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Efficacy of bioabsorbable antibiotic containing bone screw in the prevention of biomaterial-related infection due to *Staphylococcus aureus*

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Abstract

Impregnation of antimicrobial agents within biodegradable orthopedic implants provides a possibility for local antimicrobial prophylaxis of biomaterial-related infections. The objective of this study was to evaluate the efficacy of a bioabsorbable ciprofloxacin containing bone screw (Ab-PLGA) in the prevention of biomaterial-related infection due to *Staphylococcus aureus* in a rabbit model. Animals in Group I (n = 8) received a Ab-PLGA screw contaminated with *S. aureus*, while animals in Group II (n = 8) received a stainless steel (SS) screw contaminated with *S. aureus*. In two negative control groups, the animals received a Ab-PLGA screw (Group III, n = 4) or a SS screw (Group IV, n = 4) without bacterial contamination. ^{18}F-FDG-PET imaging, performed at 6 weeks, was applied as a novel quantitative in vivo imaging modality of implant-related infection. Infection was verified by swab cultures, direct cultures of the retrieved implant, and quantitative cultures of pulverized bone. The concentrations of ciprofloxacin in serum and local bone tissue were determined by a high performance liquid chromatographic (HPLC) method with fluorescence (FLD) detection. In the group of contaminated Ab-PLGA screws, all cultures were negative. In the group of contaminated SS screws, all cultures of retrieved implants and six cultures out of eight of pulverized bone were positive for inoculated *S. aureus*. In negative control groups, all cultures were negative except one contaminant (*S. cohnii*) found in a SS screw culture. Verified infection of contaminated SS screws was collaborated by the increased ^{18}F-FDG-PET uptake (P = 0.004 compared with the group of contaminated Ab-PLGA screws). The mean bone tissue concentration of ciprofloxacin varied from 2.54 to 0.83 μg/g bone as a function of distance from the implantation site. The serum concentration of ciprofloxacin remained undetectable and below the resolution of the analytic method (<5.0 ng/ml). This study confirmed the in vivo efficacy of bioabsorbable antibiotic containing bone screw in the prevention of biomaterial-related infection due to *S. aureus*.

Keywords: Osteomyelitis; Implant-related infection; *Staphylococcus aureus*; Bioabsorbable; Ciprofloxacin

Introduction

Treatment of biomaterial-related infections remains one of the greatest challenges both in trauma and elective orthopedic surgery [1,2]. Antimicrobials alone do not eradicate pathogens attached on implant surfaces, and such infections often persist until the implant is removed [3,4]. This emphasizes the paramount importance of infection prevention [4].

There is a high demand for novel prevention techniques for biomaterial-related infections. Although the administration of systemic prophylactic antibiotics has been found to be the most important single factor in prevention of implant infections [5,6], it has been associated with the increasing prevalence of resistant strains such as MRSA [7,8]. Therefore, alternative prophylactic strategies must be explored. There is a belief that use of local antimicrobial prophylaxis may carry a less risk of inducing resistant strains than the

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current practice of routine systemic administration of antibiotics for 24–48 h after implant surgery.

Local antimicrobial prophylaxis is based on the controlled release of the antibiotic aimed to achieve adequate local bactericidal tissue concentrations for a prolonged time without significant systemic exposure [9]. In metal implants, one option is to use thin biodegradable polymer coatings as a local drug delivery system [10–12]. The use of biodegradable orthopedic implants provides a possibility for direct impregnation of antimicrobial agents within the polymer matrix of the implant.

The current study was designed to evaluate the efficacy of a bioabsorbable antibiotic containing bone screw in the prevention of biomaterial-related infection due to *Staphylococcus aureus*. Positron emission tomography using ^{18}F-fluorodeoxyglucose (^{18}F-FDG-PET) was applied as a noninvasive method for imaging of biomaterial-related infection.

Materials and methods

Implants

Bioabsorbable antibiotic-containing bone screws (Ab-PLGA) were manufactured from self-reinforced (SR) ciprofloxacin containing poly(lactide-co-glycolide) 80:20 (PLGA) (PuraSorb®PLG, Purac Biochem bv., Gorinchem, Netherlands) rods. The selection of the antibiotic content was based on detailed in vitro studies, which had delineated the release characteristics of the antibiotic from screws with different ciprofloxacin contents. In the screws selected for this study, the measured initial ciprofloxacin content was 6.6 ± 0.2 wt%. In in vitro immersion testing of these screws, after the initial burst, the sustained release of ciprofloxacin reached maximum level (15.87 μg/ml/day) by 6 weeks. Subsequently, the release decreased to the level of 4.25 μg/ml/day by 12 weeks. Expressed as cumulative drug release, the amount of ciprofloxacin release was 41% by 6 weeks and 86% by 12 weeks. The shear strength of the screw was 100 ± 10 MPa. The shear strength of the screws decreased as a function of in vitro immersion time in phosphate buffer solution. The shear strength of screws was decreased 57% by 6 weeks and 82% by 12 weeks. The results were based on testing five parallel samples at each time point. Shear strength of the screw was determined using standard method ASTM B 790-87 with modified tool and in vitro studies were carried out according to ISO 15814 (1999).

