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Original Article

Efficacy of boric acid used to treat experimental vascular graft infection by methicillin-resistant *Staphylococcus aureus*

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Abstract

Introduction: We aimed to investigate the efficacy of local boric acid (BA) and teicoplanin in prosthetic vascular graft infection (PVGI) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in a rat model.

Methodology: Fourty rats were divided into five groups. Group 1 received no treatments (control group); group 2 was uncontaminated polytetrafluoroethylene (PTFE) graft group; group 3 was untreated and the PTFE graft was contaminated with 2×10^7 CFU/mL MRSA; group 4 received local BA (8 mg/kg) and was contaminated with with 2×10^7 CFU/mL MRSA; group 5 received local BA (8 mg/kg) and intraperitoneal teikoplanin (10 mg/kg), and was contaminated with 2×10^7 CFU/mL MRSA; On the 3rd day, grafts and serums were removed for microbiological, histological and serological tests.

Results: The amounts of culture growth in groups 4 and 5 were significantly lower compared to group 3 ($p < 0.001$). TNF-α was significantly higher in Group 3 than the other groups ($p = 0.001$). There was no significant difference between the groups in serum IL-1 levels ($p = 0.138$). Monocyte chemotactic protein-1 (MCP-1) was not significantly different between groups 3, 4, and 5, but it was significantly higher than groups 1 and 2 ($p < 0.001$). The severity of inflammation was significantly higher in group 3 than the other groups, and fibroblastic proliferation, granulation tissue and collagen synthesis were significantly lower ($p < 0.05$).

Conclusions: Our study showed that local BA and combined teicoplanin treatment is effective in preventing PVGI.

Key words: Teicoplanin; boric acid; *S. aureus*; cytokines; rat model.

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Introduction

Although prosthetic vascular graft infection (PVGI) is an uncommon condition, it is an important life-threatening complication in vascular surgery. The incidence of PVGI in different vascular surgery clinics has been reported to be between 1% and 6% [1,2]. Previous studies have also shown that limb amputation rates and mortality were between 5-70% and 20-70%, respectively [3,4].

PVGI may develop due to insufficient attention during the perioperative skin disinfection, contamination of postoperative incision sites, or systemic bacteremia. In the case of vascular graft infection, aggressive surgical debridement of infected tissues with graft excision, extra anatomic revascularization and high-dose systemic antibiotics are recommended as traditional treatment methods [1]. However, these treatment approaches are not always successful in preventing morbidity and mortality. The most common microorganisms that cause PVGI are *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) [5]. Since these bacteria form biofilms around the infected graft, many antibiotics cannot pass through this layer [4]. The most

effective way for the prevention of graft infection is to provide asepsis conventionally and to apply systemic antibiotics in the postoperative period. A guideline for the treatment approach in PVGI has been published previously [5].

Boric acid (BA) is a white crystalline solid with the molecular formula of H_3BO_3. It is a weak acid found in nature and can be produced by the reaction of borate minerals with sulfuric acid. BA plays an important role in cell replication in animals, ensuring the completion of the life cycle [6]. Insufficient intake of BA significantly impairs bone health, brain function and immune response [7]. It reduces the incidence and severity of inflammatory diseases [8]. Boron, found in nature, is a type of BA salt that affects the activity of different enzymes in animals, plants and chemicals [9]. BA decreases nitric oxide production in a dose-dependent manner and suppresses inflammatory mediator genes, inducible nitric oxide synthase and cyclooxygenase-2 [10,11]. BA is used in diluted form in eye infections, as an antiseptic in small incisions and burns, and as a medical treatment in acne, aphthous and ulcerated diphtheria lesions. It is also used to treat gonorrhea vaginitis and cystitis, bacterial and fungal otitis media [12-14]. It is often used as a 4% solution prepared with 70% alcohol or distilled water, and also as a pure boric acid powder formulation.

The aim of our study, was to investigate the in vivo efficacy of local boric acid and teicoplanin in experimental graft infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in a rat model.

