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Activity of fixed direct electrical current in experimental *Staphylococcus aureus* foreign-body osteomyelitis

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ABSTRACT

Fixed DC was compared to ceftriaxone, ceftriaxone with 200 μA fixed DC, or no treatment in a rat model of methicillin-susceptible *Staphylococcus aureus* foreign-body osteomyelitis. After 3 weeks, fewer bacteria were present in bones of the ceftriaxone group (5.71 $\log_{10}$cfu/g [$P = 0.0004$]) and the ceftriaxone/DC group (3.53 $\log_{10}$cfu/g [$P = 0.0002$]) than untreated controls (6.70 $\log_{10}$cfu/g). Fewer bacteria were present in the ceftriaxone/DC group than in the ceftriaxone-alone and DC-alone groups ($P = 0.0012$ and 0.0008, respectively). There were also fewer bacteria on the implanted wires in the groups treated with ceftriaxone (5.47 $\log_{10}$cfu/cm^2) or ceftriaxone/DC (2.82 $\log_{10}$cfu/cm^2) than in the untreated controls (6.44 $\log_{10}$cfu/cm^2 [$P = 0.0003$ and 0.0002, respectively]). There were fewer bacteria in the ceftriaxone/DC rats than in the ceftriaxone-alone– and fixed DC-alone–treated rats ($P = 0.0017$ and 0.0016, respectively). Fixed DC with an antibiotic may be useful for treating foreign-body infections caused by *S. aureus*.

1. Introduction

In today's medical practice, it is commonplace for patients to have surgically implanted devices ranging from prosthetic joints to catheters and to pacemakers, with many more possibilities. Arthroplasty implantation is among the most common surgical procedures performed in the United States, with over 1 million total hip and knee arthroplasties placed in 2010 (Kremers et al., 2015; Tande and Patel, 2014) and with this number expected to rise exponentially to over 500,000 and over 3.4 million total hip and knee arthroplasties, respectively, by the year 2030 (Kurtz et al., 2007). Along with the increased use of prosthetic joints come an increased number of infections and thus significant economic impact (Tande and Patel, 2014). Prosthetic joint infections (PJIs) are difficult to treat given that the bacteria related with these infections exist in the biofilm state, in which bacteria are surrounded by an extrapolymeric substance, composed of polysaccharides, proteins, lipids, and extracellular DNA, and are in their own niche wherein the availability of nutrients and growth and death rates are altered (Gbejuade et al., 2015; Høiby et al., 2010). In biofilms, bacteria are 100–1000 times more resistant to antimicrobial treatment compared to their planktonic counterparts (Gbejuade et al., 2015; Høiby et al., 2010; Tande and Patel, 2014). In the majority of PJIs, Gram-positive cocci are the causal agents, with *Staphylococcus aureus* and coagulase-negative staphylococci being the main culprits (Benito et al., 2016; Tande and Patel, 2014).

Because of the poor activity of most currently available antibiotics, novel treatment strategies need to be explored. In previous studies, we have shown that low-amperage fixed direct electrical current (DC) reduced *S. aureus* biofilms formed on implant-associated materials *in vitro* (Schmidt-Malan et al., 2015) and also *in vivo* in a novel model of foreign-body osteomyelitis caused by *Cutibacterium acnes* (Schmidt-Malan et al., 2017). In the present study, we tested *S. aureus* in the same *in vivo* model, comparing fixed DC alone, ceftriaxone alone, and fixed DC combined with ceftriaxone to each other and to no treatment.

2. Materials and methods

2.1. Microorganism

Methicillin-susceptible *S. aureus* Infectious Diseases Research Laboratory (IDRL)-4284, a clinical isolate, was studied. The isolate was saved in a Microbank™ (Pro-Lab Diagnostics, Round Rock, TX) at −80°C. The MIC of oxacillin was 0.25 μg/mL (susceptible) and that of penicillin was 2 μg/mL (resistant); the cefoxitin disc zone size was 26.5 mm (susceptible). Susceptibility to oxacillin and cefoxitin infers susceptibility to ceftriaxone (CLSI, 2018a, 2018b).

