Foreign body infection: a new rat model for prophylaxis and treatment

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A subcutaneous catheter model in the rat was developed that allowed the study of prevention and treatment strategies for foreign body infection. In contrast to earlier models, the foreign body was inoculated with a low inoculum of *Staphylococcus epidermidis* just before implantation, thus mimicking intraoperative contamination with skin flora. Reproducible infection of all catheters followed if no prophylaxis was given. However, foreign body infection could be prevented or treated with antibiotics such as teicoplanin, which was marginally effective, and rifampicin, which proved very effective.

**Introduction**

Infection of medical devices, or foreign body infection (FBI), is an important problem in modern medicine.\(^1\) Antibiotic prophylaxis just before implantation of the device has become widely established, but there are only limited data from controlled clinical trials on the efficacy of particular antibiotic regimens.\(^2\) For this reason, models of FBI are an important tool to assess the relative efficacy of prophylaxis with antibiotics that differ with regard to their antibacterial spectrum, pharmacokinetic parameters, toxicity and cost.

Several animal models of infection with specific types of prosthetic device, such as vascular grafts, heart valves, joint prostheses and osteosynthesis materials, have been designed.\(^3\)–\(^8\) Their value lies mainly in the study of device-specific issues. For a more general approach to antibiotic prophylaxis, simpler models have been developed.\(^9\)–\(^15\) However, these models present some problems such as the choice of the infecting organisms, which have mostly been *Staphylococcus aureus*, and not coagulase-negative staphylococci such as *Staphylococcus epidermidis*, the organism most frequently encountered in FBI. A further problem is the timing of the infection, which is delayed until after device implantation in nearly all models. In this respect these models differ from the clinical situation, since most FBIs are acquired perioperatively. A nitrobiotic prophylaxis is aimed at the prevention of infection that originates from perioperative bacterial seeding of the device. Here, we describe a newly developed rat model of FBI that addresses these problems.

**Materials and methods**

**Experimental animals**

Three rat strains were used: outbred Wistar rats raised at the animal facility, University of Leuven, Leuven, Belgium; a commercially available inbred Fisher rat strain from the Broekman Institute, Someren, The Netherlands and a Fisher rat strain that had been inbred and maintained under germ-free conditions in the Rega Institute for Medical Research, University of Leuven, since 1965. This last rat strain was used both under germ-free conditions and after exposure to normal rat flora over a 4-week period. Rats ‘conventionalized’ in this manner were allowed to breed, and a new generation of rats was obtained, born and reared under conventional conditions. These rats, exposed to rat flora from birth, were labelled EGF rats (for ex-germ-free Fisher rats). A nimal experimentation guidelines were followed throughout this study.

**Bacterial strains and inoculum preparation**

Strains of *S. epidermidis* (Table) were obtained from clinical cases of FBI. A nitrobiotic susceptibilities were determined by an agar dilution method for all antibiotics tested and subsequently, MIC values were determined either by...
Etest (AB Biodisk, Solna, Sweden) for teicoplanin or broth microdilution for rifampicin according to NCCLS guidelines.16 Two clinical strains of S. aureus, one belonging to the epidemic methicillin-resistant S. aureus clone that is dominant in Belgian hospitals,17 and the other, a randomly selected methicillin-sensitive S. aureus strain, were also assessed.

Bacterial suspensions (1 mL) in brain–heart infusion broth (BHI, Oxoid Ltd, Basingstoke, Hampshire, UK) with 20% glycerol were stored at –70°C. For each experiment a fresh aliquot was taken and used to inoculate 5 mL of BHI broth. Cultures were grown overnight at 36°C and 1 mL was centrifuged for 3 min and resuspended in 1 mL NaCl 0.9%. Bacterial density was measured at 600 nm and diluted to a defined final cell concentration in BHI broth.

Segments (1 cm) of polyurethane triple lumen iv catheters (Arrow International, Reading, PA, USA) were used. All catheter segments from the same experiment were incubated for 2 h at –70°C in a single container with 5 mL of BHI broth. Cultures were grown overnight at 36°C and 1 mL was centrifuged for 3 min and resuspended in 1 mL NaCl 0.9%. Bacterial density was measured at 600 nm and diluted to a defined final cell concentration in BHI broth.

Segments (1 cm) of polyurethane triple lumen iv catheters (Arrow International, Reading, PA, USA) were used. All catheter segments from the same experiment were incubated for 2 h at 0°C in a single container with 5 mL of the diluted bacterial suspension. In each experiment, three catheter segments were not implanted but processed as described below for explanted catheters, plated on Mueller–Hinton agar and incubated for 24 h at 36°C. The mean of the cfu obtained from these catheters was recorded as the inoculum size for each experiment.