The geometry of the Ab-PLGA screws was same as that of commercially available SmartScrew® 2.7 screws (Linvatec Biomaterials, Tampere, Finland). Outer diameter and length of the screws were 2.7 and 24 mm, respectively. The pitch of the screws was 1.0 mm. Standard stainless steel (SS) cortical screws (2.7-mm diameter, 14.0-mm length, 1.0-mm pitch, Synthes®, STRATEC Medical, Oberdorf, Switzerland) were used as control implants.

Animals

Twenty-four adult male New Zealand White rabbits (Harlan, Netherlands) weighting mean 3530 g (range 3045–4225 g) were used in the current study. Before surgery, rabbits were acclimated to their new environment and fed a standard laboratory diet. The animals were caged individually with a constant temperature. The Ethical Committee of the University of Turku and the Provincial State Office of Western Finland approved the study protocol. All experiments were carried out in accordance with the guidelines of the local Animal Welfare Committee.

Study protocol

One bicortical bone screw, a resorbable Ab-PLGA screw, or a SS screw (Fig. 1) was surgically implanted into the right proximal tibia with or without *S. aureus* contamination in each animal. Animals in Group I (n = 8) received ciprofloxacin containing Ab-PLGA screw contaminated with *S. aureus*. Animals in Group II (n = 8) received a SS screw contaminated with *S. aureus*. In the two negative control groups, animals received a Ab-PLGA screw (Group III, n = 4) or a SS screw (Group IV, n = 4) without bacterial contamination. In Groups III and IV, both administration of prophylactic systemic antibiotics and wound irrigation with saline containing an antibiotic were employed in order to simulate the current clinical practice of infection prophylaxis in implant surgery. In Groups I and II, no systemic or local antimicrobial prophylactic measures were used. Six weeks after surgery, detailed studies were performed to delineate the rate of implant-related infections in each group of animals and to verify the release of ciprofloxacin from Ab-PLGA screws in vivo.

Preparation of bacterial suspension

S. aureus (strain 52/52A/80, provided by Dr. Jon T. Mader) was used as pathogen. The strain was cultured overnight on blood agar plate. Bacterial cells were suspended in sterile saline until the final optical density of 0.18 was achieved by measuring the absorbance at 600 nm using spectrophotometer (Smart Spec™ 3000, Bio-Rad, USA). A 10-fold dilution series was prepared in sterile saline and the 1:10,000 dilution, corresponding to 3×10^4 colony-forming units (CFU)/ml of *S. aureus*, was used for colonization of the implanted screws. The selection of the bacterial dose was based on our recent experience in induction of experimental osteomyelitis in the rabbit tibia using the same bacterial strain [13]. The bacterial suspension was stored at 4°C and used on the day of preparation. In order to verify the actual number of bacteria in the

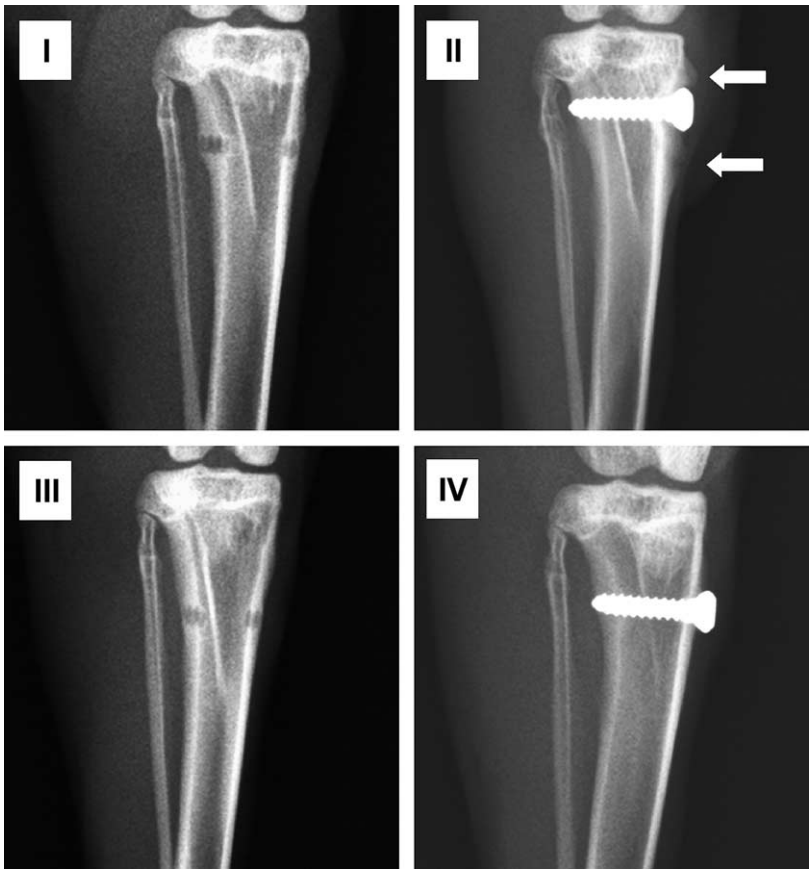

Fig. 1. Plain radiographs of bioabsorbable (Groups I and III) and stainless steel (Groups II and IV) bone screws in the rabbit tibia at 6 weeks. Radiological signs of implant-related infection (peri-implant reaction, mild destruction of bone and soft-tissue swelling, shown as white arrows) observed only in an animal of Group II.

