Antibacterial effects of electrically activated vertebral implants

Kutsal Devrim Secinti a, Murat Ayten a, Gokmen Kahilogullari a,*, Gulsah Kaygusuz b, Hasan Caglar Ugur a, Ayhan Attar a

a Department of Neurosurgery, Ankara University School of Medicine, Ankara, Turkey
b Department of Pathology, Ankara University School of Medicine, Ankara, Turkey

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Abstract

Bio-implants in the human body act as passive surfaces that are prone to bacterial adhesion and may cause deep body infections. Pedicle screws made of titanium (Ti) alloy and their silver-coated forms (Ag-Ti) were used both in vitro and in vivo to determine whether silver-coated materials are antimicrobial when they are anodized. Twenty-four New Zealand Albino rabbits were divided into four groups with six in each. In Group 1, the rabbits were exposed to 8 μA direct current (DC) via silver-coated screws. In Group 2, the rabbits were not exposed to any electrical current, but silver-coated screws were used. In Group 3, the rabbits were exposed to 8 μA DC by the use of Ti alloy screws. In Group 4, the rabbits were not exposed to any electrical current, but Ti alloy screws were used. Staphylococcus aureus (10^6 colony-forming units) was inoculated into the rabbits before any electrical current was used. All the animals were sacrificed, and screw areas were histologically and microbiologically examined. Silver-coated titanium screws prevented implant-associated deep bone infections when they were polarized anodically. Antibacterial effects of the same kind of screws with the same bacterium were confirmed through in vitro test. When the screws were anodized with the same electrical parameters in vitro, a marked inhibition zone was detected around the silver-coated screws on agar plates but not around the traditional Ti alloy screws. Our findings suggested that silver-coated titanium implants can be used to prevent implant-associated deep bone infections when they are polarized anodically.

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Keywords: Electric current; Silver electrode; Pedicle screw; Antiseptic bone screw; Animal model

1. Introduction

Silver ions have been known to be antibacterial since the time of Avicenna. However, there is no evidence to date that silver has an essential function in plant and animal metabolism.1

The inhibition of bacterial growth by the use of a weak electrical current has been reported.2–7 If a pure (>99.9%) silver wire is connected to a weak direct current (0.1–10 micro ampere) in a semi-solid culture medium, a bacteria-free inhibition zone appears near the anode after an incubation period, but not near the cathode. This antibacterial effect is persistent even after the electric current is cut off and sustains for at least 3 weeks.8 This procedure is called silver anode treatment. In the relevant literature, electrically induced silver ions have been shown to be antifungal, antibacterial, antiviral, non-allergenic and even nontoxic for mammalian cells.6,9

Inhibitory concentrations of electrically induced silver ions are approximately 100 times lower than they are for silver sulfadiazine.6 This shows that electrical polarization provides extensive bactericidal specificity to silver. No other metal provides antimicrobial effect as much as silver does when it is anodized. The specificity of silver suggests that electricity is not a unique key factor for silver anode antisepsis. The mechanism of anodic silver antisepsis originates from either free silver ions scattering from the anode surface to the medium or from electricity. It is well known that this is not silver iontophoresis.6

* Corresponding author. Present address: Ankara Universitesi Ibni Sina Hastanesi, Beyin ve Sinir Cerrahisi Anabilim Dalı, 06100 Sihhiye-Ankara-Turkey. Tel.: +90 312 3103333 2598; fax: +90 312 3094340.
E-mail address: gokmenkahil@hotmail.com (G. Kahilogullari).

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2. Material and methods

2.1. Screws

Ninety-six standard titanium (Ti) alloy bone screws (3 mm diameter, 20 mm length) were commercially provided (Trimed, M 36340, Istanbul, Turkey). The surface area of the inserted part of each screw was estimated to be 1.84 cm².

Forty-eight of 96 screws were coated with silver by using an electroplating method (LPW Ag, Spiegelglanz® H137 T, LPW Chemie GmbH, Neuss, Germany). The thickness of the silver layer of the coated (Ag-Ti) screws was calculated as 5–8 μm. The enhancement in the surface area by silver covering of screws remained negligible. Durability of the screws for insertion was tested by inserting some of the screws into previously used in fresh frozen cadaveric sheep vertebrae. The same screws were then tested in agar media in vitro, as described below, and it was confirmed that they could withstand insertion.

2.2. Bacteria

Coagulase positive Staphylococcus aureus was provided from the stock solutions of Department of Infectious Diseases of Ankara University, School of Medicine, Ankara, Turkey. The bacteria were kept in a 5-mL broth medium at 4 °C. Freshly cultured bacterial cells obtained from stock solutions were used during the experiment.

