The Electricidal Effect Is Active in an Experimental Model of *Staphylococcus epidermidis* Chronic Foreign Body Osteomyelitis[▽]

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Treatment with low-amperage (200 μ A) electrical current was compared to intravenous doxycycline treatment or no treatment in a rabbit model of *Staphylococcus epidermidis* chronic foreign body osteomyelitis to determine if the electricidal effect is active in vivo. A stainless steel implant and 10^4 CFU of planktonic *S. epidermidis* were placed into the medullary cavity of the tibia. Four weeks later, rabbits were assigned to one of three groups with treatment administered for 21 days. The groups included those receiving no treatment ($n = 10$), intravenous doxycycline ($n = 14$; 8 mg/kg of body weight three times per day), and electrical current ($n = 15$; 200 μ A continuous delivery). Following treatment, rabbits were sacrificed and the tibias quantitatively cultured. Bacterial load was significantly reduced in the doxycycline (median, 2.55 [range, 0.50 to 6.13] $\log_{10}$ CFU/g of bone) and electrical-current (median, 1.09 [range, 0.50 to 2.99] $\log_{10}$ CFU/g of bone) groups, compared to the level for the control group (median, 4.16 [range, 3.70 to 5.66] $\log_{10}$ CFU/g of bone) ($P < 0.0001$). Moreover, treatment with electrical current was statistically significantly more efficacious ($P = 0.035$) than doxycycline treatment. The electricidal effect (the bactericidal activity of low-amperage electrical current against bacterial biofilms) is active in vivo in the treatment of experimental *S. epidermidis* chronic foreign body osteomyelitis.

The pathogenesis of foreign body infection, including osteomyelitis, is related to the presence of bacteria in biofilms (7). *Staphylococcus epidermidis* is the leading cause of infections associated with biofilm formation on indwelling medical devices (22). Biofilm-related infections are challenging to treat with conventional antimicrobial agents, and removal of the implant is often necessary to effect a cure (3). Accordingly, a novel therapeutic approach that permits implant retention is desirable.

Although electrical current has been used clinically to treat bone nonunion (1, 8, 9, 17), there is a lack of published in vivo data on the potential therapeutic use of electrical current in foreign-body-related infection. In a recently published in vitro study performed by our group, dose- and time-dependent killing of *S. epidermidis* biofilms was observed after prolonged (i.e., up to 7 days) exposure to low-intensity (i.e., 20, 200 and 2,000 microamperes) direct electrical current, a phenomenon that we labeled the electricidal effect (5). This is distinguished from the bioelectric effect, which is in vitro enhancement by electrical current of the activity of antimicrobial agents against certain bacteria in biofilms (4). If effective in vivo, electrical current might be a potential therapeutic alternative to conventional antimicrobial agents.

To our knowledge, the electricidal effect in experimental chronic foreign body osteomyelitis has not been evaluated. The purpose of this study was to compare the activities of locally

delivered electrical current, intravenous doxycycline treatment, and no treatment in a rabbit model of *S. epidermidis* chronic foreign body osteomyelitis (modified from an in vivo model of chronic osteomyelitis developed in our laboratory [11]) to determine if the in vitro electricidal effect also occurs in vivo. Chronic foreign-body-associated osteomyelitis was chosen for study because it is a biofilm-mediated infection and because there is a human equivalent to which this approach can be extrapolated (i.e., infections associated with orthopedic hardware).

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MATERIALS AND METHODS

Microorganism. *S. epidermidis* Xen 43 (a kind gift from Xenogen Corp., Hopkinton, MA), a bioluminescent strain derived from the parental strain *S. epidermidis* 1457 (a clinical isolate able to form in vivo biofilms), was studied.

Antimicrobial agent. Doxycycline was obtained from Ben Venue Laboratories, Inc. (Bedford, OH). The doxycycline MIC and minimum bactericidal concentration were determined by broth microdilution using an inoculum of 5×10^5 CFU/ml, according to the Clinical and Laboratory Standards Institute guidelines (2).

Pharmacokinetic studies. Doxycycline powder was dissolved in sterile water and administered intravenously at 8 mg/kg of body weight three times daily, a dosage selected to simulate serum concentrations achieved in humans. The doxycycline concentrations in serum were determined for three noninfected rabbits after a single dose and after three doses administered every 8 h. Doxycycline serum levels were also determined after 4 and 21 days of treatment for infected animals treated with doxycycline. Blood was obtained via left marginal ear vein puncture 30 min after doxycycline administration. Serum doxycycline levels were assayed in triplicate using a bioassay on Mueller-Hinton agar seeded with *Bacillus subtilis* as the indicator organism. Paper disks with 80 μ l of serum were placed on the bioassay plates and incubated for 18 h at room temperature. The zone sizes were measured with calipers, and concentrations were calculated

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FIG. 1. Illustration of the experimental chronic foreign body osteomyelitis model. (Copyright, Mayo Foundation for Medical Education and Research; reproduced with permission.)

against a five-point standard curve by linear regression (23). The detection range of the assay was 1 to 16 $\mu\text{g/ml}$. The calculated intra-assay (each specimen) and interassay (day-to-day standard curves) coefficients of variation were 15.2% and 1.9%, respectively.