suspension, 100 μl of each dilution was plated on blood agar plates to calculate CFU/ml.

Implantation surgery

Animals were premedicated by a subcutaneous injection of 1 mg/kg atropine (Atropin®, Oy Leiras Ab, Turku, Finland). Anesthesia was induced by subcutaneous injection of 0.3 ml/kg fentanyl citrate-fluanisone (Hypnorm®, 0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone, Janssen Pharmaceutica, Beerse, Belgium). Before surgery, another injection of 0.2–0.4 ml/kg fentanyl citrate-fluanisone was given. The animals of the negative control groups (Groups III and IV) received preoperatively a single prophylactic dose of 500,000 IU benzylpenicillin (Geepenil®, Orion Oyj, Espoo, Finland) intramuscularly as the systemic antimicrobial prophylaxis. The choice of the antibiotic was based on the current species-specific recommendations on the use of prophylactic antibiotics (consultation of P. Keränen, DVM, Small Animal Surgery, Faculty of Veterinary Medicine, University of Helsinki). The animals of Groups I and II received no systemic antibiotic prophylaxis. Surgery was performed under strict sterile conditions in a veterinary operating room reserved for implant surgery and standard surgical techniques were employed. Skin preparation involved careful shaving and scrubbing with 20% chlorhexidine (Klorhexol®, Oy Leiras Ab) followed by disinfection with 5% chlorhexidine and surgical draping. The anteromedial aspect of the right proximal tibial metaphysis was exposed and a bicortical hole of 2.0-mm diameter was hand-drilled and tapped. Before screw insertion, in Groups I and II, the screw was colonized with bacteria by incubating it in a solution of 3×10^4 CFU/ml of *S. aureus* for 6 min. All screws were hand-tightened, but no insertion torque was measured. High-speed blade was used to cut the Ab-PLGA screw at the proximal end with an offset of 2 mm to provide optimal screw length. A new bacterial solution was prepared for each Ab-PLGA screw. The surgical field was lavaged with 100 ml of sterile saline in Groups I and II before wound closure. In negative control animals (Groups III and IV), a similar lavage of the wound space was performed, but 150 mg of cefuroxime sodium (Zinacef, GlaxoSmithKline, Verona, Italy) was added into saline in order to simulate standard surgical lavage with an antimicrobial solution. Finally, the wound was closed in layers and an intramuscular injection of 0.1 mg/kg naloxone (Narcanti®, Bristol-Myers Squibb S.p.A., Anagni, Italy) was given. After surgery, the animals were closely monitored. Functional activity was not limited. The animals received standard postoperative pain medication (4 mg/kg of

carprofen, Rimadyl® Vet, Pfizer, Vericore Ltd., Dundee, UK) for 3 days.

^{18}F-FDG-PET imaging

^{18}F-FDG was synthesized with an automatic apparatus by modification of the method of Hamacher et al. [14], leading to a specific activity >75 GBq μmol^{-1} and the radiochemical purity >99%. All animals underwent PET imaging at 6 weeks after surgery before sacrifice. The animals were sedated by subcutaneous injection of 0.3 ml/kg fentanyl citrate-fluanisone. Mean 82 MBq of ^{18}F-FDG (range 50−104 MBq) was injected in the ear artery of the rabbit. Imaging was performed using a GE Advance Whole-body PET scanner (General Electric Medical Systems, Milwaukee, WI, USA), which acquires 35 contiguous slices with an axial field of view of 15.2 cm [15]. Slice thickness of the scanner was 4.25 mm and the spatial resolution at full-width-half-maximum (FWHM) in the center of the field view was 5 mm. Dynamic acquisition consisting of 4 × 5 min frames and lasting for 20 min was started 40 min after ^{18}F-FDG injection. A 5-min transmission scan for attenuation correction was obtained after emission imaging using two rod sources containing germanium-68. Data were corrected for dead time, decay, and photon attenuation, and the images were reconstructed in a 128 × 128 matrix employing a Hanning filter with a cut-off frequency of 4.6 mm. Quantitative analysis was performed on standardized elliptical regions of interest (ROI, 5 mm × 10 mm) of the screw area of the right tibia and the corresponding region of the contralateral intact tibia. ^{18}F-FDG accumulation was reported as the standardized uptake value (SUV). The SUV was calculated as the radioactivity of the ROI divided by the relative injected dose expressed per kilogram of body weight. SUV ratio between the operated and nonoperated sides was calculated as the normalized value [16].