2.3. Animals

Twenty-four New Zealand rabbits (10 weeks old; weight range: 2000–2500 g; mean weight: 2250 g) were randomly divided into four groups of six rabbits (Table 1). The study was approved by the ethics committee of Ankara University in accordance with the Helsinki Declaration of Animal Rights.

Group 1 was exposed to 8 μA direct current (DC) via silver-coated screws. Group 2 was not exposed to any electrical current, but silver-coated screws were used in this group. Group 3 was exposed to 8 μA DC via titanium alloy screws. Group 4 was not exposed to any electrical current, but titanium alloy screws were used in that group.

The animals were anesthetized with commercially provided ketamin-hydrochloride (Pfizer, New York, USA) and 2% xilazin-hydrochloride (Bayer, Monheim, Germany). The animals’ lower backs were shaved and surgically scrubbed. Then, a 4-cm skin incision was made on the midline starting from the level of the iliac crest. After the bilateral exposure of the iliac bones, two burr holes (diameter: 2.0 mm) were opened on each iliac bone with appropriate drills (Aesculap, FF055R, FF068R, Tuttlingen, Germany). The distance between two successive holes was adjusted to be approximately 15 mm. Each hole was irrigated with 3 mL of sterile saline. Cotton balls embedded into 0.1 mL of bacterial solution (10⁶ CFU) were inserted into each hole, and then removed. All the screws were tightly inserted into the holes. Well-insulated (Teflon-coated) titanium alloy wires were soldered to the tip of each screw. After the operation area was closed with surgical sutures, the skin was covered with sterile dressings moistened with 4 mL of benzalconiumhydrochloride (0.3% w/v). The same researcher operated upon all the animals in order to standardize screw placement.

Plain x-rays of all the animals were obtained immediately after the procedure to determine the positions of the screws (Fig. 1). The free endings of wires were kept outside; all the screws represented one electrode (anode). The upper back skin of the animals was shaved, and a pure silver wire (0.5 mm) was provided.

Table 1

<table>
<thead>
<tr>
<th>Animals</th>
<th>Screws</th>
<th>Actual charge (μA)</th>
<th>Exposure time (min)</th>
<th>Total charge delivered (Coulomb)</th>
<th>Charge density (C/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ag-Ti</td>
<td>Ti</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Group (n = 6)</td>
<td>24</td>
<td>0 24 48</td>
<td>8 360</td>
<td>0.17 0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>2. Group (n = 6)</td>
<td>24</td>
<td>0 24 48</td>
<td>0 0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>3. Group (n = 6)</td>
<td>0 24 48</td>
<td>8 360</td>
<td>0.17 0.015</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>4. Group (n = 6)</td>
<td>0 24 48</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>Total: 24</td>
<td>48 48 48</td>
<td>360</td>
<td>0.17 0.015</td>
<td>0.015</td>
<td></td>
</tr>
</tbody>
</table>

Only Groups 1 and 2 animals were exposed to electric current (2 h/day). Ti = titanium, Ag-Ti = silver-coated titanium.

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Bone was divided into two pieces. Eventually, each bone specimen was immediately fixed in 10% neutral buffered formalin and decalcified in 20% formic acid. Paraffin-embedded 5-μm serial tissue sections were stained with haematoxylin-eosin and examined under a light microscope (Courtesy, Zeiss, Oberkochen, Germany). Histological evaluation was performed by two double-blinded pathologists (Fig. 2a–d). The degree of inflammation was semiquantitatively scored as none, weak, moderate and severe, depending on the intensity of the neutrophile leukocytic infiltration, the presence and extent of the necrosis, and devitalized bone tissue. Bone marrow cellularity, bone tissue inflammation, presence of devitalized bone, necrotic changes in the bone marrow, new bone formation, and inflammatory cell counts were recorded for each screw.

2.3. In vitro confirmation

Silver anode application procedure was performed in vitro. Sterilized Ag-Ti electrodes and Ti electrodes were embedded into four Petri dishes (1th and 2nd Petri dishes had a pair of silver-coated Ti alloy electrodes; 3th and 4th, a pair of uncoated titanium alloy electrodes). After electrical connections were established, 5 mL of 1.3 × 10^6 CFU S. aureus-containing solution was poured into 50 mL of cooling agar media. The temperature of the agar was 40 °C. Then, the agar was vortexed and poured into the Petri dishes, as was previously described by Spadaro et al. In this experiment, the same bacteria and same kind of materials (as electrodes) were used to confirm in vitro anodic silver antisepsis. The electrodes were polarized applying the same electrical regimen (8 μA for 2 h/day for 3 days) by the use of the same in vivo apparatus. Agar impedance was 16 K ohms (±1.8 K ohms); total charge was 1.2 Coulomb. The distance between the electrodes was 4 cm. Finally, inhibition zone radius was measured, and Petri dishes were photographed. The data were analyzed statistically by the use of Kruskal-Wallis variant analysis test. The comparisons of the parameters for each group were performed using multiple comparison test. \( P < 0.05 \) was considered statistically significant.