Implantation procedure

Rats weighing 120–200 g were anaesthetized using diethyl ether inhalation for the duration of the implantation procedure, which was performed under laminar airflow. The back of each rat was shaved over a large area and the skin was disinfected using chlorhexidine 0.5% in alcohol 70% and allowed to dry for 30 s. A 10–12 mm skin incision was made and the subcutis was bluntly dissected to create tunnels in which catheter segments were inserted at a distance of at least 2 cm from the incision, covered by intact skin. Three catheter segments were inserted in each animal. The incision was closed with surgical staples.

Antibiotic regimens

Treatment and prophylaxis experiments were performed using two antibiotics. In prophylaxis experiments, teicoplanin (Hoechst Marion Roussel, Frankfurt, Germany) was administered im in a dose of 10 mg/kg, 4 h before implantation. In the teicoplanin treatment regimen, an im dose of 50 mg/kg was administered bid starting 5 h after implantation and continuing until 16 h before explantation, for a total of seven doses. Teicoplanin trough serum levels were determined using TDX (Abbott Laboratories, Diagnostics Division, Irving, TX, USA).

In prophylaxis experiments, rifampicin (Hoechst Marion Roussel), was administered ip in a dose of 25 mg/kg 4 h before implantation. In the treatment experiments, rifampicin was administered at the same dose bid for a total of seven doses at the same time as teicoplanin. During rifampicin prophylaxis experiments, rats were kept in wire-floor cages to avoid prolonged rifampicin exposure through coprophagia.

Explantation procedure and quantitative cultures

In the prophylaxis and treatment experiments, animals were killed 72 and 96 h, respectively, after implantation. The skin was disinfected with chlorhexidine 0.5% in alcohol 70% and allowed to dry. A new skin incision was made at approximately 2 cm from the implantation wound, the catheter segments were gently removed from the subcutaneous tissue. Each catheter was placed in a separate tube containing 1 mL of NaCl 0.9% and stored on melting ice. All catheter-containing tubes were vortexed for 5 s, sonicated at 40,000 Hz in a water bath for 5 min (B ranson 2200, B ranson Ultrasound Co., Danburg, CT, USA) and again vortexed for 5 s. Plating of the sonicate was performed on Mueller–Hinton agar (Oxoid) with an automated Spiral Plater Model B (Spiral Systems, Cincinnati, OH, USA) according to the manufacturer’s guidelines. Incubation was at 36°C for 24 h. Plates inoculated from control tubes with saline only were included in each experiment to check for sterility.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Inoculum</th>
<th>oxacillin</th>
<th>clindamycin</th>
<th>rifampicin</th>
<th>ciprofloxacin</th>
<th>teicoplanin</th>
</tr>
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<tbody>
<tr>
<td>S. epidermidis 10b</td>
<td>2.7</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S. epidermidis X</td>
<td>2.9</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S. epidermidis 1</td>
<td>3.5</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S. epidermidis 111</td>
<td>3.9</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S. aureus MRSA</td>
<td>≤3.0</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>S. aureus MSSA</td>
<td>≤3.0</td>
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Colonies were counted using the Bacteria Colony Counter, Model 500 A and Data Processor, Model 800 (Spiral Systems). All plates were visually checked and recounted when less than 20 cfu were detected by the Bacteria Colony Counter. Results of undiluted samples were used unless counts higher than 5 log_{10} cfu/mL were obtained. For those samples, counting was done on a 100-fold diluted sample.

All data were logarithmically transformed. Statistical analysis was performed with the two-tailed Student’s t-test for unpaired variables.

Results

Choice of the animal and bacterial strain

Catheters inoculated with 6 log_{10} cfu of S. epidermidis strain 10b were implanted in the three different rat strains. In outbred Wistar rats, spontaneous eradication of the inoculum occurred in two out of 10 catheters at day 2 and in six out of 20 at day 6. Furthermore, the levels of bacterial infection were highly variable (variance: 4.3 log_{10} cfu). In the commercially available Fisher rat strain, spontaneous eradication occurred in one out of 24 catheters at day 2 and in two out of 24 at day 6. The variability of the levels of infection were again substantial (variance: 2.5 log_{10} cfu). In germ-free rats, conventionalized rats from the same strain and in EGF rats, a 100% infection rate was obtained at days 2, 3 and 6 (n > 100 for EGF rats, data not shown). In EGF rats using an inoculum of 2.9 log_{10} cfu, the infection rate was 100% with 5.6 log_{10} cfu (variance of 1.1 log_{10} cfu) recovered at day 3.