Methodology

Experimental animals and housing conditions

A total of 40 adult Wistar albino rats, weighing 200-250 g, were used in the study. After the study was approved by the animal ethics committee of Kahramanmaraş Sütçü İmam University (approval date: July 22, 2020; issue: 2), the study was carried out in the Animal Experiments Laboratory of Kahramanmaraş Sütçü İmam University. Acclimatization for the study conditions was allowed for one week prior to the start of the study. All experiments were conducted in strict accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. The rats were housed and maintained at 22 °C, 60 ± 5% humidity, and a 12:12 hours light/dark cycle, with free access to food and water ad libitum.

Experimental surgical design

A total number of 40 rats were randomly divided into 5 groups (n = 8). The experimental groups were as follows: Group 1: control (no BA and no other medication); Group 2: uncontaminated polytetrafluoroethylene (PTFE); Group 3: untreated contaminated PTFE; Group 4: Local BA (8 mg/kg) + contaminated PTFE; Group 5: Local BA (8 mg/kg) + intraperitoneal teikoplanin (10 mg/kg) + contaminated PTFE.

In Group 1, no graft was settled. Instead, a 1.5 cm-pocket was opened on the right dorsum, and then 5/0 polypropylene sutures (Dogsan, Istanbul, Turkey) were used to close the incision (the control group). In Group 2, a 1.5 cm pocket was opened on the right dorsum, and a PTFE graft (Gore-Tex; W.L. Gore & Associates Inc, Arizona, USA) was placed aseptically and the incision closed. In Group 3, a 1.5 cm pocket was opened on the right dorsum, and PTFE graft was placed aseptically and closed. The MRSA strain was then inoculated to the surface of the graft in a tuberculin syringe at a concentration of 2×10^7 CFU/mL and in 1 mL of saline solution. In Group 4, a 1.5 cm pocket was opened on the right dorsal side and PTFE graft was placed aseptically. 8 mg/kg of BA was administered locally into the graft tissue and closed. Then the MRSA strain was inoculated on the surface of the graft in a tuberculin syringe at a concentration of 2×10^7 CFU/mL and in 1 mL of saline solution [15]. In Group 5, a 1.5 cm-pocket was opened on the right dorsal side and PTFE graft was placed aseptically. 8 mg/kg of BA was administered locally into the graft tissue and closed. Then the MRSA strain was inoculated on the surface of the graft in a tuberculin syringe at a concentration of 2×10^7 CFU / mL and in 1 mL of saline solution. Additionally, 10 mg/kg teicoplanin was administered intraperitoneally once a day for 3 days [16].

The animals were placed in individual cages and followed daily. Under sterile conditions, all grafts were removed for bacteriological study. Perigraft tissue was debrided for histological examinations. In addition, blood samples were taken by cardiopuncture for the measurement of tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1) and monocyte chemoattractant protein-1 (MCP-1).

Microbiological Strain

The MRSA strain used in this study was isolated from a clinical sample sent to the Microbiology Department of Kahramanmaraş Sütçü İmam University Faculty of Medicine for routine bacteriological research. Commercially available *Staphylococcus*

aureus ATCC 43300 was used as a control strain of the methicillin susceptibility test. The organism was incubated overnight on sheep blood agar. The number of bacteria was determined by turbidimetry and confirmed by culture results. The antimicrobial susceptibilities of MRSA strains were determined using the microfluid dilution method according to the procedures specified by the National Clinical Laboratory Standards Committee [17].

Assessment of the infection

Explanted grafts were placed in sterile tubes, washed in sterile saline solution. They were, placed in tubes containing 10 mL of phosphate buffered saline solution, and ultrasonicated for 5 minutes to remove sticky bacteria from the grafts. Quantification of viable bacteria was carried out by preparing serial 10-fold dilutions (0.1 mL) of bacterial suspensions in 10 mM buffer and culturing each dilution on blood agar plates to minimize the contamination effect. All plates were incubated at 37 °C for 48 hours and evaluated for the presence of the MRSA strain. Organisms were measured by counting the number of colony-forming units (CFU) per plate. The detection limit for this method was approximately 5×10^1 CFU/cm^2 of graft tissue.