Experimental rabbit model. The experimental model described was developed and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Mayo Clinic (Rochester, MN). Experimental chronic foreign body osteomyelitis was established in 2.5- to 3.5-kg male New Zealand White rabbits (Fig. 1). The rabbits' lower legs were shaved with an electric clipper, and the skin was disinfected with povidone-iodine, USP, 10% topical solution (prep solution; Novaplus, Novation, Inc., Irving, TX). Surgical anesthesia was induced with ketamine (60 mg/kg) (Ketasat, Fort Dodge, IA), xylazine (6 mg/kg) (Vetrek, Phoenix Scientific Inc., St. Joseph, MO), and acepromazine (1 mg/kg) (Boehringer Ingelheim, St. Joseph, MO) administered intramuscularly. The proximal third of the left tibia was surgically exposed, and a 3-mm hole was bored through the cortical bone. One hundred microliters of a bacterial suspension containing 10^5 CFU/ml of *S. epidermidis* was injected into the intramedullary cavity. A 10-mm (length) by 3-mm (outside diameter) stainless steel electrode was fit into the medullary cavity. The electrode was attached to an insulated power cable that exited the bone via the bone defect. This electrode functioned as the cathode. The bone defect was sealed with viscous bone cement (Simplex P; Stryker, Inc., Mahwah, NJ) that polymerized into a solid plug. The anode was an uninsulated 0.5-mm (outside diameter) by 25-mm (length) stainless steel wire surgically wrapped around the tibia and connected to an insulated power cable. The power cables terminated with an insulated power connector. The power cables were subcutaneously tunneled to the midscapular region and externalized and all the wounds closed.

After four weeks, the electrodes were attached to an external power source/current regulator (battery and electronic circuitry for regulation of the electrical circuit delivered to the electrodes) to continuously deliver 200 microamperes of direct current between the electrodes. The current generators used were designed and fabricated by Mayo Division of Engineering. The electrical-current generator was powered by two 3-V lithium batteries. The electronics in the battery pack sensed impedance in vivo and adjusted voltage output to maintain the current between the electrodes at 200 microamperes. By design, the electronics were self-limited to deliver 200 ± 10 microamperes. On a daily basis,

delivery of the correct amount of electric current was verified using an ammeter. The system was implanted in all animals regardless of whether or not current was delivered. Prior to initiation of therapy, the correct placement of the wires was radiographically confirmed.

Four weeks after infection, treatment was initiated. Rabbits were allocated to one of three study arms (i.e., control, intravenous doxycycline, or electrical current), and treatment was administered for 21 days. Data were collected in 12 runs of one to seven animals each. Untreated control animals were included in each experiment.

At the end of treatment, animals were sacrificed with a lethal dose (100 mg/kg) of intravenous sodium pentobarbital (Sleepaway, Fort Dodge, IA). The tibia was aseptically removed, and the center section, including the intramedullary electrode (Fig. 1), was quantitatively cultured. Quantitative cultures were performed by weighing the specimens and freezing them at -20°C . The frozen bone was pulverized to a fine powder and suspended together with the intramedullary electrode in 2 ml of trypticase soy broth. This was vortexed for 30 s and then placed in an ultrasonic bath (model 750D; Zenith Ultrasonics, Norwood, NJ), exposing it to 40 kHz at 320 mW/cm² for 5 min to remove and disaggregate biofilm bacteria. The broth suspension was serially diluted (10-fold) and 100 μl of each dilution spread on the surfaces of blood agar plates. The plates and broth were incubated in 5% CO₂ at 35°C for 48 h. After incubation, the plates were inspected for growth; the CFU were counted on the plate, yielding 0 to 100 CFU; and then, with the dilution plated on that plate taken into consideration, counts were expressed as log₁₀ numbers of CFU per gram of bone. If the quantitative culture result was negative, the qualitative broth culture of the pulverized bone homogenate was checked for growth. For statistical purposes, if the qualitative culture was positive and the quantitative culture was negative, the result was expressed as 0.5 log₁₀ CFU per gram of bone. If the qualitative culture was negative, the result was expressed as 0.45 log₁₀ CFU per gram of bone. Four rabbits, lost due to tibial fracture during the experiment (two each from the control and doxycycline groups), were excluded from analysis. Nine separate rabbits were sacrificed 4 weeks following infection to determine baseline quantities of bacteria in the tibia at the time treatment was initiated.