In addition to S. epidermidis strain 10b, other staphylococcal strains with different antibiotic susceptibilities were tested for their ability to establish a FBI in the EGF rat model. The minimal inoculum required to establish infection varied between 2.7 and 3.9 log_{10} cfu for the different strains (Table). For the S. aureus strains, inocula below 3.0 log_{10} cfu were not assessed.

All experiments reported below were performed with S. epidermidis strain 10b. For this strain the MICs of teicoplanin and rifampicin were 4.0 mg/L and 0.003 mg/L, respectively.

Validation of procedures

Uninfected catheters implanted in rats for 2 and 6 days (n = 9 in each group) were processed according to the protocol. All catheters tested sterile after explantation. Sterility checks of catheters and suspensions were performed to exclude contamination.

The plates in all experiments were visually observed. Colonies with a different morphology were infrequently encountered. No more than three aberrant colonies were ever present in any of the plates, indicating an insignificant level of contamination.

The effect of sonication on S. epidermidis viability was tested. No decrease in cfu could be demonstrated using bacteria in suspension after sonication for 5 and 20 min (n = 5, data not shown). Similarly, sonication of explanted catheters for 40 min versus 5 min did not decrease bacterial counts (n = 6, data not shown). This demonstrates that the sonication process did not lead to substantial bacterial killing.

Prophylaxis of infection

Teicoplanin (10 mg/kg) or rifampicin (25 mg/kg) was administered 4 h before implantation. The mean (s.d.) teicoplanin serum level at the time of implantation was 8.9 (1.4) mg/L (n = 6).

In studies with an initial inoculum of 2.9 log_{10} cfu, the mean (s.d.) log_{10} cfu recovered from untreated rats (n = 48) was 5.6 (1.1). For rats pre-treated with teicoplanin (n = 60), the mean (s.d.) log_{10} cfu recovered was 5.2 (1.3), while for rats receiving rifampicin (n = 9), it was 2.4 (1.0). The difference in bacterial load in untreated and pre-treated rats was statistically significant (P = 0.05 and P < 0.001 for teicoplanin and rifampicin, respectively). The difference in bacterial load between rats not receiving prophylaxis and those receiving teicoplanin prophylaxis, however, amounted to less than half a log_{10}.

Treatment of infection

In the treatment experiments, teicoplanin was administered at a dose of 50 mg/kg bid. Mean (s.d.) teicoplanin trough serum levels (n = 4) were 20.4 (5.2) mg/L 16 h after the seventh and last dose. The same teicoplanin regimen was combined with rifampicin 25 mg/kg bid. Catheters carrying an inoculum of 6 log_{10} cfu S. epidermidis strain 10b were implanted. The mean (s.d.) log_{10} cfu recovered was 5.8 (0.6) in the control group (n = 36) and 5.0 (1.2) in the teicoplanin-treated rats (n = 36). This difference was statistically significant (P < 0.001). In the teicoplanin plus rifampicin-treated rats (n = 12), all catheter cultures were sterile.

Discussion

The main conclusion from this study is that the subcutaneous catheter model in the EGF rat is a simple and reproducible model to study the perioperative prophylaxis and treatment of FBI. An infection rate of 100% was reached in untreated animals and the FBI responds to antibiotics with known activity in a predictable manner.

Several other FBI models have been used to study antibiotic prophylaxis, but they suffer from conceptual weaknesses. Transcutaneous models are less suitable because of the risk of external contamination. In studies in which catheters were implanted either intraperi-
or subcutaneously, the catheters were infected after implantation, either early or one to several days later which does not mirror perioperative infection. In the tissue cage model of Zimmerli et al., the foreign body was in place for several weeks before bacterial inoculation, with the result that coating by host proteins and the host reaction to the foreign body were developing before infection was induced.

A foreign body inoculated just before introduction into the host is more appropriate for several reasons. If perioperative antibiotic prophylaxis is to be studied, then establishment of the infection on the ‘naked’ foreign body (i.e. before its surface has been modified by the host) is probably clinically most relevant. It is logical to assume that inoculation occurs when exposure to microorganisms is maximal, i.e. during manipulation and before the material is covered by host tissue. Moreover, the material is most vulnerable to bacterial colonization before host-derived albumin coating is established. This suggests that early intraoperative bacterial contamination of the foreign body from the skin of the surgeon or the patient is the main target of perioperative prophylaxis. A foreign body tissue reaction is absent at that time and coating by host proteins only starts after insertion. These conditions were not fulfilled in previous models described in the literature, but are achieved by our model. In the model described by Lambe et al. the catheter was also inoculated before implantation, but this was achieved by a 48 h incubation at 37°C in broth, allowing for extensive bacterial biofilm formation on the foreign body surface before implantation. In addition, a second large inoculum was given after implantation, which is again different from the clinical situation.

