Effect of Treatment with Methicillin and Gentamicin in a New Experimental Mouse Model of Foreign Body Infection

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Received 20 December 1993/Returned for modification 3 May 1994/Accepted 28 June 1994

A new mouse model of foreign body infection has been developed. Intraperitoneal placement of a silicone catheter followed by injection of 108 Staphylococcus aureus organisms resulted in a reproducible, localized foreign body infection. The infection persisted as an intra-abdominal abscess surrounding the catheter for at least 30 days. Treatment with up to nine doses of methicillin or gentamicin or both was started 3 days after infection. The treatment showed a significant effect (P < 0.05), measured as reduction of bacteria on the foreign body, for all three regimens with a reduction of up to 2 log units, but no synergism was observed. The result of the treatment was poor, despite the facts that the local concentrations of methicillin were greater than the MIC for at least 72 h and that nine peak concentrations of gentamicin of >13 μg/ml were obtained. The poor result of the treatment was not caused by development of antibiotic resistance or influenced by protein concentration, pH, or local presence in the pus of inhibitors of antibiotics. Both antibiotics showed good effects in time-kill studies in vitro on bacteria on catheters taken out of infected mice and catheters infected in vitro. During treatment, the proportion of intracellular bacteria increased in all treated mice to 60 to 75% compared with 20 to 30% in nontreated mice (P < 0.05). This indicates that intracellular survival of staphylococci may influence the outcome of the treatment in foreign body infections.

A variety of foreign bodies are today implanted in humans. Their extended use is often a great benefit for the patients, but a major limitation to their use is the development of bacterial infections. These foreign body infections, which most often are caused by coagulase-negative staphylococci or Staphylococcus aureus (27, 29, 35), have in the last decades increased in frequency, and they are now the leading hospital-acquired infections (15, 39).

Foreign body infections are characterized by a low infective inoculum, the persistence of the pathogen at the site of the infection, and great difficulties in antibiotic treatment (10, 14, 18, 45). The major problem when a treatment regimen has to be tested is proving the antibiotic effect without removal of the foreign body.

A number of animal models have been developed for the purpose of studying the different biomaterials, pathogenesis, treatment, and prophylaxis of foreign body infection. The classical study in humans by Elek and Conen (18) was followed by the development of animal models, in which a superficial suture is infected either before or after application. The superficial skin lesions are evaluated (1, 2, 30). An extension of these models is the tissue cage model, in which a small cage (or chamber) is implanted subcutaneously in the animal (9, 26, 44). The major advance of this model is the capacity for repeated punctures of tissue cage fluid, which makes it possible to study in more detail the pharmacodynamics of antibiotic treatment and the local host response in foreign body infections (26, 44). More sophisticated models often necessitate the use of larger animals but offer the possibility of a close similarity to the clinical situation (6, 36, 40).

The purpose of the present study was to develop an easy, reliable, and inexpensive foreign body infection model, which also should allow the possibility of obtaining local samples useful for antibiotic measurements and for the evaluation of the local host response. We have developed a foreign body mouse model, and in this study we evaluate the results of treatment of S. aureus foreign body infection with systemic administration of methicillin and/or gentamicin.

MATERIALS AND METHODS

Antibiotics. Methicillin (Lucopenin) and gentamicin (Garamycin) were obtained from ASTRA (Glostrup, Denmark) and Schering-Plough (Farum, Denmark), respectively. The antibiotics were prepared fresh for each day of an experiment by dilution in saline.

Bacteria. S. aureus E2371, isolated from a blood culture (20), was used throughout the study. For bioassays, Micrococcus luteus ATCC 9341 was used for measurement of methicillin and Staphylococcus epidermidis (collection no. P903/76) was used for gentamicin.