Agents and drugs

Teicoplanin (Targocid®) was obtained from Aventis Pharma (Istanbul, Turkey). The drug was dissolved in sterile distilled water at a concentration of 1 mg/mL. Boric acid was obtained from Sigma-Aldrich (Istanbul, Turkey). The solutions were freshly prepared on the day of the experiments. All rats in the study were anesthetized with ketamine hydrochloride (Ketalar, 50 mg/kg, intramuscularly, Parquet-Davis, Eczacıbaşı, İstanbul, Turkey). Additional ketamine hydrochloride was administered intramuscularly (25 mg/kg) for anesthesia during the procedure.

Enzyme-linked immunosorbent assay (ELISA)

At the end of the experimental process, all blood samples were centrifuged at 3000 rpm for 10 minutes. Biochemical evaluation of local infection in rats was evaluated by measuring TNF-α, IL-1 and MCP-1. Rat serum TNF-α, IL-1 and MCP-1 concentrations were measured using ELISA kits (TNF-α, Catalog No.: YLA0118RA; IL-1, Catalog No.: YLA0153RA; MCP-1, Catalog No.: YLA0041RA; Shanghai YL Biotech Co. Ltd., Shanghai, China) according to the manufacturer's recommendations. All the results are expressed as pg/mL of serum. The intra- and inter-assay coefficients of variation were < 6.9 and < 9.0 %, < 5.8 and < 8.8 %, and < 4.7 and < 8.7 % for TNF-α, IL1 and MCP-1, respectively.

Histopathological evaluation

Subcutaneous tissue samples taken from each rat were fixed in 10% buffered formalin and processed for paraffin block preparation. Five micrometers tissue sections were cut and stained with hematoxylin and eosin (H&E) for morphological examination and Masson's Trichrome for collagen deposition. The slides were randomly analyzed blindly by a histologist. Granulation tissue formation, collagen organization, inflammatory cell infiltration and fibroblastic proliferation were evaluated according to the scoring system reported previously [18,19].

Statistical analysis

The compatibility of the variables to normal distribution was examined using the Shapiro-Wilk test. Comparisons between groups in variables with normal distribution were analyzed using the one-way ANOVA test. Tukey HSD and Dunnett tests were used for post-hoc analysis. Kruskal Wallis H test was used for the comparison of the groups for variables that did not show normal distribution. Dunn test was used for post-hoc analysis. Statistical parameters are expressed as mean ± SD and median (min-max). The data were evaluated using IBM SPSS statistics for Windows version 22 (IBM SPSS for Windows version 22, IBM Corparation, Armonk, New York, United States). Statistical significance was accepted at $p < 0.05$.

Ethics committee approval

The study was approved by the Institutional Ethics Committee (approval date: July 03, 2019, document number: 3).

Results

None of the rats died during the study. No side effects of drugs such as local symptoms of perigraft inflammation, anorexia, diarrhea, or a behavior disorder were observed.

Microbiology results

There was no anatomical or microbiological evidence to suggest graft infection in either the control group or the graft group. In group 3, bacterial growth of 10. 2×10^4 CFU/cm^2 was observed. In group 4, 4. 5×10^3 CFU/cm^2 bacterial growth was observed. In group 5, there was no significant bacterial growth. Group 1 and 2 had a significant difference compared to group 3 and 4 ($p < 0.001$).

Figure 1. *Haematoxylin-Eosin (H&E)* staining of control (A), control uninfected (B), infected untreated (C), infected and treated with boric acid (D), infected and treated with boric acid and teicoplanin groups (E). A, B, C, D, E x 200.

