the bacterial suspensions on blood agar plates. All plates were incubated at 37° C for 48 hours and evaluated for presence of the inoculated staphylococci. The microbiological test was performed by counting the number of colony-forming units (CFUs) per plate. The detection limit for this method was 10 CFU/ml. Microbiological samples were also obtained from liver, spleen and blood, and the assessments were carried out using standard microbiological tests.

Statistical Analysis

The primary variable of interest in the present study was the bacterial count in an experimental graft model. The purpose of the study was to evaluate the impact of graft type (Dacron vs ePTEE), and type and route of antibiotic application on the bacterial load of the experimentally contaminated grafts. Prior to statistical analysis, an analysis of outliers and extremes values was performed through visual inspection of the boxplots. Normality of residuals was examined by the Shapiro-Wilks test and normality plots-histograms and quantile-quantile plots. Levene's test and examining of residual plot were used to explore the homogeneity of variation. The assumptions of normality and homogeneity of variance were not met. Therefore, the data were transformed by natural logarithm to achieve model assumptions. Statistical analysis of data was conducted using factorial analysis of variance with two between subject factors. The Bonferroni's test was used for post hoc analysis to adjust for multiple comparisons. The results were expressed as mean ± standard error. A value of $p < 0.05$ was considered significant. Statistical analyses were carried out using PASW Statistics (Version 18, SPSS Inc., Chicago, Illinois, USA).

Results

No local signs of inflammation were seen in gross inspection of graft area and systemic infection signs were not observed in any of experimental groups. Bacterial cultures from liver, spleen and blood did not show any growth in experimental groups and none of the animals were died. There was no microbiological evidence of the graft infection in the animals of the uncontaminated control subgroups ($Dacron_1$ and $ePTFE_1$). In contrast, all rats of the untreated contaminated control subgroups ($Dacron_2$ and $ePTFE_2$) demonstrated evidence of graft infection with mean quantitative culture results showing $4.7 \times 10^6 \pm 1.1 \times 10^6$ CFU/ml and $3.8 \times 10^5 \pm 1.2 \times 10^5$ CFU/ml, respectively.

Table 1 represents graft culture (CFUs/ml) of *S. aureus* based on type of the grafts and the type and route of administration of antibiotics. The bacterial growth count (CFU/ml) was significantly higher in Dacron grafts than that of ePTFE grafts in the untreated contaminated control subgroups-$ePTFE_2$ and $Dacron_2$. The bacterial count in implanted Dacron grafts which impregnated with vancomycin ($Dacron_3$) was significantly lower than that of implanted Dacron grafts impregnated with cefepime ($Dacron_5$) ($p < 0.05$). Also, the bacterial count in Dacron grafts impregnated with vancomycin ($Dacron_3$) was significantly lower than that of implanted Dacron grafts treated with intraperitoneal cefepime ($Dacron_6$) ($p < 0.05$).

There was no any significant difference between the bacterial count in implanted ePTFE grafts impregnated with vancomycin ($ePTFE_3$) or cefepime ($ePTFE_5$) and implanted ePTFE grafts treated with intraperitoneal vancomycin ($ePTFE_4$). However, the bacterial count in implanted ePTFE grafts treated with intraperitoneal cefepime ($ePTFE_5$) was significantly higher than that of implanted ePTFE grafts impregnated with vancomycin ($ePTFE_3$) or cefepime ($ePTFE_5$) and implanted ePTFE grafts treated with intraperitoneal vancomycin ($ePTFE_4$).

Table 1. The quantitative bacterial culture (CFUs/ml) of *S. aureus* in implanted Dacron and ePTFE grafts following intraperitoneal, local application or in combination of vancomycin and cefepime.