Emergence of resistance studies. The MIC of *S. epidermidis* recovered from tissues after doxycycline treatment was determined.

TABLE 1. Intravenous doxycycline pharmacokinetics

Doxycycline dose	Concn in serum ($\mu\text{g/ml}$) at:				
	0.5 h	1 h	2 h	4 h	8 h
8 mg/kg (1 dose) ^a	8.4 $\pm$ 2	3.8 $\pm$ 0.94	2.6 $\pm$ 0.31	1.5 $\pm$ 0.06	<1
8 mg/kg/8 h					
3 doses ^a	9.9 $\pm$ 0.42	4.1 $\pm$ 0.34	3 $\pm$ 0.61	1.7 $\pm$ 0.24	<1
4 days ^b	10.5 $\pm$ 0.85				
21 days ^c	8.6 $\pm$ 2.03				

^a Mean concentration in serum samples from three healthy rabbits.^b Mean concentration in serum samples from five infected rabbits.^c Mean concentration in serum samples from 12 infected rabbits.

Statistical methods. Descriptive statistics for *S. epidermidis* log₁₀ numbers of CFU per gram of bone were summarized as median (minimum to maximum). Differences in median log₁₀ numbers of CFU of *S. epidermidis* per gram of bone among the three groups were compared using the Kruskal-Wallis test. The Wilcoxon rank sum test was used for comparison of median log₁₀ numbers of CFU of *S. epidermidis* per gram of bone between the two treatment groups. All tests were two sided; *P* values of <0.05 were considered statistically significant. Analysis was performed using SAS software (version 9; SAS Institute, Inc., Cary, NC).

RESULTS

The *S. epidermidis* MIC and minimum bactericidal concentration for doxycycline were ≤ 0.125 and 64 $\mu\text{g/ml}$, respectively. The doxycycline serum concentrations are shown in Table 1.

Four weeks after the animals were challenged with *S. epidermidis*, the median count (minimum to maximum) of *S. epidermidis* was 5.44 (4.06 to 6.16) log₁₀ CFU per gram of bone in the nine animals in which this was studied.

Results of quantitative cultures of bone observed after treatment are summarized in Fig. 2. *S. epidermidis* was cultured from all harvested bones in the control group at a median of 4.16 (range, 3.70 to 5.66) log₁₀ CFU/gram of bone. After treatment, the median counts of *S. epidermidis* per gram of bone were 2.55 (range, 0.50 to 6.13) log₁₀ CFU/gram of bone in the doxycycline group and 1.09 (range, 0.50 to 2.99) log₁₀ CFU/gram of bone in the electrical-current group. Differences in colony counts from control rabbits were significant in the doxycycline- and electrical-current-treated groups (*P* < 0.0001). The colony counts for the electrical-current-treated rabbits were significantly lower than those for the doxycycline-treated rabbits (*P* = 0.035).

Emergence of resistance (doxycycline MIC after treatment, $\geq 16 \mu\text{g/ml}$) was detected in isolates from five animals (35.7%), including the two animals with the highest bone counts of *S. epidermidis*. A MIC of 0.5 $\mu\text{g/ml}$ was detected in isolates from two animals.

DISCUSSION

Herein, we demonstrated that local delivery of electrical current, without concomitant antimicrobial therapy, is an effective treatment for experimental *S. epidermidis* chronic foreign body osteomyelitis, based on the results for quantitative cultures. The amount of direct current studied (i.e., 200 μA) appeared to be well tolerated by the animals studied. Formal study of toxicity was, however, outside the scope of this study. We did observe some discoloration of the bones exposed to

electrical current. We did not perform histopathological studies to detect morphological changes or perform strength testing to detect functional changes in the exposed bones.

We selected doxycycline as a comparator on the basis of the following rationale. Our initial plan had been to determine whether the bioelectric effect was active in vivo. Because we had observed a more prominent in vitro bioelectric effect with tetracycline plus electrical current than we had with more-conventional antistaphylococcal agents plus electrical current in prior short-term studies, we planned our studies with doxycycline (4). However, during the course of our precedent in vitro experiments, we also showed that prolonged electrical current alone substantially reduces in vitro biofilms (5). Then, in this study, we observed sterile cultures in 7 of 15 animals treated with electrical current alone, in our opinion obviating the study of doxycycline combined with electric current by using the parameters studied for each of these alone, since there would be limited statistical power for detection of results of treatment more efficacious than electrical current alone. In future studies, it will be interesting to examine different doses, dosing intervals, and durations of electrical current and delivery of lesser regimens of electrical current with antimicrobial agents.