Determination of MIC and MBC. The MIC and MBC were determined by broth dilution with an inoculum of 106 CFU/ml. The medium was either Mueller-Hinton broth or beef broth (Statens Seruminstitut, Copenhagen, Denmark) supplemented with 20% (vol/vol) freshly prepared human citrated blood. The MIC was defined as the lowest concentration of antibiotic that resulted in no visual turbidity after incubation for 24 h at 35°C. The MBC was determined by subculture of 0.1 ml from tubes with no visual turbidity on 5% blood agar plates (Statens Seruminstitut). The MBC was defined as the lowest concentration of antibiotic that reduced the inoculum by at least 99.9%. All assays were performed in triplicate.

Catheters. Silicone catheters (Hedima, Glostrup, Denmark) with an internal diameter of 2.8 mm and an external diameter of 4.9 mm were cut into segments of 1.5 cm.

Preparation of inoculum. A few colonies of strain E2371

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were grown overnight in Mueller-Hinton broth, harvested, and washed three times in saline (1,600 × g for 10 min). The bacteria were then adjusted to an optical density at 540 nm of 0.3, as measured with a colorimeter, and diluted 1:1 in saline. The bacteria were then diluted to the appropriate concentration in saline (corresponding to approximately 10⁸ CFU/ml), which was confirmed by colony counts.

Experimental foreign body infection. Ten- to 12-week-old CF-1 female mice (Statens Seruminstitut) weighing approximately 25 g were operated on under ether anesthesia. A 1-cm incision was made in the lateral abdominal wall opposite to the operation wound. A group of mice received bacterial challenge without any prior operation, and one group was operated on but not challenged. The mice were allowed free access to food and water. At different times, the animals were sacrificed as follows. Under ether anesthesia, blood was obtained by orbital cutdown, and then peritoneal washing was performed by injection of 2 ml of saline into the peritoneal cavity; after gentle massage of the abdomen between the thumb and forefinger, the peritoneum was opened and 1 ml of fluid was aspirated (peritoneal washings). Blood was either used directly for quantitative bacteriology or allowed to clot at 37°C for 30 min and centrifuged at 1,500 × g for 10 min and the serum was stored at −80°C. Fluids from peritoneal washings were either used directly for quantitative bacteriology or stored at −80°C. The peritoneal cavity was inspected, aspirates from the foreign body were collected, and the foreign body was removed for culture. Aspirates from the foreign body were obtained by puncture of the abscess around the catheter with a thin pipette and aspiration of the fluid inside the catheter. The aspirate was either used directly for quantitative bacteriology and microscopy or stored at −80°C. Finally, the foreign body was suspended in 1 ml of saline and vortex mixed for 10 s and quantitative culturing of the saline was performed (catheter washings). The smallest amounts of blood and aspirate obtained were 0.1 and 0.05 ml, respectively. This gives a lower limit of detection of 10 bacteria per ml of blood and 20 bacteria per ml of aspirate.

Three days after bacterial challenge, groups of mice were treated with methicillin, gentamicin, both methicillin and gentamicin, or no antibiotics (control group). The treatment was given three times a day (at 0800, 1100, and 1400) for up to 3 days. Each dose of methicillin was 75 mg per mouse (3 g/kg of body weight), and the gentamicin dose was 0.75 mg per mouse (0.03 g/kg). The antibiotics were given subcutaneously in 0.1 or 0.5 ml of saline. Preliminary experiments were performed to define the dosage and the schedule for both drugs. During the treatment, groups of mice were sacrificed and serum and aspirate samples were collected 20 min after doses 1 and 3 (peak levels), just before doses 2, 3, 4, and 7, and when sacrificed (trough levels).

Bioassays. Concentrations of antibiotics in serum and aspirates were measured by bioassay after appropriate dilution of samples in human serum (17). Standard curves were obtained by using antibiotics diluted in normal human serum. The lower limits were 0.6 μg of gentamicin per ml and 0.4 μg of methicillin per ml. The analytical variation was determined by retesting of 10 samples on different days. The interday variations were 11% ± 28% (95% confidence interval) for methicillin and 14% ± 36% for gentamicin, and intraday variations were 13% ± 33% and 16% ± 41%, respectively.