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2.2. Experimental rat model

This study followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978) and was approved by Mayo Clinic Institutional Animal Care and Use Committee in Rochester, MN. Foreign-body osteomyelitis was established in male Wistar rats (approximately 300 g) (Envigo, Indianapolis, IN), as previously described (Schmidt-Malan et al., 2017). Animals underwent general anesthesia by intramuscular application of ketamine (60 mg/kg), xylazine (6 mg/kg), and acepromazine (1.5 mg/kg). For each animal, the left leg and midscapular area were clipped and washed with Hibiclens (4% chlorhexidine gluconate) (Mölnlycke Health Care, Norcross, GA). The left knee was exposed, after which a median parapatellar incision was made. A hole was created between the condyles of the femur using a 16G needle followed by insertion of a 14G needle to allow space for the wire to be implanted. Ten microliters of arachidonic acid sodium salt (a sclerosing agent) (99%; Sigma-Aldrich Co., St. Louis, MO) at 50 μg/mL was injected into the femur. A platinum wire (10 mm × 3 mm) with a preformed *S. aureus* biofilm was implanted into the femur. The platinum wire was seeded with *S. aureus* biofilm (~10^5 colony forming units (cfu)/cm^2, as determined by quantitative culture on a separate set of wires) by incubation in trypticase soy broth (TSB) for approximately 2 h. The remaining space in the bone was covered with dental gypsum after the wire was implanted. The cables used to supply power were tunneled under the skin to the prepared midscapular area and exposed (Fig. 1); the incisions on the leg and in the midscapular area were closed with 3-0 Vicryl (Ethicon, Inc., Somerville, NJ), and the exposed wires were secured to the skin with 3-0 silk (Ethicon, Inc.) (Fig. 1). The leg was sprayed with AluSpray (Neogen Corporation, Lansing, MI) and Chew-Guard (Summit Hill Laboratories, Tinton Falls, NJ). Buprenorphine (slow release at 60 mg/kg) and meloxicam (slow release at 4 mg/kg) were used for analgesia.

One week after establishing infection, treatment was started. Thirty-six animals were randomly assigned to 1 of 4 study arms: no treatment ($n = 13$), ceftriaxone treatment ($n = 7$), continuous 200 μA fixed DC ($n = 8$), or ceftriaxone with 200 μA fixed DC ($n = 8$). The DC amperage was chosen based on our prior studies of *in vitro* effects on biofilms when administered in combination with antimicrobial agents (Del Pozo et al., 2009). One animal from the ceftriaxone treatment group was removed from the study because it chewed the jacket harboring the power source and lost one of its bottom incisors. Ceftriaxone was administered at 50 mg/kg intramuscularly once daily; we have previously published pharmacokinetic data using this ceftriaxone dose (Schmidt-Malan et al., 2017). Battery packs, set to deliver 200 μA of fixed DC, were connected to the exposed wires (Fig. 1). Treatment was administered for 21 days. Twenty-four hours after the last dose of ceftriaxone, rats were sacrificed with CO_2 inhalation, and the left femur with the platinum implant was removed and placed into a sterile 50-mL conical tube. The femur was frozen to −80 °C. Bone surrounding the implanted wire was cut (a 5-mm section), weighed, refrozen to −80°C, and then pulverized for quantitative bacterial culture. The crushed bone was placed in 2 mL of TSB, vortexed for 30 s, sonicated at 40 kHz for 5 min, vortexed for 30 s, serially diluted, and plated on trypticase soy agar plates containing 5% sheep blood (TSA II, Becton Dickinson, Franklin Lakes, NJ). The wire was removed from the bone and placed in 1 mL of TSB and cultured, as described above. Quantitative culture results for bone and wire were obtained after 48 h of incubation at 37 °C and expressed as $\log_{10}$ cfu/g or $\log_{10}$ cfu/cm^2, respectively.

2.3. Statistical methods

Statistical analyses were performed using SAS software (SAS Institute, Inc., Cary, NC). Using the Wilcoxon rank sum test, we compared the $\log_{10}$ cfu per gram of bone or cm^2 of wire for the no-treatment, ceftriaxone treatment, 200 μA fixed DC treatment, and ceftriaxone with 200 μA fixed DC treatment groups. All tests were 2 sided; *P* values of <0.05 were considered statistically significant.

3. Results

3.1. Experimental rat model

Continuous low-amperage fixed DC alone and in combination with ceftriaxone as well as ceftriaxone alone was used to treat experimental femoral foreign-body osteomyelitis.