Sacrifice and radiography

Six weeks after surgery, the animals were sedated by subcutaneous injection of fentanyl citrate-fluanisone and blood was collected via cardiac puncture for evaluation of ciprofloxacin release into systemic circulation. Serum was separated at 4000 rpm for 10 min and stored at −20°C before measurement of ciprofloxacin concentration. The animals were sacrificed with an intravenous administration of sodium pentobarbital (Mebunat®, Orion Oyj). Standard anterior–posterior and lateral radiographs of the right hind limb were taken. Digital image plates (Fuji IP cassette, type C, Fuji Photo Film co., LTD, Japan) and a standard stationary X-ray unit (Philips, Holland) were used. The radiographs were evaluated using a grading system that was originally developed by Kraft et al. [17]. Seven parameters (sequestral bone formation, periosteal new bone formation, destruction of bone, screw loosening, peri-implant reaction, soft-tissue calcification, soft-tissue swelling) were evaluated for each animal, and the numerical score was assigned for each variable (Table 1). In this grading system, the highest total score was 10.0.

Bacteriological analysis

After radiography, the proximal metaphysis of the right tibia was surgically exposed using sterile techniques. Swab cultures were taken from subfascial soft tissues and from the exposed head of the SS or Ab-PLGA screw. The SS screw was removed and separately cultured 4 days at 35°C on brain–heart infusion (BHI) solution (BBL™, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). After removing the SS screw, additional swab culture was taken from the screw tract. All swab specimens were cultured 20 h at 35°C in blood agar plates. The distal end of the Ab-PLGA screw was cut with high-speed blade and placed in a tube containing BHI solution. The tube was rinsed vigorously for 15 s in order to dislodge any adherent bacteria and the Ab-PLGA screw specimen was transferred into BHI solution. The original BHI solution and the BHI solution with the Ab-PLGA screw were cultured 4 days at 35°C. For quantitative culture, cortico-cancellous bone specimens were excised from the proximal side of the screw tract. The specimens were chilled with liquid nitrogen, crushed into fragments, and pulverized with a homogenizator (Ultra-Turrax® T25, Janke and Kunkel GmbH and Co. KG, Staufen, Germany). Bone powder was diluted in 5 ml of sterile saline and samples of 100 μl were withdrawn for serial (10-fold) dilutions. Samples were

Table 1
Classification system for radiological changes of implant infection (originally presented by Kraft et al. [17])

Radiographic parameter	Definition	Point score
Sequestral bone formation	present	1
	absent	0
Periosteal new bone formation	present	1
	equivocal	0.5
	absent	0
Destruction of bone	severe, multiple areas involved	2
	moderate, only one area involved	1
	mild, only one area involved	0.5
	no destruction	0
Screw loosening	severe, multiple areas involved	2
	moderate, only one area involved	1
	mild, only one area involved	0.5
	no destruction	0
Peri-implant reaction	severe, multiple areas involved	2
	moderate, only one area involved	1
	mild, only one area involved	0.5
	no destruction	0
Soft-tissue calcification	present	1
	equivocal	0.5
	absent	0
Soft-tissue swelling	present	1
	equivocal	0.5
	absent	0
Maximum total score		10

cultured 20 h at 35°C in blood agar plates and analyzed for the amount of CFU *S. aureus*/g bone. Slidex Staph Plus latex agglutination test (bioMériux, Marcy-I'Etoile, France), as described by van Griethuysen et al. [18], was used for the identification of isolated *S. aureus* strains. *S. aureus* (ATCC 29213) was used as positive control and *E. faecalis* (ATCC 29212) as negative control.

Histology

Three histological specimens were taken from each animal. A soft tissue specimen was removed from tissues covering the head of the screw. Two bone tissue specimens were removed, one for hard-tissue sectioning and one for decalcified histology. The bone specimens were excised from the proximal and distal part of the screw tract. The distal bone specimen was fixed in 70% ethanol, dehydrated in a graded series of ethanol, cleared in xylene, and embedded in isobornylmethacrylate (Technovit 1200 VLC, Kulzer, Germany). Using a water-cooled, high-speed, low-feed saw equipped with a diamond-impregnated blade, the specimen was cut in the cross-sectional plane along the screw tract. Sections of 20-μm thickness were prepared with a cutting and grinding technique (Exakt Apparatebau, Hamburg, Germany) and stained with a modified van Gieson method. The proximal bone specimen and soft tissue specimen were fixed in neutral buffered formalin, decalcified, dehydrated, and embedded in paraffin. Sections of 4-μm thickness were prepared and stained with hematoxylin and eosin.