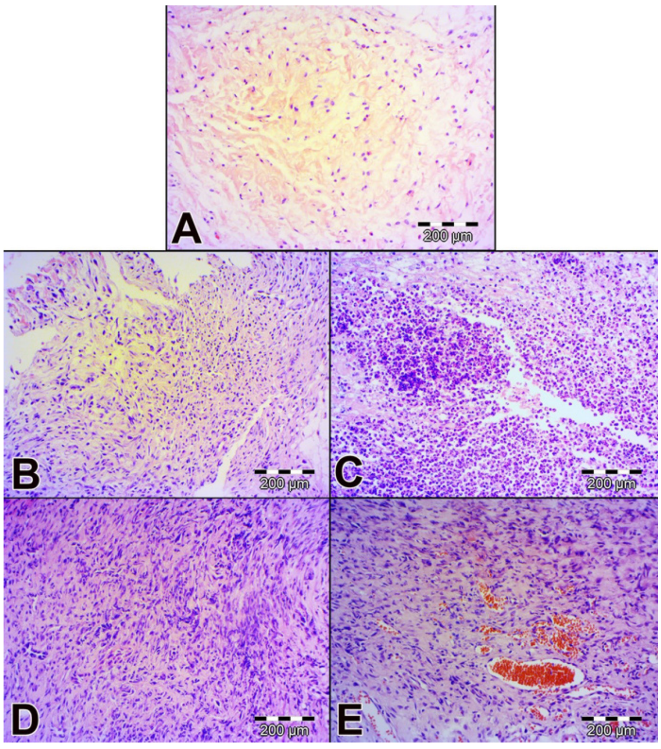

Figure 2. *Masson's trichrome* staining of control (A), control uninfected (B), infected untreated (C), infected and treated with boric acid (D), infected and treated with boric acid and teicoplanin groups (E). A, B, C, D, E x 200.

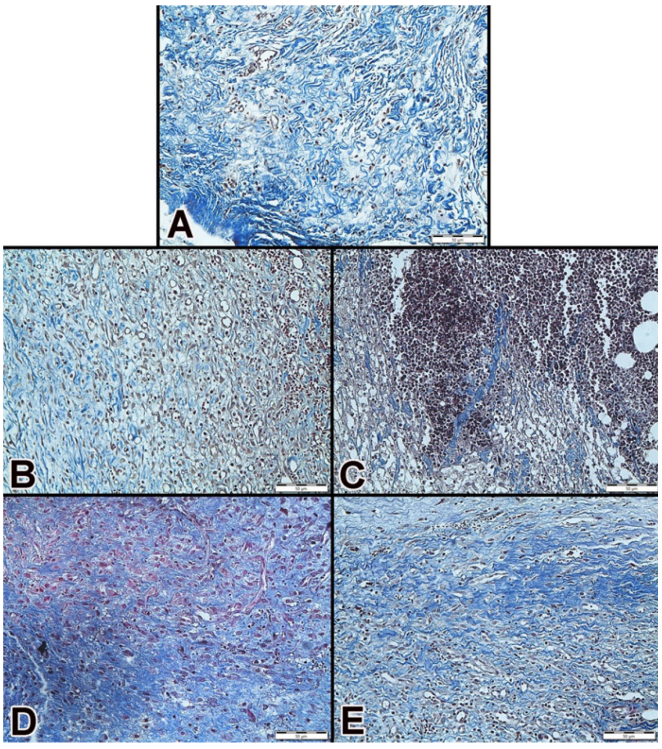

Table 1. Study groups and quantitative microbiological results of in vivo experiments.

	Group 1	Group 2	Group 3	Group 4	Group 5	*p*
	Control	Graft	Graft + MRSA	Graft + BA + MRSA	Graft + BA + MRSA + T	
Production amount, Median (Min-Max)	0.00 (0.00-0.00)^{c,d}	0.00 (0.00-0.00)^{c,d}	102000.00 (21000.00-350000.00)^{a,b,d,e}	4500.00 (0.00-60000.00),^{a,b,c}	0.00 (0.00-20000.00)^{c}	$p < 0.001$*

Kruskal Wallis H test; Post-hoc Dunn Test; a: 0.05; *The difference is statistically significant; [a]Significant difference with the control group; [b]Significant difference with graft group; [c]Significant difference with graft + MRSA group; [d] Significant difference with graft + BA + MRSA group; [e] Significant difference with graft + BA + MRSA + T group. BA: Boric Acid; MRSA: Methicillin resistant *Staphylococcus aureus*; T: Teicoplanin.

Table 2. Study groups and quantitative serum results of in vivo experiments.