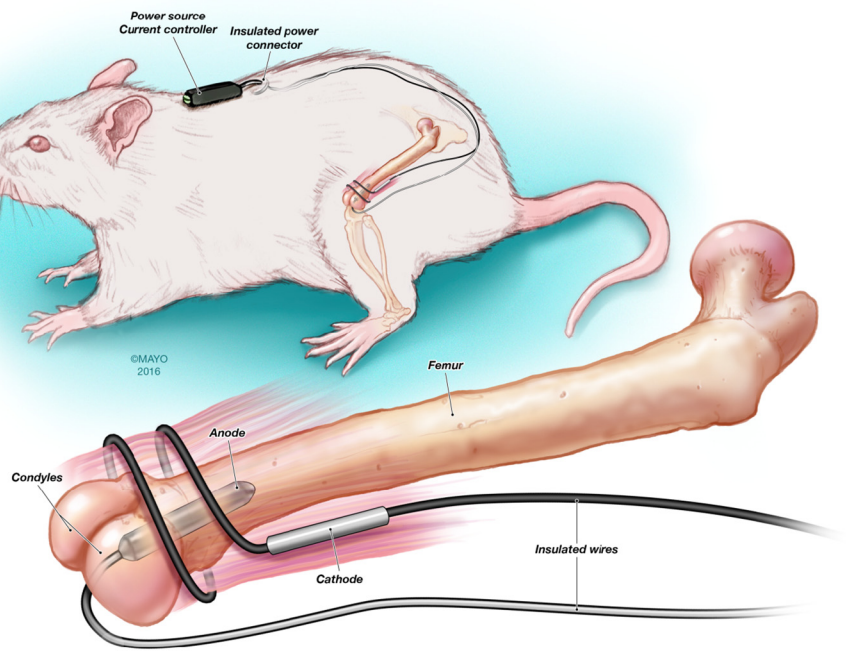

Fig. 1. Graphic illustrating the experimental foreign-body osteomyelitis model [Reproduced with permission from (Schmidt-Malan et al., 2017)].

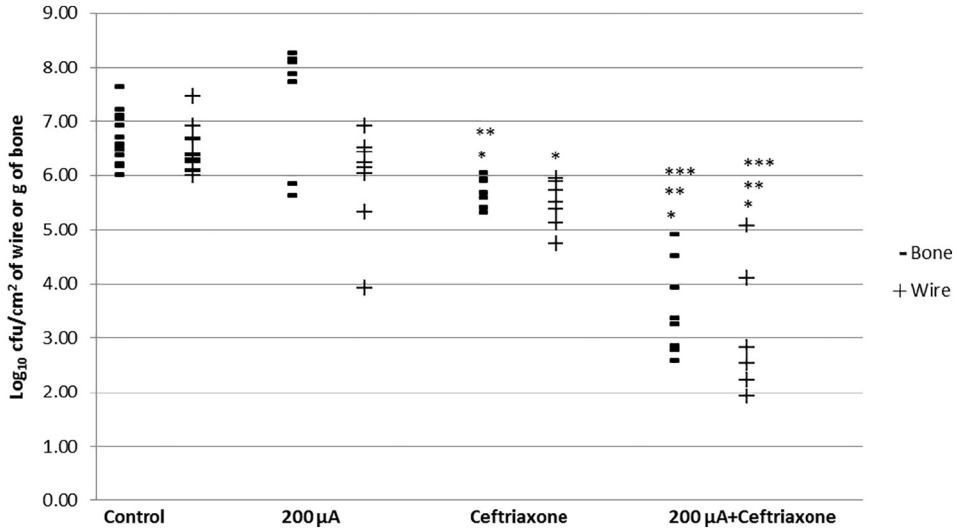

Fig. 2. Treatment of foreign-body osteomyelitis caused by *S. aureus* IDRL-4284. *Denotes significance compared to control, **denotes significance compared to 200 μA alone, ***denotes significance compared to ceftriaxone alone; $P < 0.05$.

The median quantities of bacteria were 6.59 (range 6.01–7.64) $\log_{10}$ cfu/g of bone in the untreated group, 5.70 (range 5.33–6.05) $\log_{10}$ cfu/g of bone in the ceftriaxone group, 8.00 (range 5.64–8.27) $\log_{10}$ cfu/g of bone in the 200 μA fixed DC group, and 3.31 (range 2.58–4.92) $\log_{10}$ cfu/g of bone in the ceftriaxone with 200 μA fixed DC group. The bones of animals treated with ceftriaxone or ceftriaxone with 200 μA fixed DC had statistically significantly fewer bacteria than the bones of untreated animals ($P = 0.0004$ or 0.0002, respectively). There were fewer bacteria in the ceftriaxone with 200 μA fixed DC animals than in the ceftriaxone-alone– and 200 μA fixed DC-alone–treated animals ($P = 0.0012$ and 0.0008, respectively) (Fig. 2).