Microscopy. Smears were made from aspirates and catheter washings, and light microscopy was performed after Gram or methylene blue stain. For groups of mice allocated to different treatment regimens, a quantitative assessment of the number of intracellular bacteria was performed by microscopy of methylene blue-stained smears. The number of leukocytes per high-power field (×1,000) and the number of intracellular and extracellular bacteria were determined. Fields were counted until at least 75 leukocytes were identified.

Infection of catheter segments in vitro. Catheter segments were infected in vitro by addition of a few colonies of strain E2371 to a tube containing one catheter segment in 2 ml of beef broth supplemented with 20% (vol/vol) citrated human blood and incubation at 37°C for 3 days.

Time-kill studies. The effect of methicillin and/or gentamicin on S. aureus in suspension was compared with the effect on bacteria after infection of catheters in vivo and in vitro. Catheters infected in vivo, i.e., taken from the mice 3 days after challenge, or in vitro were gently washed by shaking for a few seconds in three successive volumes of 3 ml of saline. Then the catheters were placed in 2 ml of beef broth supplemented with 20% (vol/vol) citrated human blood containing either methicillin, gentamicin, methicillin and gentamicin, or no antibiotics. The final concentration of the antibiotics was twice the MIC. Incubation was performed in a water bath at 37°C with (250 cycles per min) or without agitation. After incubation for 0.5, 2, or 24 h, the catheters were removed and rinsed three times by gentle shaking for a few seconds in 5 ml of saline. The catheters were then placed in 1 ml of saline and vortex mixed for 10 s, and then quantitative culturing of the saline was done. Kill curves for bacteria in suspension in the same medium were made simultaneously. All experiments were performed in triplicate. Synergy was defined as a decrease in the log CFU per milliliter of more than a factor of 2 for the combination of drugs compared with the value for the most active constituent after 24 h.

Development of resistance. Four groups of mice were either treated with methicillin, gentamicin, or methicillin and gentamicin or received no treatment. Catheter washings from these groups of four mice each were diluted 1:9 in saline, and 100 µl was cultivated on 5% blood agar plates containing twofold dilutions (12.8 to 0.1 μg/ml) of either methicillin or gentamicin.

Determination of pH and protein concentration. The pH of pools of aspirates was determined with a pH-meter, and protein concentrations were estimated by the method of Lowry et al. (33).

Statistical methods. The numbers of bacteria in different groups of animals were compared by the Mann-Whitney U test. A P of <0.05 was considered significant.

RESULTS

MIC and MBC. In Mueller-Hinton broth, the MIC and MBC of methicillin for S. aureus E2371 were 1.6 and 3.2 μg/ml, respectively, and in beef broth supplemented with human blood the MIC and MBC were 0.8 and 1.6 μg/ml, respectively. The MIC and MBC of gentamicin were 0.8 and 3.2 μg/ml, respectively, in Mueller-Hinton broth and 0.4 and 0.8 μg/ml, respectively, in medium supplemented with blood.

Foreign body infection model. Table 1 shows the effect of an increasing inoculum. A challenge dose of greater than 1 × 10⁵

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CFU gave nearly 100% infection rates, i.e., infection defined as growth in catheter washings 3 days after challenge, while an inoculum of greater than $5 \times 10^6$ CFU resulted in mortality (Table 1). Therefore, an inoculum of $0.5 \times 10^6$ to $1.0 \times 10^6$ CFU was used for further study.

With an inoculum of $1.0 \times 10^8$ CFU, catheter washings showed an increase from $6.1 \times 10^6$ 3 h after challenge to $9.2 \times 10^6$ 30 days later (Fig. 1). After 30 days of infection, the mice showed severe wasting. During the first day of infection, the mice had bacteremia with a maximum of $10^3$ to $10^4$ bacteria per ml of blood (Fig. 1). The number of bacteria in peritoneal washings decreased rapidly 3 days after challenge (Fig. 1). Almost identical curves for peritoneal washings and blood were found for mice challenged after an operation in which no catheter was implanted (data not shown).