We treated the animals for 21 days because previous studies performed in our laboratory had demonstrated that this duration is optimal in an analogous model of non-foreign-body-associated osteomyelitis (11). We administered doxycycline parenterally since preliminary experiments showed that administration of doxycycline in drinking water was associated with low to nondetectable levels of drug in serum, even at high dosage (i.e., 150 mg/kg), as has been described for tetracycline (12). In humans, the mean serum doxycycline concentration after 3 months of therapy for Q fever endocarditis was $3.02 \pm 1.89 \text{ mg/ml}$ (range, 0.06 to 8.5 mg/ml) (14). The serum doxycycline concentrations in our model were slightly higher (i.e., $8.58 \pm 2.03 \text{ g/ml}$ after 21 days of treatment).

As indicated by Vuong et al. (21), Xen 43 is suitable tool for

FIG. 2. Results for quantitative cultures of bone after treatment; the log₁₀ numbers of CFU per gram of bone in the control, doxycycline, and electrical-current groups are shown (the median and interquartile range are shown for every group). Bacterial growth was significantly reduced in the doxycycline and electrical-current groups in comparison to the level for the control group (*P* < 0.0001); statistically significant differences were detected between the doxycycline and electrical-current groups (*P* = 0.035).

studying *S. epidermidis* device-associated infections in vivo using biophotonic imaging. Since the metabolic activity of viable cells can be detected in a noninvasive manner, this method is especially appealing for the study of chronic biofilm infections and drug efficacy in vivo. Unfortunately, in preliminary experiments, we found a low intensity of bioluminescent signal in the infected animals, rendering the use of in vivo biophotonic imaging unfeasible in this study. This is likely related to the low inoculum ($\sim 5 \log_{10}$ CFU per gram of bone) at the infection site.

In vitro experiments have demonstrated that *Staphylococcus* species adhering to stainless steel in a biofilm mode of growth detach with the application of low direct electrical currents (25 to 125 μ A) (20). Poortinga et al. (13) demonstrated that it is possible to stimulate *Streptococcus oralis* detachment from conducting indium tin oxide by applying electrical currents of 10 μ A/cm². Anodic and cathodic surfaces were almost entirely cleaned, even in the presence of an adsorbed conditioning film. van der Borden et al. (19) showed, in an experimental caprine model, that *S. epidermidis* infection of percutaneous pin sites of external fixators in reconstructive bone surgery could be prevented by the application of a 100- μ A direct electric current.

An obvious potential human application of the electricidal effect would be in the management of infections associated with orthopedic hardware. This would be a potential strategy for overcoming the reduced susceptibility of biofilm microorganisms to conventional antimicrobial agents. The mechanism of the antibacterial activity of electrical current is unknown; however, this activity has been suggested to result from toxic substances produced as a result of electrolysis (e.g., H₂O₂, oxidizing radicals, and chlorine molecules) (10, 18), oxidation of enzymes and coenzymes, membrane damage leading to leakage of essential cytoplasmic constituents (15), and/or altered bacterial respiratory rate (6). In one study (13), an ionic strength-dependent transfer of electrons during the initial bacterial adhesion mechanism that had to be reversed in order for detachment to occur was proposed.

Although the mechanism of the electricidal effect remains unclear, it is a potentially attractive therapeutic option for foreign body osteomyelitis in humans. However, this approach needs more studies using experimental models before translation into clinical practice. There are potential advantages in comparison with antimicrobial treatments, including lack of selection of resistance to conventional antimicrobial agents. We observed emergence of resistance to doxycycline in approximately one-third of doxycycline-treated animals. Bacteria may become resistant to tetracycline by limiting access of tetracycline to ribosomes; by altering ribosomes, thereby preventing tetracycline binding; or by producing tetracycline-inactivating enzymes; all three types of resistance have been described to occur in clinical isolates (16). We did not study the resistance mechanism present in the isolates in this study; whether a combination of electrical-current treatment and antimicrobial therapy would prevent emergence of resistance is not known.

We conclude that direct electric current (200 μ A) significantly reduces the numbers of viable bacteria in a staphylococcal experimental model of chronic foreign body osteomyelitis. The efficacy was higher than that of intravenous doxycycline.

The in vivo efficacy of lower current intensities and shorter treatment lengths; the efficacy against other genera, species, and strains of microorganisms; and the role of the electricidal effect in infection prevention deserve further evaluation. Finally, noninvasive approaches to the delivery of electrical current deserve further study.

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R. Patel has an unlicensed U.S. patent pending for a method and an apparatus for sonication; she has, however, foregone her right to receive royalties in the event that the patent is licensed.

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