Type	Uncontaminated control	Untreated control	Local vancomycin	Systemic vancomycin	Local cefepime
Dacron	$Dacron_1$ 0.0	$Dacron_2$ 4720000.0 ± 1118928.1*a	$Dacron_3$ 3480.0 ± 356.4b	$Dacron_4$ 28000.0 ± 3240.4*ce	$Dacron_5$ 30800.0 ± 11861.7*ce
ePTFE	$ePTFE_1$ 0.0	$ePTFE_2$ 384000.0 ± 127200.6a	$ePTFE_3$ 2580.0 ± 238.7b	$ePTFE_4$ 2820.0 ± 535.7b	$ePTFE_5$ 1660.0 ± 502.9be

Type	Systemic cefepime	Local vancomycin +Systemic cefepime	Systemic vancomycin+ Local cefepime	Systemic cefepime+Systemic vancomycin
Dacron	$Dacron_6$ 45000.0 ± 10977.2*fe	$Dacron_7$ 2.2 ± 0.8g	$Dacron_8$ 3460.0 ± 1625.7*b	$Dacron_9$ 6920.0 ± 1937.0*d
ePTFE	$ePTFE_6$ 28800.0 ± 8105.6c	$ePTFE_7$ 2.00 ± 0.7d	$ePTFE_8$ 1400.0 ± 339.1e	$ePTFE_9$ 1420.0 ± 303.3e

a,b,c,… Values with different superscripts are significantly different ($p < 0.05$). * Results were significantly different between two grafts in each column ($p< 0.05$). Values are given as mean ± SE. Subscript numbers denote number of groups.

Combinations of cefepime with vancomycin resulted in enhanced efficiency of the antibiotics in the bacterial growth inhibition. There was no any significant difference in the bacterial count between Dacron or ePTFE ($Dacron_7$ and $ePTFE_7$) implanted grafts impregnated with vancomycin and in combination with intraperitoneal cefepime. The bacterial count in both $Dacron_7$-$ePTFE_7$ subgroups were significantly lower than that of $ePTFE_8$-$Dacron_8$ and $ePTFE_9$–$Dacron_9$ subgroups. The bacterial count in implanted Dacron grafts impregnated with cefepime in combination with intraperitoneal vancomycin was significantly lower than that of implanted Dacron grafts treated with combination of intraperitoneal cefepime and vancomycin ($p < 0.05$).

Discussion

In general, the results of the present study showed that prevention of the vascular graft infected with *S. aureus* could be affected by the type of graft and the type and the route of the antibiotics administration. The significantly higher bacterial growth count in implanted Dacron grafts compared to ePTFE grafts in the untreated contaminated control rats ($Dacron_2$ and $ePTFE_2$) demonstrated evidence of more graft infection of the Dacron grafts compared to ePTFE grafts. The quantitative graft culture showed that impregnation of the Dacron graft with vancomycin was more effective than impregnation of this graft with cefepime in reduction of the graft's contamination. Also, the combinations of cefepime with vancomycin resulted in enhanced efficiency of these antibiotics in inhibition of *S. aureus* growth on the implanted grafts. Prevention is the optimal treatment for vascular graft infections. The significant lower bacterial count in all treated rats, $Dacron_3$–$Dacron_9$ and $ePTFE_3$–$ePTFE_9$, compared to untreated contaminated control rats ($Dacron_2$ and $ePTFE_2$) could be suggested as a standpoint concerning vascular graft implantation. Therefore, systemic antibiotic administration and impregnation of the implanted graft with appropriate antibiotic could be an effective prophylactic strategy for prevention of vascular graft infection despite meticulous asepsis and proper surgical technique.[10-13]

Dacron and ePTFE are the most frequently used prosthetic vascular grafts. All type of the prosthetic grafts are prone to various degrees of bacterial infections. Infection could be occurred due to direct contamination through the graft implantation or through the post-operation bacteremia.[25] In present study, the significantly lower bacterial count in the untreated contaminated ePTFE grafts in comparison with the untreated contaminated Dacron grafts could be resulted from differences in molecular structure and surface area between the two grafts. Reportedly, the graft material of ePTFE is relatively nonporous when it is compared to multifilament Dacron graft. The material of ePTFE graft is more hydrophobic than Dacron. Also, the hydrophobic properties of bacterial cell wall may influence the bacterial adherence to the graft. Hence, the bacterial affinity is less likely for bonding to the ePTFE graft compared to the Dacron grafts.[26,27] The findings of present study were consistent with those of previous studies reporting that *S. aureus*, *S. epidermidis*

and *Escherichia coli* had greater affinity to Dacron graft when compared to ePTFE graft.[25,28,29]