Ciprofloxacin concentration in bone and serum

A high performance liquid chromatographic (HPLC) method with fluorescence (FLD) detection and internal standard method was applied to the determination of concentrations of ciprofloxacin in bone and serum (CRST Bioanalytics, Turku, Finland). Ofloxacin was used as an internal standard. The concentration of ciprofloxacin was measured from proximal bone tissue facing the screw tract and also from a distal bone tissue specimen excised 5 mm distally from the tract. The bone specimens were ground with a homogenisator (Mikro-Dismembrator S, B. Brown, Melsungen, Germany). Fine-ground bone was weighed and mixed with internal standard solution (1.0 μg/ml in methanol), methanol, water, and perchloric acid in water. The suspension was centrifuged and the supernatant was filtered with 0.45-μm membrane filter. Finally, 20 μl of the sample was injected into the HPLC column. Standard samples and quality control samples were handled identically, but instead of methanol, certain amounts of solutions containing either ciprofloxacin in methanol or methanol were added. The frozen serum sample was thawed in a refrigerator, and 0.5 ml of serum was mixed with perchloric acid and internal standard. After centrifugation, the supernatant was transferred into an autosampler vial from which 20 μl was injected into the HPLC column. HPLC-FLD analysis was carried out using Waters 2695 Separations Module, Waters 2475 multi λ Fluorescence detector and Millennium version 4.0 software. The column used for separation of the ciprofloxacin was a Nova-Pak C_8 150 × 3.9 mm i.d. 60 Å column (Waters Co., Milford, MA, USA). The mobile phase consisted of 9% acetonitrile and 91% of buffer. The buffer was filtered before use through a 0.45-μm HV filter (Millipore Corporation, Bedford, MA). The flow rate of mobile phase was 1.0 ml/min. The excitation wavelength was 290 nm and the emission wavelength 470 nm. The standard curve was generated using weighted ($1/x$) linear regression. The measured concentration of ciprofloxacin was expressed per weight of bone tissue (μg/g).

Statistical analysis

The significance of differences observed in the change of mean body weight, SUVs, and SUV ratios between the four groups was calculated using one-way ANOVA with post hoc Tukey's test. The results of radiographic scoring were analyzed by means of nonparametric Kruskall–Wallis ANOVA with post hoc Dunn's test. The microbiological results were analyzed by Chi-square test. A P value of 0.05 was considered significant. All statistical analyses were performed with SigmaStat 2.03 software (SPSS Inc., Chicago, IL, USA).

Results

The animals of contaminated SS screws (Group II) tended to loose some of their body weight (5–6%) for 3 weeks after surgery and were approaching the level of the original body weight by 6 weeks. In contrast, the animals of contaminated Ab-PLGA screws (Group I) and the negative control animals (Group III and IV) maintained their original body weight after surgery and even gained more weight during the 6-week follow-up. Clinical signs of local infection (erythema and moderate soft-tissue swelling) were observed in animals of contaminated SS screws (Group II) mainly for the 2nd to 4th weeks, but were still present in five animals out of eight at 6 weeks. The animals of other groups did not have local signs of infection.

^{18}F-FDG-PET imaging

PET imaging showed an increased tracer uptake of ^{18}F-FDG over the screw implantation area of the proximal tibia in animals with contaminated SS screws (Group II) (Fig. 2). Expressed as the SUV ratio, the uptake of ^{18}F-FDG was significantly higher in Group II than in other groups (Fig. 3). The mean SUVs of Groups I and II were 0.44 (SD 0.10) and 0.76 (SD 0.34), respectively (P = 0.036 for the group difference). The corresponding mean SUVs of Groups III and IV were 0.59 (SD 0.10) and 0.52 (SD 0.07).

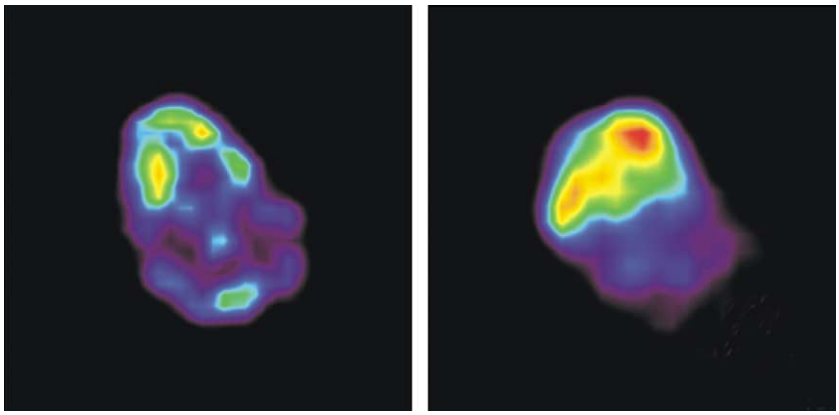

Fig. 2. ^{18}F-FDG-PET transaxial images of rabbit tibias with a contaminated Ab-PLGA screw (left) and a contaminated SS screw (right). The contaminated SS screw showed a high tracer uptake (SUV 1.12) compared with the contaminated Ab-PLGA screw (SUV 0.36).