3. Results

3.1. In vivo experiment

The surgical procedure was well-tolerated by all the animals. Tissue impedance between inner surface of iliac bone and shaved skin was 75 K ohms (SD: ±8.4 K ohms; median: 74.4 K ohms, \( n = 24 \)). Current levels tended to decline with time in each session.
Multifocal exudate accumulation and bone marrow cellularity (hypercellular or normocellular) are the early signals of inflammation. Such microscopic findings were interpreted in favor of severe inflammation. The count of inflammatory cells (such as lymphocytes and macrophages) in each microscope area was recorded.

The mean bacterial count of Group 1 was 2.4 CFUs. The mean inflammatory cell count was 3.0, and the bone marrow cellularity was normal.

The mean bacterial count of Group 2 was 76.0 CFUs. The mean inflammatory cell count was 3.0. The bone marrow cellularity was normal, and there was minimal bone tissue inflammation.

The mean bacterial count of Group 3 was 161.5 CFUs. The mean inflammatory cell count was 10.29. Significantly increased bone marrow cellularity and severe bone tissue inflammation were observed. Necrotic changes in the bone marrow were noticed in two screws.

The mean bacterial count of Group 4 was 248.2 CFUs, and the mean inflammatory cell count was 13.0. There was significantly increased bone marrow cellularity, and severe bone tissue inflammation and exudate formation were observed.

There were no devitalized bone tissue, necrotic changes in bone marrow, or new bone formation in any of the groups.

By using multiple comparison tests, the results of each group were compared with the results of the other groups for bacterial count (as CFUs) and inflammatory cell count (those with bacterial count over 300 were considered to have a count of 300).

The comparison of Group 1 with Group 2 for inflammatory cell count showed no difference between the mean inflammatory counts of the two groups. However, in the paired comparisons of all the other groups, including Groups 1 and 2, significant differences were detected between the mean inflammatory counts of the two groups.

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Statistical significance</th>
<th>Interpretation</th>
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</thead>
<tbody>
<tr>
<td>1–2</td>
<td>$p &gt; 0.05$</td>
<td>No difference</td>
</tr>
<tr>
<td>1–3</td>
<td>$p &lt; 0.001$</td>
<td>Significant difference</td>
</tr>
<tr>
<td>1–4</td>
<td>$p &lt; 0.001$</td>
<td>Significant difference</td>
</tr>
<tr>
<td>2–3</td>
<td>$p &lt; 0.001$</td>
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</tr>
<tr>
<td>2–4</td>
<td>$p &lt; 0.001$</td>
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</tr>
<tr>
<td>3–4</td>
<td>$p &lt; 0.001$</td>
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Fig. 2. (a) Mild smooth tissue inflammation around the silver anode (Group 1). (b) Mild inflammatory reaction and fibrosis in the soft tissue around silver electrode, sham group (Group 2). (c) Severe inflammation characterized by the presence of multifocal neutrophilic micro-abscesses around titanium anode (Group 3). (d) Severe acute inflammation and devitalized bone tissue around the titanium electrode, sham group (Group 4) (H&E ×400).

Table 2
Paired comparisons of all groups for inflammatory cell count

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between the inflammatory cell counts of the groups compared (Table 2). The paired comparisons of all the groups for bacterial count revealed that the bacterial count of Group 4 was the highest, while it was the lowest in Group 1. There were significant differences between the mean bacterial counts of all the groups compared (Table 3).

3.2. In vitro experiment

On the Petri dishes, Ag-Ti anodes (screws or other parts of traditionally used stabilization systems) showed 23 mm of inhibition zone, but not the uncoated ones (Fig. 3a–b). Microbiologic samples obtained from both inhibition zones did not show bacterial growth. No bacterial growth was detected beneath Ti electrodes and even on the electrode surface.

