Efficacy of a novel rifamycin ABI-0043 against *Staphylococcus aureus* in an experimental model of foreign-body infection

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ABSTRACT

We compared the efficacy of a novel rifamycin derivative ABI-0043 with that of rifampin, alone and in combination with levofloxacin, against methicillin-susceptible *S. aureus* ATCC 29213 in a guinea pig tissue cage infection model. The MIC, the logarithmic growth-phase MBC and stationary growth-phase MBC values of ABI-0043 were 0.001, 0.008 and 0.25 µg/ml, respectively; the corresponding values of rifampin were 0.016, 0.8 and 3.6 µg/ml, respectively. After a single 12.5 mg/kg-intraperitoneal dose, the peak concentration in cage fluid was 1.13 µg/ml for ABI-0043 and 0.98 µg/ml for rifampin. Five days after completion of treatment, levofloxacin administered alone (5 mg/kg/12 h) showed similar bacterial counts in cage fluid as untreated controls (>8.0 log_{10} CFU/ml), whereas rifampin and ABI-0043 administered alone (12.5 mg/kg/12 h) decreased the mean titer of bacteria ± SD in cage fluid to 1.43 ± 0.28 log_{10} and 1.57 ± 0.53 log_{10} CFU/ml, respectively. In combination with levofloxacin, both rifamycins cleared bacteria from the cage fluid. Cure rates of cage-associated infections with rifampin and ABI-0043 administered alone were 46% and 58%, respectively, which increased to 88% and 92% in combination with levofloxacin. Emergence of rifamycin resistance was observed in all 5 failures after ABI-0043 and in 9 of 13 failures after rifampin therapy; no emergence of resistance occurred in combination treatment with levofloxacin. In conclusion, ABI-0043 had cure rates comparable to that of the rifampin. ABI-0043 in combination with a quinolone has the potential for treatment of implant-associated infections caused by susceptible strains of *S. aureus*, potentially without drug-drug interactions.
INTRODUCTION

Implanted devices are increasingly used in modern medicine to improve function (e.g. internal fixation, neurosurgical shunts, prosthetic heart valves), to alleviate pain (e.g. prosthetic joints), or for aesthetic reasons (e.g. breast implants) (7). Despite the use of perioperative antimicrobial prophylaxis, infection of the implant can occur, since less than 100 CFU of microorganisms can cause an infection when a foreign body is present (37). If infection occurs, it causes high morbidity and substantial cost, especially in orthopedic surgery (23). Since bacteria grow and persist as biofilms on the implant surface, they are difficult to eradicate (25). Consequently, until recently, standard treatment approaches to these infections in orthopedic surgery included removal or replacement of all foreign-body material (4). Since this surgical concept is invasive, may cause considerable tissue damage and requires prolonged hospital stay, it has been challenged (26, 28, 36).

Successful chemotherapeutic treatment of device-associated infection requires an antimicrobial agent that acts against surface-adhering microorganisms in the stationary growth phase (1, 6, 29, 31, 34). Rifampin fulfills this requirement for staphylococci based on experimental animal models (21, 29, 34) and on clinical studies (10, 30, 38).

However, rifampin has several drawbacks, including important drug interactions due to liver enzyme induction, poor clinical tolerance (nausea, fever, myalgia), and rapid emergence of resistance (20, 32). Therefore a novel rifamycin derivative that overcomes these shortcomings will be of interest.
Rifalazil, the most advanced of a new generation of rifamycins, the benzoxazinorifamycins, has the benefit of a long terminal half-life, a large volume of distribution, good tissue penetration, and the lack of significant interactions on liver microsomal enzymes (cytochrome P450 system) (19). In a human clinical trial, rifalazil has shown efficacy in the treatment of sexually transmitted disease caused by *Chlamydia trachomatis* (22). Rifalazil is currently undergoing clinical testing for peripheral arterial disease, based on the hypothesis that this condition is associated with infection in the vasculature caused by *Chlamydia pneumoniae* (19). Derivative compounds, new chemical entities (NCEs) such as ABI-0043, ABI-0369, and ABI-0699, are of particular interest in the treatment of serious Gram-positive infections. These compounds have even more potent activity *in vitro* than rifampin or rifalazil and retain some activity against rifampin-resistant strains (14). They are also effective in animal models at lower doses than rifampin or rifalazil in murine models of infection (17). Like rifalazil, the NCEs do not interact with P450 CYP 3A4 (19), whereas induction of P450 enzymes by rifampin has been shown to be responsible for a number of drug-drug interactions (16, 32).