	Group 1	Group 2	Group 3	Group 4	Group 5	*p*
	Control	Graft	Graft + MRSA	Graft + BA + MRSA	Graft + BA + MRSA + T	
TNF-α Mean ± SD	85.70 ± 1.01	85.59 ± 0.71	110.64 ± 27.73^{a,b,d,e}	83.85 ± 1.18	84.68 ± 8.78	0.001*
IL-1 Mean ± SD	17.79 ± 0.54	18.17 ± 0.28	19.21 ± 2.57	17.67 ± 0.69	17.98 ± 0.80	0.138
MCP-1 Mean ± SD	57.93 ± 1.31^{c,d,e}	59.04 ± 1.31^{c,d,e}	65.67 ± 6.74^{a,b}	70.45 ± 4.42^{a,b}	70.89 ± 1.63^{a,b}	$p < 0.001$*

One Way ANOVA Post-hoc: Tukey HSD Test; Dunnett Test; a: 0.05; *The difference is statistically significant; [a]Significant difference with the control group; [b]Significant difference with graft group; [c]Significant difference with graft + MRSA group; [d]Significant difference with graft + BA + MRSA group; [e]Significant difference with Greft + BA + MRSA + T group. BA: Boric Acid; MRSA: Methicillin resistant *Staphylococcus aureus*; T: Teicoplanin.

Table 3. Study groups for in vivo experiments and histological findings.

	Group 1	Grup 2	Group 3	Group 4	Group 5	*p*
	Control	Graft	Graft + MRSA	Graft + BA + MRSA	Graft + BA + MRSA + T	
Granulation Tissue, Mean±SD	0.55 ± 0.51^{a,b,c,d}	2.40 ± 0.50^{a,c,d,e}	1.77 ± 0.62^{a,b,d,e}	3.52 ± 0.51^{a,b,c}	3.18 ± 0.64^{a,b,c}	$p < 0.05$*
Collagen, Mean±SD	3.80 ± 0.41^{b,c,e}	2.10 ± 0.64^{a,c,d,e}	1.53 ± 0.60^{a,b,d,e}	3.70 ± 0.52^{b,c,e}	2.85 ± 0.70^{a,b,c,d}	$p < 0.05$*
Inflammation, Mean±SD	0.35 ± 0.49^{a,b,c,d}	2.60 ± 0.50^{a,c,d,e}	3.70 ± 0.46^{a,b,d,e}	1.93 ± 0.53^{a,b,c}	1.98 ± 0.58^{a,b,c}	$p < 0.05$*
Fibroblastic Proliferation, Mean±SD	0.40 ± 0.50^{a,b,c,d}	3.00 ± 0.73^{a,c,d,e}	2.40 ± 0.50^{a,b,d,e}	3.67 ± 0.47^{a,b,c}	3.58 ± 0.50^{a,b,c}	$p < 0.05$*

Kruskal Wallis H test; Post-hoc Dunn Test; a: 0.05; *The difference is statistically significant; [a]Significant difference with the control group; [b]Significant difference with graft group; [c]Significant difference with graft + MRSA group; [d]Significant difference with graft + BA + MRSA group; [e]Significant difference with graft + BA + MRSA + T group. BA: Boric Acid; MRSA: Methicillin resistant *Staphylococcus aureus*; T: Teicoplanin.

There was also a significant difference between group 3 and the other groups ($p < 0.001$). There was a significant difference between group 5 and group 3 ($p < 0.001$). There was a significant difference between group 4 compared to groups 1, 2 and 3 ($p < 0.001$). Groups, treatment protocols and quantitative results of microbiological examinations are shown in Table 1.

Serum results

When serum TNF-α values were compared between the groups, there was a significant difference between Group 3 and the other groups ($p = 0.001$). When serum IL-1 levels were compared between groups, there was no statistically significant difference ($p = 0.138$). When serum MCP-1 values were compared between the groups, there was no significant difference between groups 3, 4, and 5, but they were significantly higher than groups 1 and 2 ($p < 0.001$). (Table 2)

Histopathological results

Histopathological observations of subcutaneous tissue of control rats revealed normal architecture of the dermis (Figure 1A, 2A). In samples taken from control uninfected rats, mature granulation tissue with numerous fibroblasts and moderate deposition of collagen fibers were observed (Figure 1B, 2B). The infected untreated rat group displayed most evidence of inflammatory response and loosely organized and reduced collagen deposition and granulation tissue accumulation with few cells compared to all the other groups. (Figure 1C, 2C). There were also focal necrotic areas in this group. The average intensity of inflammation was significantly more in the infected untreated group (Table 3). The granulation tissue formation with many fibroblasts and collagen deposition was increased in infected rats receiving boric acid (Figure 1D, 2D). Histological comparison of tissues showed that boric acid administration positively affected granulation tissue and wound collagen formation in infected and treated rats. The infected rats treated with boric acid and teicoplanin showed similar features and the congestion in the vessels was remarkable in this group (Figure 1E, 2E).