4. Discussion

The idea to insert silver electrodes into living tissue is not new in orthopedics and dentistry. Becker et al.12 treated infected nonunions in orthopedics with silver anodes. Similarly, Aydın et al.3 treated infected teeth with inserted silver anodes. In such cases, actual current passes through the tissue and total charge amount is based on tissue changes. In the literature, threshold of electricity for living eucariotic cells was described as 20 μA for actual current and 2 Coulombs/day for total charge.2 In this study, these electrical parameters were maintained to avoid irreversible tissue damage. However, 6 peri-implant tissues developed severe inflammation due to the electrical current used. On the other hand, the same electrical parameters caused lesser inflammation with silver anode use, which shows that silver ions emitted from the anode surface are more easily tolerated than are Ti ions. When inflammatory cell counts of Groups 1 (Ag-Ti) and 3 (Ti) were compared, anodized silver caused less inflammation in living tissue. In the control groups (Group 2 vs. 4), Ag-Ti screws showed less inflammation; however, Ti screws showed moderate inflammation. Darouiche reported that no differences were seen among the silver-coated and uncoated metal and other implants, such as silver-impregnated silicon catheters or silver-coated external fixation pins etc.14 However, in our study, the microbiological analysis of sham screws (Groups 2 and 4) showed significant differences.

Our microbiological results were in favor of silver material. Silver is known to be antibacterial (oligodynamic effect). When it is anodized, silver ions emitted from the metal surface deeply penetrate into the bone tissue. This may be the reason for less bacterial growth in peri-implant tissue of silver-coated screws. In this study, we did not measure silver ion concentration in deep bone tissue; however, the literature shows that silver ions are capable of penetrating into bone structures as deep as 1 cm12 or 1.38 mm in dentinal tissues.3 Penetration depth of silver is correlated with electric current given, while antibacterial effect of silver anode is mostly independent of electricity. In the liquid medium, even 1 μA was sufficient for bacterial inhibition of silver anode.9 If the electric current had directly influenced the bacteria, we would have detected less bacterial growth when anodes were charged with higher electric current. However, there was significant microbicidal difference between silver and titanium anodes.

It is well known that bacterial biofilm formation on the surface of implanted metal is one of the major clinical problems.15,16 Bio-implants in the human body act as passive surfaces that are prone to bacterial adhesion and thus,

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Fig. 3. (a) Silver-coated titanium anode and cathode; note the inhibition zone near the anode but not near the cathode. (b) Uncoated titanium anode; note the lack of any inhibition zone around the electrodes.
may cause implant-associated infections (peri-implantitis). Antibiotic treatment of infected implants is not fully successful because biofilm architecture protects the adhering organisms. Minimal inhibitory concentration of the antibiotics to inhibit free-floating bacteria has been reported to be approximately 50 or 500 times lower than required for bacteria in a biofilm. Such infections that are associated with foreign materials are expensive to manage. For example, findings from the Dutch Trauma Trial indicated a mean cost of SUS22,000 (in 1991) to treat a patient who developed a deep tissue infection after surgical fixation of a closed fracture. Postoperative surgical site infections remain a major source of illness but a less frequent cause of death in the surgical patient. These infections number approximately 500,000/year, among an estimated 27 million surgical procedures, and account for approximately one-quarter of the estimated 2 million nosocomial infections in the US each year. Infections result in longer hospitalization and higher costs. However, major complications such as deep body infections continue to have a grave impact, increasing the duration of hospitalization as much as 20-fold and the cost of hospitalization five-fold.

Biofilms may cause antibiotic resistance by harboring pathogen micro-organisms. Electricity has been used to easily remove biofilms from medical surfaces. Pootringa et al. have stated that it is possible to stimulate bacterial detachment from conducting indium tin oxide-coated glass by using 10 μA/cm² electrical current. Similarly, Borden et al. have reported electric current-induced detachment of *S. epidermidis* from surgical implants. A 100-μA DC yielded 78% detachment, whereas a 100-μA block current under the same experimental conditions yielded only 31% detachment. The same trend was found for 60 μA, with 37% detachment for a DC and 24% detachment for a block current. These results suggest that DCs are preferred in preventing infections. By a moderate speculation, in our study, external electrical intervention to screws contributed to the removal of bacterial biofilm from the metal surface. On the other hand, Aydin et al. described antibiotic sensitivity for bacteria after they were exposed to silver anode. When Ag-Ti screws are anodized, antibiotics are expected to be more effective. This property may be useful in prevention and management of implant-based spinal infections or may make traditional antibiotic therapy possible.

One question remains as to how long antibacterial effect of polarized silver is sustained on peri-implant tissue. It is interesting that once silver is anodized in culture medium (1 μA for 1 h), no bacteria can grow within at least 3 weeks despite frequently washed culture medium and cessation of electrical current. In our opinion, anodic silver ions may remain longer than 3 weeks in trabecular bony structures, providing a clinical advantage.

In conclusion, our results obtained with electrically activated Ag-Ti implants strongly encourage insertion into any infected nonunion vertebra but with use of less electricity. Judging by silver cathodes having osteoblastic activity, inserted Ag-Ti screws should be anodized for a while. Then, it should be cathodized throughout the treatment. We suggest the use of silver-coated titanium to prevent implant-associated deep bone infections when they are polarized anodically.

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