In this work, one representative NCE, ABI-0043 (Fig. 1) was tested against *S. aureus in vitro*. Subsequently the potential of ABI-0043 was investigated alone and in combination with levofloxacin for the treatment of implant-associated infections caused by a methicillin-susceptible *S. aureus* in guinea pigs.
MATERIALS AND METHODS

Study microorganism. *S. aureus* ATCC 29213, susceptible to methicillin, levofloxacin and rifampin was used in this study. The strain was stored at -70°C using the cryovial bead preservation system (Microbank, Pro-Lab Diagnostics, Richmond Hill, ON, Canada). One cryoculture bead was incubated in 1 ml brain heart infusion (BHI) broth for 7 h at 37°C without shaking. Cultures were then diluted 1:100 in 5 ml BHI broth and incubated overnight at 37°C without shaking. Subsequently bacteria were centrifuged at 4000 x g for 10 min, pellet was washed twice and resuspended in pyrogen free 0.9% NaCl before use.

Antimicrobial agents. Levofloxacin intravenous solution 5 mg/ml (Aventis Pharma AG, Zurich, Switzerland) and rifampin in powder form (Medika AG, Aesch, Switzerland) were purchased from the manufacturer. ABI-0043 was provided by ActivBiotics Inc. (Lexington, MA, USA) in powder form. ABI-0043 powder was dissolved in 100% dimethyl sulfoxide (DMSO, Merck KGaA, Darmstadt, Germany) using bath sonication at 40 kHz ± 5 kHz for 20 min to prepare a stock solution of 50 mg/ml. One volume of stock solution was added to four volume of diluted liquid fill (18) (375 g Etocas 35NF, 4.4 g pluronic acid F68, 50.8 g PEG 400, and 10.8 mL water; 15 volumes mixed with 33 volumes of water) resulting in a dosing solution containing ABI-0043 in 20% DMSO. The 20% DMSO diluent alone showed no antibacterial effect on *S. aureus*.

Antimicrobial susceptibility testing. All in vitro susceptibility tests were performed in triplicate with a standard inoculum of 5 x 10^5 CFU *S. aureus*/ml adjusted from an
overnight culture, and are reported as median values. The MIC was determined in cation-adjusted Mueller-Hinton broth (MHB) by the macrotube dilution method according to Clinical Laboratory Standards Institute guidelines (formerly the National Committee for Clinical Laboratory Standards) (5). The MIC was the lowest drug concentration that resulted in no detectable growth by visual inspection. During preparation of serial dilutions, pipette tips were changed after each dilution to ensure minimal carryover of antimicrobial agents. The minimal bactericidal concentration for the logarithmic growth phase ($\text{MBC}_{\text{log}}$) was defined as the antimicrobial concentration reducing the initial bacterial concentration after incubation in cation-adjusted MHB for 24 h by $>99.9\%$ (i.e. $3 \log_{10} \text{CFU/ml}$), as described in the Manual of Clinical Microbiology (15). In addition, the MBC was determined also for the stationary growth phase ($\text{MBC}_{\text{stat}}$), as previously described (34). For this purpose, the overnight culture of $S. \text{aureus}$ was centrifuged (2000 x g for 10 min) and resuspended in 0.01 M phosphate-buffered saline (PBS, pH 7.4). In this medium, bacterial counts remained within $\pm15\%$ of the original inoculum in the antimicrobial-free culture for $>36$ h (data not shown).