Radiography

In animals with contaminated SS screws (Group II), radiographic examination showed signs of osseous destruction, peri-implant reaction, periosteal new bone formation, and soft-tissue swelling (Fig. 1). Using the grading system (Table 1) for radiological signs of bone infection, the mean score was 3.4 (SD 1.8, range 1–6). In animals with contaminated Ab-PLGA screws (Group I) and in negative control animals (Groups III and IV), radiographs did not present any of these indices of implant-related infection (mean score 0.0). Statistically, the radiographic changes of Group II differed significantly from the other groups ($P < 0.001$).

Bacteriological analysis

In animals with contaminated Ab-PLGA screws (Group I), all bacterial cultures were negative. No bacteria could be cultured from pulverized bone or direct BHI culture solutions of extracted screws (Table 2).

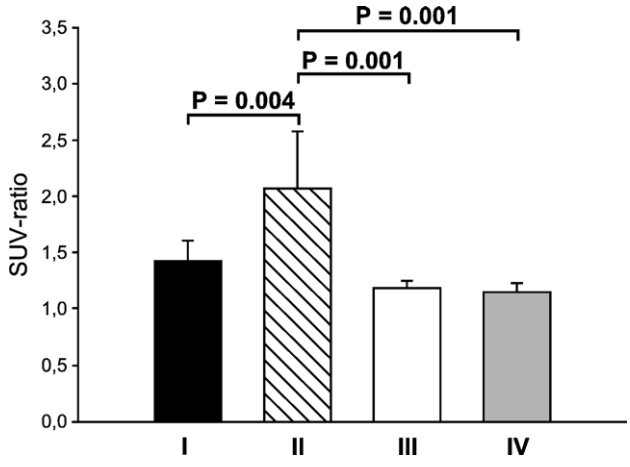

Fig. 3. ^{18}F-FDG-PET imaging (SUV ratio) of the implant area showed an increased tracer uptake in animals operated with contaminated SS screw (Group II), which was significantly higher than that of other groups. The bars represent the means and standard deviations (n = 4–8).

In animals with contaminated SS screws (Group II), all direct cultures of the SS screw in BHI solution were positive for the inoculated *S. aureus* (strain 52/52A/80) ($P < 0.001$, compared with other groups, Chi-square test). In addition, six cultures out of eight from the screw head, screw tract, soft tissues, and bone specimens were positive for same *S. aureus* strain ($P = 0.001$). The average amount of CFU determined per gram of bone was 3.3×10^5 (SD 9.2×10^5).

In negative control animals (Group III and IV), all bacterial cultures were negative. However, one SS screw in Group IV had a positive bacterial culture (*S. cohnii*) on BHI solution, which was regarded as a contaminant.

Histology

Histological sections did not show clear differences in the quality of reactive new bone formation surrounding the pin tracts. The shape of the threads tended to be more uniform around the contaminated Ab-PLGA screws and the control Ab-PLGA and SS screws compared with those of contaminated SS screws. However, no meaningful comparative quantitative analysis of pin tract morphology could be performed.

Table 2
The number of positive bacterial cultures determined on the day of sacrifice

	Group I (Ab-PLGA + *S. aureus*)	Group II (SS + *S. aureus*)	Group III (Ab-PLGA)	Group IV (SS)
Culture from screw	0/8	8/8	0/4	1/4
Culture from screw head	0/8	6/8	0/4	0/4
Culture from screw tract	ND[a]	6/8	ND[a]	0/4
Culture from soft-tissues	0/8	6/8	0/4	0/4
Culture from pulverized bone	0/8	6/8	0/4	0/4

[a] ND = not determined.

Ciprofloxacin concentration in bone and serum

In bone tissue (proximal area of the screw tract), the mean concentration of ciprofloxacin was 2.54 μg/g (SD 2.76 μg/g, $n = 12$). This concentration is higher (about 3-fold) than the bone tissue concentration (0.8 μg/g) measured in a rabbit tibia 120 min after a single intravenous infusion (10 mg/kg) of ciprofloxacin (unpublished data, Mäkinen et al.). In distal bone samples, the mean concentration of ciprofloxacin was 0.83 μg/g (SD 1.09 μg/g, $n = 12$). The serum concentration of ciprofloxacin remained undetectable and below the resolution of the HPLC-FLD method (<5.0 ng/ml) in all samples ($n = 12$).

Discussion

Impregnation of antimicrobial agents within biodegradable orthopedic implants provides a possibility for local antimicrobial prophylaxis of biomaterial-related infections. This experimental study evaluated the efficacy of a bioabsorbable antibiotic containing bone screw in the prevention of biomaterial-related infection due to *S. aureus*. The results showed that ciprofloxacin-containing PLGA screws prevented implant-related infection. Negative bacterial cultures of contaminated ciprofloxacin-containing PLGA screws were collaborated by a low ^{18}F-FDG uptake in PET imaging. Control SS screws with *S. aureus* contamination developed uniform infection under equal conditions with a high ^{18}F-FDG-PET uptake. The use of ciprofloxacin-containing PLGA screws resulted in adequate concentration of the antibiotic in bone without detectable systemic exposure.