The untreated group had a median quantity of 6.30 (range 6.00–7.46) $\log_{10}$ cfu/cm^2 on the wires, with 5.52 (range 4.73–5.94) $\log_{10}$ cfu/cm^2 of wire in the ceftriaxone group, 6.18 (range 3.92–6.92) $\log_{10}$ cfu/cm^2 of wire in the 200 μA fixed DC group, and 2.38 (range 1.92–5.07) $\log_{10}$ cfu/cm^2 of wire in the ceftriaxone with 200 μA fixed DC group. Compared to the untreated group, the ceftriaxone-alone and the ceftriaxone with 200 μA groups had statistically significant fewer bacteria on the wires ($P = 0.0003$ and 0.0002, respectively). There were fewer bacteria in the ceftriaxone with 200 μA fixed DC animals than in the ceftriaxone-alone– and 200 μA fixed DC-alone–treated animals ($P = 0.0017$ and 0.0016, respectively) (Fig. 2).

We have presented the results using Wilcoxon rank sum test. The false discovery rate approach is more powerful than methods like the Bonferroni correction that control for multiple comparisons (Glickman et al., 2014). However, after adjusting the *P* values for multiple comparisons using approaches such as false discovery rate and Bonferroni, the findings remain unchanged (i.e., $P < 0.05$).

4. Discussion

In this study, we have shown that, when combined with ceftriaxone, fixed DC is able to significantly reduce the bacterial population in the bones and on the implants of rats infected with *S. aureus* in a femoral model of foreign-body osteomyelitis.

To date, our group and several others have tested low-amperage fixed DC in combination with antibiotics *in vitro* to reduce the bacterial load in biofilms of *Pseudomonas aeruginosa*, *S. aureus*, *S. epidermidis*, and *Klebsiella pneumoniae*. This has been termed the "bioelectric effect," in which it is proposed that electrical current assists the penetration of antibiotics into biofilms by a form of electrophoresis (Costerton et al., 1994; Del Pozo et al., 2009; Stoodley and Lappin-Scott, 1997; Wellman et al., 1996). Costerton et al. showed that amperages as low as 100 μA may be adequate in enhancing the effects of tobramycin against *P. aeruginosa* in a biofilm state, with a 6-log reduction in viable bacteria (Costerton et al., 1994). In a study, similar to the work of Costerton et al., the activity of tobramycin was heightened when in the presence of 1-mA current against *P. aeruginosa* and *K. pneumoniae* biofilms, in which a 6- to 8-log reduction was recognized (Wellman et al., 1996). Another study, by our group, found that the activity of vancomycin against methicillin-resistant *S. aureus* when combined with fixed DC was enhanced compared to vancomycin alone; in addition, we reported enhanced activity of daptomycin and erythromycin when combined with fixed DC against *S. epidermidis* (Del Pozo et al., 2009).

The evidence provided in prior *in vitro* studies, along with that of our current *in vivo* study, gives promise to the possibility of combining electrical strategies with conventional antimicrobial agents for treatment of foreign-body–associated osteomyelitis. Studies are currently under way addressing toxicity/adverse effects of the applied strategy.

A limitation of this study is that only 1 strain of *S. aureus* was tested. Testing a variety of *S. aureus* strains, as well as other PJI-associated species, would allow a better understanding of the scope of use of fixed DC in combination with antimicrobials for the treatment of PJI. A second limitation is that this model does not fully encompass the clinical conditions that are present in human patients, as a platinum electrode served as the implanted material (whereas ceramic constituents, metal alloys, and stainless steel are the most commonly used in humans). A third limitation is that only 1 antimicrobial agent was tested; the combined effect of antimicrobial agent and fixed DC may be further enhanced if fixed DC were combined with an antimicrobial agent with better antibiofilm activity than ceftriaxone. Other metals and amperages should be tested, along with different combinations of antimicrobials, in future studies. When treating with DC alone, there was an increase in the amount of bacteria in bone compared to the controls; however, this was not statistically significant. The standard deviation in the fixed DC alone bone group was higher (SD 1.08) compared to the control bone group (SD 0.47), resulting in a higher median bacterial density in the fixed DC–alone group.

In conclusion, adding fixed DC to conventional antimicrobial agent treatment may aid in the management of foreign-body–associated methicillin-susceptible *S. aureus* infections.

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