**Animal model.** A foreign-body infection model in guinea pigs was used, as previously described (2, 21, 33, 35, 37). In brief, four sterile polytetrafluorethylene (Teflon) cages (32 mm x 10 mm), perforated by 130 regularly spaced holes of 1 mm diameter (Angst-Pfister AG, Zurich, Switzerland) were subcutaneously implanted in flanks of male albino guinea pigs (Charles River; weight, 640-800 g) under aseptic conditions. Animals were anesthetized with an intramuscular injection of ketamine (20 mg/kg) and xylazine (4 mg/kg). Two weeks after surgery and complete healing of the wounds, sterility of cages was verified by culture of aspirated cage fluid. For pharmacokinetic studies, non-infected
animals were used. For treatment evaluation, cages were infected by percutaneous inoculation of 200 µl overnight culture containing $2 \times 10^4$ CFU S. aureus. Established infection was confirmed 24 h later by culture of aspirated cage fluid. Guinea pigs were kept under specific pathogen-free conditions in the Animal House of the Department of Research, University Hospital Basel, and animal experimentation guidelines were followed according to the regulations of Swiss veterinary law. The study protocol was approved by the Institutional Animal Care and Use Committee.

**Antimicrobial treatment.** 24 h after cage infection (day 1), antimicrobial treatment was initiated. Animals were randomized into eight treatment groups: control (saline), levofloxacin 5 mg/kg, rifampin 12.5 mg/kg (each dose with and without levofloxacin 5 mg/kg), ABI-0043 3 mg/kg and 12.5 mg/kg (each dose with and without levofloxacin 5 mg/kg). The volume of each dose was adjusted to a total volume 5 mL by adding saline. Each antibiotic was administered intraperitoneally every 12 h for 4 days (total 8 doses). Doses and administration intervals for rifampin were chosen to achieve serum concentrations mimicking those in humans, based on previous experience (21, 29, 34). Quantitative cultures of aspirated cage fluid were performed immediately before the initiation of antimicrobial treatment (day 1), during the treatment before the last antimicrobial dose (day 4) and 5 days after completion of treatment (day 9). The animals were then anesthetized with fentanyl-droperidol and cages were removed under aseptic conditions. Each cage was placed in 10 ml trypticase soy broth (TSB), vortexed for 30 s and incubated at 37°C. After 24 h, 50 µl of this TSB were plated on 5% sheep blood agar plates (Becton Dickinson, Heidelberg, Germany) to determine the presence of...
bacteria. Guinea pigs dosed with 20% DMSO prepared with the diluted liquid fill but without ABI-0043, showed no visible symptoms of illness, including loss of weight, or hematological or liver biopsy irregularities over the full course of the study.

Each antimicrobial regimen was evaluated in at least 12 cages (i.e. 3 animals with 4 cages each) by determining (i) the mean log CFU count during the treatment before the last antimicrobial dose (day 4) or 5 days after completion of treatment (day 9) compared to the bacterial counts 24 h after infection immediate before initiation of treatment (day 1), and (ii) the cure rate, i.e. fraction of cages in which the infection was eradicated, defined as the absence of growth of \textit{S. aureus} in TSB containing the explanted cages. Comparisons between categorical variables were performed using \( \chi^2 \) or Fisher’s exact tests, as appropriate.

\textbf{Determination of rifamycin-resistant strains.} Positive cultures from TSB containing explanted cages (i.e. in treatment failures) were screened for rifamycin resistance. For this purpose, multiple colonies of each morphologically distinct colony type were collected from an agar subculture. A standardized inoculum of \( 5 \times 10^5 \) CFU \textit{S. aureus} was plated on Mueller-Hinton agar plates containing 1 µg/ml ABI-0043 or rifampin. Plates were incubated at 37°C and screened for growth after 24 h.