Every effort should be made to prevent implant-related infections, including systemic antibiotic prophylaxis, clean air systems, and the use of body exhaust suits in operating rooms. The appealing new approach is to develop carrier systems for local delivery of antibiotics in implant surgery. The use of bioresorbable implants also makes it possible to create true multifunctional implants. Different antibiotics and even growth factors can be incorporated into the biodegradable matrix during the manufacturing process [10,19]. Based on several in vitro and in vivo studies, the biodegradable materials have been shown to provide high local drug concentrations for prolonged time [20–23]. The release characteristics of the selected drug from a biodegradable matrix can be tailored to meet special needs using polymers with different composition and structure.

The main problem with the use of biodegradable implant materials is their lower mechanical strength compared to metallic ones [24]. This has limited, for example, the use of bioresorbable fracture fixation devices mainly to metaphyseal and periarticular fractures. Incorporating antimicrobial agents or growth factors to biodegradable materials may further impair their mechanical strength. Our in vitro immersion testing confirmed that the shear strength of the current antibiotic containing screws decreased by a half within 6 weeks. This certainly may limit clinical applications of the screws.

This study had certain limitations. The applied model was based on bone screw fixation without any fracture or osteotomy. This was done to minimize the number of variables, such as stability of the fixation, influencing the rate of implant-related infections. The screw insertion torque was not measured, although the measurement could have excluded any subclinical difference between groups in the initial stability of screw implantation. In the current experiment, only one pathogen (*S. aureus*) was examined. *S. aureus* is the traditional pathogen of surgical wound infections and one of two leading pathogens causing implant infections. The other one is *S. epidermidis*, which is claimed to be the most frequent causative organism causing infections of polymer-containing implants [25]. Without doubt, similar in vivo studies should be conducted to clarify the efficacy of the drug delivery system in prevention of implant-related infections caused by different pathogens. Fluoroquinolones, such as ciprofloxacin, have long been the drugs of choice for treatment of bone infections [26] and also proposed as prophylactic agents [27]. The choice of the antibiotic for local prophylaxis is multifactorial, and one option is to use a combination of two antibiotics. Further studies are needed to compare the efficacy of different antibiotics and their combinations and also to delineate the activity of the released antibiotic against different bacteria.

Without doubt, the antibiotic release from Ab-PLGA screw brought a challenge for microbiological analyses. The released antibiotic could have masked positive cultures. This was the reason the current study applied the novel technique of ^{18}F-FDG-PET imaging for the detection of local infection, which is based on intensive use of glucose by granulocytes and mononuclear cells [28]. In a previous experimental study, ^{18}F-FDG-PET imaging allowed the differentiation of uneventful bone healing from that complicated by localized osteomyelitis in a rabbit model [13]. Our results showed that negative bacterial cultures of contaminated Ab-PLGA screws were collaborated by a low ^{18}F-FDG uptake in PET imaging while those SS screws with positive bacterial cultures had a high ^{18}F-FDG uptake. This finding speaks strongly against false-negative cultures in the group of contaminated Ab-PLGA screws.

Aside from bone tissue and swab cultures, we used direct cultures of the contaminated screws for retrieval of the pathogen. The method was found to be sensitive in detection of the inoculated pathogen in the group of contaminated SS screws. Thus, the use of mechanical techniques, such as immersion in ultrasonic bath [29], was not necessary for dislodgment of bacteria attached to the implant surfaces. On the other hand, prolonged immersion of Ab-PLGA screw in ultrasonic bath could only have promoted a release of the impregnated antibiotic and thereby disturb detection of any possible surface-attached pathogens.

In conclusion, this study confirmed the efficacy of bioabsorbable antibiotic-containing bone screw in the prevention of biomaterial-related infection due to *S. aureus*. This type of bone screw might reduce the incidence of implant infections especially in high-risk patient groups. Clinical trials are also needed to verify if local antibiotic prophylaxis could replace administration of systemic antibiotics or act as an adjunct prophylactic measure of implant-related infections.