Discussion

Graft infections are serious complications of vascular surgery. All prosthetic vascular grafts are susceptible to varying degrees of infection via direct contamination at implantation or post-operative bacteremia. MRSA is becoming an increasing problem in cardiovascular surgery units, and most graft infections are believed to occur at the time of graft placement [20,21]. Prevention of prosthetic vascular graft infection is important because infection often results in graft excision. Despite constantly improving antimicrobial therapies, PVGI remains a problem for a vascular surgery, resulting in a high number of limb amputations and deaths [1-3]. Both methicillin-sensitive and methicillin-resistant strains of *S. aureus* are responsible for early and late-onset of PVGIs and are increasingly developing resistance to antibiotics such as cefazolin [3,22].

Another thing that makes it difficult to cope with staphylococcal PVGI is the ability of bacteria to form biofilms in vascular prostheses as well as in other medical implants [23-25]. Several studies have shown that systemic antibiotic prophylaxis reduced the incidence of PVGI, but did not prevent it. Due to the emergence of resistance to antibiotics, various antibiotics and prophylaxis protocols have been investigated. Teicoplanin appears to be one of the obvious options in systemic therapy to prevent such infections. Teicoplanin, one of the glycopeptides, is a bactericidal agent capable of inhibiting bacterial cell wall synthesis. Teicoplanin is preferred because its side effects such as nephrotoxicity and ototoxicity are less than vancomycin, which is also a glycopeptide [26]. Antibiotic resistance makes it difficult to treat PVGI in all infections. MRSA increases the need for new antibiotic regimens and/or new local and systemic treatment methods.

Previous studies showed that boric acid positively supported the antioxidant system and basic metabolic parameters [27,28]. Boron compounds, especially BA, have been shown to reduce the oxidative stress in different toxicity models, thereby contributing to treatment [29-31]. The boron-containing products are common in many different areas of medicine, from cancer therapeutics to oral antidiabetics, anticoagulants and anti-infective drugs [32]. However, there are several phase 1-3 studies on the use of borinic compounds as antiviral, antifungal, antituberculosis and antibacterial agents [32]. Boronic acid derivatives can be used effectively to overcome the problem of beta-lactamase resistance. Boron atoms mimic the carbon of the beta-lactam ring and selectively inhibit the serine protease family of beta-lactamases [33]. Borinic esters can also inhibit menaquinone methyl transferase, making them new control measures for Gram-positive bacteria [34]. Therefore, there are studies aiming to determine the doses of boric acid against strains such as *Staphylococcus aureus*, *Acinetobacter septicus*, *Escherichia coli* and *Pseudomonas aeruginosa* [35].

In our study we used rats which are the preferred method to test treatment sensitivity before human testing. We investigated the effects of boric acid as a possible local treatment option for PVGI. We also utilized teicoplanin, a systemic antibiotic on PVGI. It is important to select the correct inoculum dose in animal models because inadequate inoculum may be insufficient to stop a wound infection and wound colonization, trigger the host's inflammatory response and thus promote faster wound healing. On the other hand, injecting large amounts may lead to high mortality rates [36]. Since we created a closed wound in our study, no erythema was observed on the sutured incision edges as described in previous animal models with open infected wounds [37].

In our study we found that there was a significantly higher rate of growth in group 3 where MRSA was injected on the graft compared to all groups. In group 4, grafts were placed and only BA was given locally to the incision area. We saw that the amount of production in culture was significantly decreased compared to group 3, and suggested that local BA application prevented the development of PVGI. It was also statistically shown in group 5 which received intraperitoneal teicoplanin prophylaxis with local BA, that the production rate in culture was significantly reduced. Similarly, Guzel *et al*. created an osteomyelitis rat model infected with MRSA strain in their study. They administered local and systemic BA and reported that the production rate in culture decreased significantly with BA [15].