\textbf{Pharmacokinetic studies.} Samples of cage fluid, corresponding to interstitial fluid, were aspirated by percutaneous cage puncture from non-infected animals at various times for 12 hours following intraperitoneal administration of a single dose of ABI-0043 or rifampin. In addition samples were taken once daily on subsequent days, just prior to
dosing for rifampin and ABI-0043 for determination of trough concentrations of antimicrobials. Aliquots of 150 µl cage fluid were transferred to tubes containing 15 µl of filter sterilized 1.5% EDTA (pH 7.4), mixed by hand to avoid clotting, and centrifuged (2000 x g for 10 min); the supernatant was stored at -20°C until further analysis.

Concentrations of ABI-0043 in cage fluid were determined in triplicate by agar plate diffusion bioassay using *Streptococcus pneumoniae* ATCC 49619 as indicator organism. *S. pneumoniae* was grown overnight in cation-adjusted MHB supplemented with 3% laked horse blood (Oxoid, Basingstoke, Hampshire, United Kingdom), diluted 1:50 in the same medium, and grown until turbid (i.e. for 6-7 h). MHB agar plus 5% defibrinated sheep blood was warmed to 37°C prior to adding to large assay plates (30 x 30 cm). After hardening of the base agar, 1.5 ml of the *S. pneumoniae* culture was diluted in 10 ml MHB seed agar at 48°C, poured onto the assay plates, and allowed to solidify.

Concentrations of rifampin were determined in a bioassay using *Micrococcus luteus* and the ones of levofloxacin using *Escherichia coli* V6311/65, as previously described (2). The bioassay detection limit was 0.0005 µg/ml for ABI-0043, 0.001 µg/ml for rifampin and 0.01 µg/ml for levofloxacin.

Cage fluid was serially diluted 2- and 4-fold in serum; 10 µl of each cage fluid dilution was spotted onto an assay plates and incubated overnight. The diameter of zones of inhibition was measured with calipers. The mean intra- and interassay coefficients of variation were <5%. Results were calculated by exponential regression analysis (Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA, USA). The
concentration-time data were analyzed with WinNonlin (Pharsight Corp., Mountain View, CA).

At each time point, the mean concentration of two cages from three animals was used to calculate concentrations and error bars. The area under the concentration-time curve from time zero to 12 h ($AUC_{0-12}$) was determined by the log-linear trapezoidal method.
RESULTS

Antimicrobial susceptibility. Table 1 compares the in vitro susceptibilities of *S. aureus* to the pharmacokinetic parameters (peak concentration in cage fluid). For levofloxacin, the MBC\textsubscript{stat} was 125 times higher than the MBC\textsubscript{log}, whereas rifamycins showed lesser differences between MBC\textsubscript{stat} and MBC\textsubscript{log} (5 to 31 times). ABI-0043 had the lowest MIC, MBC\textsubscript{log} and MBC\textsubscript{stat} values for *S. aureus* (0.001, 0.008 and 0.25 µg/ml, respectively), which is reflected by the highest ratio of peak/MIC and peak/MBC\textsubscript{stat} in cage fluid.

Pharmacokinetic studies. Table 2 shows the pharmacokinetics in cage fluid after a single and multiple intraperitoneal administrations in non-infected animals. As shown in Figure 2, the drug concentration of ABI-0043 in cage fluid from non-infected animals after a single dose of 12.5 mg/kg (day 1) was well above the MIC, the MBC\textsubscript{log}, and the MBC\textsubscript{stat}. Following this single dose, the AUC\textsubscript{0-12} of ABI-0043 (6.53 µg*h/ml) was similar to that of rifampin (4.56 µg*h/ml). However, the peak concentration of rifampin was achieved after a single intraperitoneal dose at 2 h, whereas ABI-0043 had a longer time to peak (T\textsubscript{max} = 8 h). The terminal half-life of rifampin was calculated to be 5.8 h. The determination of the half-life of ABI-0043 from the single-dose data is difficult because of the longer time to T\textsubscript{max}, yet is considerably longer than that of rifampin, based on trough levels following multiple dosing. ABI-0043 continued to accumulate through the 4 days of dosing, after rifampin reached steady-state (Table 2).