Reportedly, the combined application of systemic antibiotic prophylaxis and drug bonded grafts were more effective in decreasing the incidence of prosthetic vascular graft infections.[10,30] The results of present study showed using vancomycin-cefepime combination was more commonly effective compared to where they were used alone to control *S. aureus* infection in the implanted grafts. This could be resulted from the synergistic activity of the antibiotics when used in a combination form. In several *in vitro* studies, it has been shown that combination of glycopeptides and β-lactams antibiotics have synergic activity against Gram-positive cocci such as entrococci and staphylococci.[31-33] Also, Lozniewski et al., (2001) showed synergistic effect of cefepime-vancomycin combination against a majority methicillin susceptible and methicillin resistant *S. aureus* (MSSA and MRSA) of strains *in vitro* and they speculated that broad-spectrum antimicrobial activity of cefepime may cause potential enhancement of the anti-staphylococcal activity of vancomycin. Furthermore, one potential strategy to overcome bacterial resistance is using a combination of antimicrobials with synergistic activity against targeted microorganisms[14]. Therefore, vancomycin as a commonly used monotherapy for severe infections caused by methicillin-resistant staphylococci in compromised hosts may be ineffective in treatment of the infection.[21]

The results of present study showed efficiency of both intraperitoneal and local impregnation of the grafts with cefepime and/or vancomycin as prophylactic treatments in prevention of the grafts infection. Application of local vancomycin combined with intraperitoneal cefepime was significantly more effective than the application of local cefepime combined with intraperitoneal vancomycin or using a combination of intraperitoneal vancomycin with cefepime. Hence, the highest reduction in bacterial count was observed in the grafts treated with combination of intraperitoneal cefepime and local vancomycin ($ePTFE_7$–$ePTFE_7$). Therefore, this method could be suggested for prevention of *S. aureus* infection for both Dacron and ePTFE vascular grafts implantation procedure.

The base of prophylaxis is preoperative administration of systemic antibiotics.[8,34] However, systemic administration of antimicrobial agents may not be enough to prevent graft infection due to low antimicrobial concentration in tissue surrounding the implant. Hence, impregnation of graft with antimicrobial agents that bind to the prosthetic grafts as an additional prophylactic measure are arousing interest especially when risk of graft contamination is high.[30,35] In agreement with the results of previous studies,[30,35] our results showed that intraperitoneal administration of vancomycin or cefepime alone was not effective compared to the combination administration of these drugs in reduction of bacterial count. Therefore, impregnation of the graft with appropriate antimicrobial agents can be suggested in combination with systemic proper antimicrobial drugs as a reliable prophylactic method to prevent vascular graft infection.[36]

In Dacron grafts, vancomycin when used locally was more effective in reducing bacterial count compared to local cefepime or intraperitoneal cefepime and vancomycin. Therefore, vancomycin could be suggested for prevention of infection in local form in Dacron vascular graft. In ePTFE graft, cefepime when used intraperitoneally was not more effective in reducing bacterial count compared to local cefepime and local or intraperitoneal vancomycin. Therefore, it seems cefepime is not an appropriate antibiotic to help prevent *S. aureus* infection in intraperitoneal administration in ePTFE vascular graft. Overall, to get more efficacy of the prophylactic antibiotics, in each type of vascular graft the route of drug administration could be very important to help prevent graft infection even with choosing of an appropriate antibiotic.

In conclusion, as a prophylactic strategy local vancomycin-intraperitoneal cefepime combination could be suggested for both Dacron and ePTFE vascular grafts to prevent *S. aureus* infection. However, local treatment alone, in vancomycin for Dacron graft and cefepime for ePTFE graft could also be suggested. Where the intraperitoneal treatment alone is the case, vancomycin for ePTFE graft could be suggested.

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Conflict of Interest

No competing interests have been declared.

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