One objection to the current model is that inoculation exactly at implantation rather than just before is preferable. This, however, makes inoculum standardization problematic, thereby increasing variability of data, especially when low inocula are used (data not shown). By briefly incubating the catheters at 0°C, a constant inoculum was achieved with minimal metabolic activity before catheter implantation.

A second characteristic of our model is that infection can be induced in a reproducible manner with S. epidermidis, which is the bacterial species most commonly implicated in FBI. Most earlier models used S. aureus as the infecting organism. S. aureus is more virulent and thus causes experimental infection more readily, but it is a less common cause of FBI than S. epidermidis. Christensen et al. and Lambe et al. used S. epidermidis, but in the former study only half of the catheters became infected, despite a massive inoculum, while in the latter study massive and repeated inoculation was needed to obtain an infection with a sufficient degree of certainty. In the model described in this paper, low inocula were capable of producing infection, which is probably also the case in the clinical situation.

Staphylococcal strains with different susceptibility patterns can be used to induce infection in our model, thus allowing for the study of antibacterial agents of different classes. Since the results are obtained in a quantitative manner, comparison between different regimens is possible.

A third issue is the choice of experimental animal. In earlier models, rabbits, guinea pigs or mice were used. The former two are more expensive in terms of acquisition and housing. Furthermore, guinea pigs suffer occasional procedure-related death and require more time for procedures to be performed (unpublished observations). Mice are robust and their cost is low, but their small size makes procedures more complicated and iv injection and phlebotomy problematic. A gamma in favour of rats are their low cost and ease of handling. Moreover, kinetic data on new drugs are nearly always available for rats because these animals are used in preclinical pharmaceutical research. On the other hand, we anticipated problems in establishing FBI in rats because of their low susceptibility to staphylococci. When using outbred Wistar rats and a commercially available inbred Fisher rat strain, we observed spontaneous eradication of infection in a significant proportion of catheters, despite high bacterial inocula. The number of bacteria recovered from the catheters was also highly variable. The EGF rat strain, however, proved to be most useful in this model, providing a 100% infection rate and a low variance in bacterial counts. Why EGF rats behave differently in this model than the other rat strains tested is unknown. They are not neutropenic and show no gross abnormalities (unpublished observation). One can only speculate on the role of inbreeding for over 30 years in germ-free conditions and thus absence of exposure to live bacteria during earlier rat generations.

In prophylaxis experiments, the experimental set-up was validated using antibiotics of known but different activity in FBI. Teicoplanin had only moderate in-vitro activity and minimal in-vivo activity in a previous animal FBI model. In rats, it has a serum half-life of about 5 h and peak levels are attained after 4 h. Teicoplanin was administered at a dose of 10 mg/kg in the prophylaxis experiments, which is a low dose for rats and the mean teicoplanin serum level at the time of implantation was only about a two-fold concentration above the MIC for S. epidermidis strain 10b. The low antibacterial activity of teicoplanin in the prophylaxis experiment in our model was therefore not unexpected.

Rifampicin has high intrinsic activity against susceptible staphylococci and this was confirmed in an earlier animal FBI model and in the clinical setting. In our model, rifampicin was administered in a dose of 25 mg/kg, which is a normal dose in rats. It has a half-life of about 4 h in this animal. Rifampicin drug levels were not measured, but based on previous studies, the expected serum levels of about 10 mg/L at 4 h were well above the MIC for
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S. epidermidis strain 10b. In our model, rifampicin was highly active, causing a more than 3 log reduction in cfu recovered from the catheters. This proves that the catheter model in the EGF rat is a prophylaxis model that can differentiate between high and low antibacterial activities. Similarly, in the treatment experiments, infection could be quantitatively influenced with antibiotics. Teicoplanin had a limited effect but values below the detection limit were obtained in all catheters exposed to a 4-day rifampicin-plus-teicoplanin regimen. Rifampicin monotherapy was not assessed because it would not be a reasonable treatment option, so the contribution of synergy with teicoplanin could not be assessed.

We conclude that the subcutaneous catheter model in the EGF rat is a simple, reproducible and reliable model that is suited to study the effect of new strategies for perioperative prophylaxis and treatment of FDI, including new antibiotics, antimicrobial combinations or biological agents that may influence the outcome of FDI.

Acknowledgements

W.E.P. has been awarded the R. van Furth chair in Infectious Diseases at the K. U. Leuven. This study was supported by A mgm Belgium, Brussels, Belgium and A mgm Inc., Thousand Oaks, CA, USA.

References


Received 29 January 1999; returned 10 June 1999; revised 30 June 1999; accepted 16 July 1999