Antimicrobial treatment studies. Cages were sterile prior to inoculation. The titer of bacteria 24 h after infection increased to ≈10\textsuperscript{7} CFU/ml of *S. aureus* in all cage fluid.
samples (data not shown). In the cage fluid of infected, untreated control animals, bacterial counts increased during 9 days after infection to $>10^8$ CFU/ml cage fluid (Fig. 3a). No spontaneous cure of cage-associated infection occurred in 12 untreated cages through the entire 9 days of the study. Levofloxacin alone reduced the bacterial count during treatment, but there was a rebound in the bacterial titer to similar counts as untreated controls 5 days after treatment ended. Rifampin alone decreased the mean titer of bacteria after 4 days of treatment to $3.68 \pm 0.59 \log_{10}$ CFU/ml and further to $1.43 \pm 0.28 \log_{10}$ CFU/ml 5 days after completion of treatment, whereas in combination with levofloxacin the reduction reached $2.14 \pm 0.35 \log_{10}$ CFU/ml 4 days of treatment and $0$ CFU/ml 5 days after completion of treatment. ABI-0043 at 12 mg/kg/12 h alone and with levofloxacin induced a comparable reduction of bacterial titers during ($3.24 \pm 0.27 \log_{10}$ CFU/ml and $1.95 \pm 0.34 \log_{10}$ CFU/ml, respectively) and after treatment ($1.57 \pm 0.53 \log_{10}$ CFU/ml and $0 \log_{10}$ CFU/ml, respectively).

With levofloxacin alone, no cage-associated infection was cured (Fig. 3b). Rifampin as a single treatment (12.5 mg/kg/12 h) showed a cure rate of 46% (11 of 24 cages), which was further improved to 88% (21 of 24 cages) by the addition of levofloxacin ($P < 0.05$). Similarly, exposure to ABI-0043 alone at 12.5 mg/kg/12 h resulted in a cure rate of 58% (7 of 12 cages), compared with 92% (22 of 24 cages) for its combination with levofloxacin ($P < 0.05$). The low-dose therapy of ABI-0043 (3 mg/kg/12 h) had a cure rate of only 8% (1 of 12 cages), which increased to 50% (12 of 24 cages) for its combination with levofloxacin ($P < 0.05$). The high-dose therapy of ABI-0043 (12.5 mg/kg/12 h) was comparable to rifampin (12.5 mg/kg/12 h) and superior to the low-dose therapy of ABI-0043 (3 mg/kg/12 h).
Emergence of rifamycin resistance. After the 4-day therapy, emergence of resistance against ABI-0043 was observed in positive cages in 8 of 11 (73%) cultures from explanted cages after low-dose ABI-0043 (3 mg/kg/12 h) and in 5 of 5 (100%) cages cultures after high-dose ABI-0043 (12.5 mg/kg/12 h). Rifampin-resistant strains were detected after rifampin therapy in 9 of 13 (69%) cage cultures. No emergence of resistance occurred during rifampin or ABI-0043 combination-therapy with levofloxacin.
DISCUSSION

Implant-associated infections are characterized by persisting surface-adhering microorganisms and the lack of spontaneous cure (8). The biofilm mode of growth represents a basic mechanism by which microorganisms survive and persist in the presence of a foreign body (6). Depletion of metabolic substances and/or waste product accumulation within biofilms causes microbes to enter a slow- or non-growing (stationary) state, in which microbes are up to 1,000 times more resistant to growth-dependent antimicrobial agents compared with planktonic (free-living) bacteria (9, 24). Because of this, standard antimicrobial susceptibility tests do not predict the outcome in implant-associated infections (3, 29, 31, 34, 38). An animal model in which the eradication of device-associated bacteria can be monitored would allow developing valuable concepts for antimicrobial therapy of such infections. The tissue-cage infection model in guinea pigs fulfills this requirement and was validated in clinical studies (12, 13, 27, 30, 38). In addition, the susceptibility of guinea pigs to cage infection closely simulates the human situation. The clinical use of rifampin in implant-associated infections has been based mainly on experimental studies in this model (2, 21, 29, 35). Therefore, in the present study, a novel rifamycin has been tested and compared with rifampin.