Brittingham *et al*. showed that BA prevented the formation of vaginitis caused by *Trichomonas vaginalis* in topical application and reported that BA had serious antimicrobial activity [38]. Aggarwal *et al.* also reported that they treated *Trichomonas vaginitis* with a combination containing BA [39]. In another study Sayın *et al.* investigated the antibacterial and antibiofilm effects of BA on different microorganisms. In these studies, its effectiveness on many microorganisms including *S. aureus* was examined and revealed that BA exhibits antibacterial properties and inhibits biofilm formation in almost all bacteria [40].

Our study also showed that BA had antimicrobial properties. The effectiveness of teicoplanin has been demonstrated well in previous studies against MRSA in experimentally created PVGI by Yasim *et al*. and Mese *et al*. [3,41]. Our results revealed that the application of local BA in addition to intraperitoneal teicoplanin significantly reduced the production rate in the culture. We claimed that the use of local BA in addition to prophylactic teicoplanin would play an important role in preventing possible PVGI formation.

The function of the immune system largely depends on interleukins [42]. In our study, we used proinflammatory markers IL-1, TNF-α and MCP-1 to determine inflammation and infection [42]. When the TNF-α levels are examined, we found a significant increase in group 3 exposed to the MRSA strain, and a significant decrease in the TNF-α level in group 4 and 5 that were given BA and BA + teicoplanin. In the experimental osteomyelitis model analyzed by Güzel *et al.*, administration of local BA and local BA + vancomycin significantly decreased the TNF-α level [15]. TNF-α levels in our study were also decreased, suggesting that local BA and local BA + teicoplanin may be used for effective treatment in PVGI. However, we found no significant change in IL-1 levels. We suggested that it may be due to the absence of infection at a level that would affect IL-1 level. Hazman *et al.* investigated the toxicity of cisplatin used in cancer treatment and the effectiveness of BA through immunocytokines. They showed that BA at an effective dose did not alter the level of IL-1, instead it decreased TNF-α level [43]. In the experimental PVGI studies conducted by Gül *et al.,* they showed that the level of TNF-α increased in the contaminated group and decreased with teicoplanin and other antibiotics. They reported that IL-1 levels did not create a significant difference in all groups [44]. Considering these studies, our results revealed the similar approach about TNF-α and IL-1 levels.

We found that infection occured in the groups with the MRSA strain, which has a significant increase in MCP-1 levels. The fact that BA and teicoplanin did not decrease MCP-1 levels statistically may be due to the fact that the waiting period of the experiment was not sufficient for MCP-1 decrease.

In this study, the severity of inflammation was also evaluated histopathologically in terms of fibroblastic proliferation density, granulation tissue and collagen amount of wound healing. Prevention or treatment of infection is important for successful wound healing. Tissue repair is adversely affected by cytolytic enzymes, free oxygen radicals and other proinflammatory mediators due to the continuous flow of neutrophils seen in the inflammatory response. We found that the severity of inflammation was the highest in group 3, where *S. aureus* was injected into the graft site without any antibiotics. Group 3 also showed a significant decrease in fibroblastic proliferation, granulation tissue and collagen synthesis compared to other groups. These results showed that inflammation

has a negative effect on wound healing as reported in previous studies [45,46].

In another experimental PVGI model created with the MRSA strain by Atahan *et al*., they administered linezolid, vancomycin and teicoplanin as prophylaxis. They showed that teicoplanin decreased inflammation and increased fibroplastic proliferation, edema and collagen suppressed by infection [19]. In our histological results, we declared that inflammation was increased, while the amount of collagen, fibroblastic proliferation and granulation tissue were decreased in group 3 infected with the MRSA strain. We observed that local BA and/or local BA + teicoplanin treatment protocol could be effective in wound healing, as seen in the groups 4 and 5, where the treatments decreased inflammation, while collagen amount, fibroblastic proliferation and granulation tissue were increased.

Conclusions

Our study has demonstrated microbiologically, serologically and histologically, that local BA administration alone and/or in combination with intraperitoneal teicoplanin prevented PVGI induced by MRSA strain. In fact, the development of novel treatment protocols designed to prevent the antimicrobial coated grafts are the future options. BA attracts attention as an important molecule in preventing graft infections with its known antimicrobial property. Further animal studies are needed to evaluate the effectiveness of the agent in early-onset graft infections to determine the final recommendations in clinical trials.

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