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References

[1] Sugarman B. Infections and prosthetic devices. Am J Med 1986;28: 78–84.
[2] Young EJ, Sugarman B. Infections in prosthetic devices. Surg Clin North Am 1988;68:167–80.
[3] Wilde AH. Management of infected knee and hip prostheses. Curr Opin Rheumatol 1993;5:317–21.
[4] Jansen B, Peters G. Foreign body associated infection. J Antimicrob Chemother 1993;32:69–75.
[5] Court-Brown CM. Antibiotic prophylaxis in orthopaedic surgery. Scand J Infect Dis 1990;70:74–9.
[6] Blackburn Jr WD, Alarcon GS. Prosthetic joint infections. A role for prophylaxis. Arthritis Rheum 1991;34:110–7.
[7] Harbarth S, Samore MH, Lictenberg D, Carmeli Y. Prolonged antibiotic prophylaxis after cardiovascular surgery and its effect on surgical site infections and antimicrobial resistance. Circulation 2000; 101:2916–21.
[8] Eggimann P, Pittet D. Infection control in the ICU. Chest 2001;120: 2059–93.
[9] Holtom PD, Patzakis MJ. Newer methods of antimicrobial delivery for bone and joint infections. Instr Course Lect 2003;52:745–9.
[10] Price JS, Tencer AF, Arm DM, Bohach GA. Controlled release of antibiotics from coated orthopedic implants. J Biomed Mater Res 1996;30:281–6.
[11] Gollwitzer H, Ibrahim K, Meyer H, Mittelmeier W, Busch R, Stemberger A. Antibacterial poly(D,L-lactid acid) coating of medical implants using a biodegradable drug delivery technology. J Antimicrob Chemother 2003;51:585–91.
[12] Lucke M, Schmidmaier G, Sadoni S, Wildemann B, Schiller R, Haas NP, et al. Gentamicin coating of metallic implants reduces implant-related osteomyelitis in rats. Bone 2003;32:521–31.
[13] Koort JK, Mäkinen TJ, Knuuti J, Jalava J, Aro HT. Comparative ^{18}F-FDG-PET imaging of experimental *Staphylococcus aureus* osteomyelitis and normal bone healing. J Nucl Med 2004;45:1406–11.
[14] Hamacher K, Coenen HH, Stocklin G. Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 1986;27:235–8.
[15] DeGrado TR, Turkington TG, Williams JJ, Stearns CW, Hoffman JM, Coleman RE. Performance characteristics of a whole-body PET scanner. J Nucl Med 1994;35:1398–406.
[16] De Winter F, Van De Wiele C, Vogelaers D, De Smet K, Verdonk B, Dierckx RA. Fluorine-18 fluorodeoxyglucose positron emission tomography: a highly accurate imaging modality for the diagnosis of chronic musculoskeletal infections. J Bone Jt Surg 2001;83-A: 651–60.
[17] Kraft CN, Schlegel U, Pfluger D, Eijer H, Textor J, Hansis M, et al. Radiological signs of osteitis around extramedullary metal implants. A radiographic-microbiological correlative analysis in rabbit tibiae after local inoculation of *Staphylococcus aureus*. Arch Orthop Trauma Surg 2001;121:338–42.
[18] van Griethuysen A, Bes M, Etienne J, Zbinden R, Kluytmans J. International multicenter evaluation of latex agglutination tests for identification of *Staphylococcus aureus*. J Clin Microbiol 2001;39: 86–9.
[19] Schmidmaier G, Wildemann B, Bail H, Lucke M, Fuchs T, Stemberger A, et al. Local application of growth factors (insulin-like growth factor-1 and transforming growth factor-β1) from a biodegradable poly(D,L-lactide) coating of osteosynthetic implants accelerates fracture healing in rats. Bone 2001;28:341–50.
[20] Kanellakopoulou K, Kolia M, Anastassiadis A, Korakis T, Giamarellos-Bourboulis EJ, Andreopoulos A, et al. Lactic acid polymers as biodegradable carriers of fluoroquinolones: an in vitro study. Antimicrob Agents Chemother 1999;43:714–6.
[21] Ambrose CG, Gogola GR, Clyburn TA, Raymond AK, Peng AS, Mikos AG. Antibiotic microspheres: preliminary testing for potential treatment for osteomyelitis. Clin Orthop 2003;415: 279–85.
[22] Calhoun JH, Mader JT. Treatment of osteomyelitis with a biodegradable antibiotic implant. Clin Orthop 1997;341:206–14.
[23] Garvin KL, Miyano JA, Robinson D, Giger D, Novak J, Radio S. Polylactide/polyglycolide antibiotic implants in the treatment of osteomyelitis. J Bone Jt Surg 1994;76-A:1500–6.
[24] Daniels AU, Chang MKO, Andriano KP. Mechanical properties of biodegradable polymers and composites proposed for internal fixation of bone. J Appl Biomater 1990;1:57–78.
[25] von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect Dis 2002;2:677–85.
[26] Kanellakopoulou K, Giamarellos-Bourboulis EJ. Carrier systems for the local delivery of antibiotics in bone infections. Drugs 2000;59: 1223–32.
[27] Dellamonica P, Bernard E. Fluoroquinolones and surgical prophylaxis. Drugs 1993;45(Suppl. 3):102–13.
[28] Sugawara Y, Gutowski TD, Fisher SJ, Brown RS, Wahl RL. Uptake of positron emission tomography tracers in experimental bacterial infections: a comparative biodistribution study of radiolabeled FDG, thymidine, L-methionine, ^{67}Ga-citrate, and ^{125}I-HSA. Eur J Nucl Med 1999;26:333–41.
[29] Tunney MM, Sheila P, Gorman SP, Nixon JR, Anderson N, Davis RI, et al. Improved detection of infection in hip replacements. A currently underestimated problem. J Bone Jt Surg 1998; 80-B:568–72.