Rifampin has traditionally been used against mycobacteria. However, during the last 25 years it has been increasingly used against staphylococci (20). Because of its efficacy against adherent and stationary-phase staphylococci, rifampin has become a standard combination drug in patients with device-associated infection (36). Since rifampin has
several drawbacks, we tested ABI-0043, a representative of the most recent series of benzoxazinorifamycins that have more potent activity in vitro than rifampin and rifalazil against \textit{S. aureus}. This new compound differs from rifampin in that it contains a four-ring, planar structure (Fig. 1), which may account for its greater lipophilicity, tissue penetration, and longer half-life (17,18). An important distinguishing characteristic compared with rifampin is that ABI-0043 lacks significant interactions with liver microsomal enzymes (18). This is a significant advantage especially in the elderly with comorbidity requiring many different drugs. In addition, ABI-0043 retains greater activity against strains highly resistant to rifampin, although emergence of resistance by single-step-mutation occurs at a similar frequency as with rifampin (14) as we observed also in this study. It will be important to obtain data on human tolerance to fully evaluate NCEs such as ABI-0043.

In this study, ABI-0043 was bactericidal for the logarithmic growing \textit{S. aureus} at a concentration of 0.008 µg/ml. The MBC$_{\text{stat}}$ against \textit{S. aureus} was below the peak and even trough level of ABI-0043 in the cage fluid. Accordingly, ABI-0043 (12.5 mg/kg/12 h) eliminated staphylococcal infection in 58% of tissue-cages when used as single agent, and in 92% of cages when used in combination with levofloxacin. These cure rates were similar to those of rifampin, indicating its potency in device-associated infection. Related NCEs, ABI-0369 and ABI-0699 have comparable MBCs against stationary cells (data not shown). The efficacy against staphylococcal foreign-body infection observed for ABI-0043 is expected to be exhibited also from these NCEs (33).
When used as single agent, the failure rate was high with rifampin, as well as with ABI-0043. During the 4-day therapy, emergence of resistance was observed in 73% failures after low-dose ABI-0043, in 100% failures after high-dose ABI-0043, as well as in 69% failures after rifampin therapy. Since *S. aureus* strains were grown in antibiotic-free media before plated on media containing rifamycin, an unstable resistance could be lost. Nevertheless, the tendency for development of rifamycin resistance was considerably higher when rifamycin was administered alone compared to the combination treatment with levofloxacin. In contrast, no emergence of resistance occurred during rifampin or ABI-0043 combination-therapy with levofloxacin. Thus, both rifamycin compounds should be combined with another antimicrobial substance in this clinical setting.

Quinolones are suitable combination agents due to their excellent bioavailability (≈100%), spectrum of activity, safety and tolerability (38). Ciprofloxacin and ofloxacin have been extensively used in long-term therapy of bone infections and proved their efficacy against prosthetic joint infections in combination, particularly with rifampin (11, 30, 38). Newer quinolones have a better activity against gram-positive cocci than ciprofloxacin, but clinical data with levofloxacin, moxifloxacin, or gatifloxacin are still sparse.

In conclusion, ABI-0043 had similar activity as rifampin in experimental device-associated infection, making this drug a promising candidate for these infections, which lacks serious drug-drug interactions.
ACKNOWLEDGMENTS

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REFERENCES


TABLE 1. In vitro susceptibility of *S. aureus* and linked pharmacokinetic data

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>MBC&lt;sub&gt;log&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (µg/ml)</th>
<th>MBC&lt;sub&gt;stat&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt; (µg/ml)</th>
<th>MBC&lt;sub&gt;stat&lt;/sub&gt;/MBC&lt;sub&gt;log&lt;/sub&gt; ratio</th>
<th>Peak&lt;sup&gt;c&lt;/sup&gt;/MIC ratio</th>
<th>Peak&lt;sup&gt;c&lt;/sup&gt;/MBC&lt;sub&gt;stat&lt;/sub&gt; ratio</th>
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<tr>
<td>Levofloxacin</td>
<td>0.16</td>
<td>0.32</td>
<td>40</td>
<td>125</td>
<td>6.1</td>
<td>0.02</td>
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<td>Rifampin</td>
<td>0.016</td>
<td>0.8</td>
<td>3.6</td>
<td>5</td>
<td>61.2</td>
<td>0.27</td>
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<tr>
<td>ABI-0043</td>
<td>0.001</td>
<td>0.008</td>
<td>0.25</td>
<td>31</td>
<td>1130</td>
<td>4.52</td>
</tr>
</tbody>
</table>

<sup>a</sup> MBC<sub>log</sub>, minimal bactericidal concentration in the logarithmic growth phase.

<sup>b</sup> MBC<sub>stat</sub>, minimal bactericidal concentration in the stationary growth phase.

<sup>c</sup> Peak concentration after a single intraperitoneal dose of 5 mg/kg (for levofloxacin) or 12.5 mg/kg (for rifampin and ABI-0043) was used.
TABLE 2. Pharmacokinetics in cage fluid after intraperitoneal administration of antimicrobials in non-infected animals

<table>
<thead>
<tr>
<th>Dosing</th>
<th>Antibiotic</th>
<th>Dose (mg/kg)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$C_{\text{min}}$ (µg/ml)$^b$</th>
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<tbody>
<tr>
<td>Single dose</td>
<td>Levofloxacin</td>
<td>5</td>
<td>0.97 ± 0.20</td>
<td>0.01 ± 0.01</td>
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<td></td>
<td>Rifampin</td>
<td>12.5</td>
<td>0.98 ± 0.32</td>
<td>0.14 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>ABI-0043</td>
<td>3</td>
<td>0.11 ± 0.03</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>ABI-0043</td>
<td>12.5</td>
<td>1.13 ± 0.23</td>
<td>0.14 ± 0.11</td>
</tr>
<tr>
<td>Multiple dose$^c$</td>
<td>Rifampin</td>
<td>12.5</td>
<td>0.61 ± 0.13</td>
<td>0.22 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>ABI-0043</td>
<td>12.5</td>
<td>0.82 ± 0.49</td>
<td>0.41 ± 0.09</td>
</tr>
</tbody>
</table>

$^a$ Concentration values are means ± SD from 12 cage fluid aspirates.

$^b$ $C_{\text{min}}$ (trough concentration) was measured 12 h after dosing.

$^c$ The indicated dose was administered every 12 h for 4 days; the pharmacokinetic values were determined on day 4.
Legends to figures

FIG. 1. Chemical structure of rifampin and its derivative compound ABI-0043.

FIG. 2. Pharmacokinetics in cage fluid after a single intraperitoneal dose (12.5 mg/kg) of rifampin (■) and ABI-0043 (▲). Values are means ± SD. Horizontal lines indicate the corresponding MIC, MBC_{log} and MBC_{stat} values of *S. aureus* for rifampin (Rifa) and ABI-0043 (ABI).

FIG. 3. Treatment efficacy with different antimicrobial regimens. A – Mean log_{10} cfu/ml *S. aureus* in aspirated cage fluid during (day 4) and after treatment (day 9). B - Cure rates of cage-associated infection determined by the fraction of explanted cages without growth of *S. aureus* in TSB. In both panels numbers above bars represent the number of culture-negative cage specimens / total number of cage fluid specimens.