A few hours after challenge with S. aureus, the catheter was covered by the omentum, and 3 days later the infection was chronic with an abscess around the catheter. From this abscess it was possible to obtain approximately 50 to 100 μl of pus by aspiration from the inside of the catheter. Implantation of a catheter without subsequent challenge also resulted in the development of a small, thin-walled abscess containing sterile fluid. The spleen increased to approximately three times normal size 10 days after challenge with bacteria.

**Treatment of foreign body infection.** Treatment of mice was started 3 days after challenge, and the results are summarized in Table 2. From the start of treatment (i.e., day 3 after challenge) to the end of treatment (i.e., day 6), the number of bacteria in catheter washes increased approximately 10-fold in untreated mice (Table 2). Treatment of mice with methicillin or gentamicin for 3 days resulted in a low but significant reduction in bacterial counts from both the catheter and the aspirate (Table 2), although the combination of methicillin and gentamicin gave a slightly better result, as for either drug alone no major differences were found in comparison with the effect of combination treatment (Table 2). The variation in log CFU per milliliter in catheter washings for the control groups was low, as indicated by a narrow range after 6 days (7.8 to 9.1; Table 2). In the treatment groups the variation was much greater, but the median log CFU per milliliter decreased only by a factor of 1 or 2, indicating an overall poor effect of the treatment.

**Pharmacokinetics of methicillin and gentamicin.** Figure 2A and B show the concentrations of methicillin and gentamicin in serum at different intervals during treatment. Peak levels of methicillin and gentamicin were in the range of 200 to 250 and 18 to 42 μg/ml, respectively. In a few mice there was a measurable trough level, but none of the antibiotics accumulated in serum during the treatment period (Fig. 2A and B). Considering the lowest measured level of methicillin of 6 μg/ml locally during the 72 h treatment, and considering that methicillin is only 40% bound to protein, the local concentration of methicillin was greater than the MIC for at least 72 h (Fig. 2C). Peak levels, measured in aspirates from the inside of the catheter, were in the range of 58 to 155 and 13 to 28 μg/ml for methicillin and gentamicin, respectively (Fig. 2C and D). Both methicillin and gentamicin showed local accumulation, as indicated by increasing trough levels (Fig. 2C and D). It must be assumed that the peak values obtained locally during the first day of treatment (Fig. 2C and D) could be at least as high during the next 2 days. Thus, the local concentration of unbound methicillin was greater than the MIC for at least 72 h, and nine peak levels of gentamicin of >13 μg/ml inside the abscess were obtained.

**Time-kill studies.** Figure 3A through F shows the results of the kill studies. The time-kill curves obtained for broth showed nearly identical results with and without agitation and did not show synergism between methicillin and gentamicin (Fig. 3A and B). Time-kill studies after infection of catheters in vitro also resulted in good killing but no synergism (Fig. 3C and D). The kill curves obtained when catheters were infected in vivo (Fig. 3E and F) showed no major differences from those obtained in vitro (Fig. 3C and D).

**Development of resistance.** The lowest concentrations of methicillin and gentamicin that inhibited growth of S. aureus were 0.8 and 0.2 μg/ml, respectively, when catheter washings from untreated mice were cultivated directly on blood agar plates containing antibiotics. Identical results were obtained for all three groups of mice treated with antibiotics for 3 days. No single colonies or just a haze of growth was found on plates containing higher concentrations of antibiotics, indicating that resistant subpopulations of bacteria did not develop during treatment.

**Characteristics of aspirates.** The median protein concentration of 35 different aspirates was determined to be 40 g/liter (range, 32 to 72 g/liter). The median pH was 7.6 (range, 7.3 to 8.0) ($n = 14$). Aspirates from 60 mice (untreated mice monitored for 3 to 6 days) were pooled and filtered through a 0.42-μm-pore-size filter. The MIC and MBC of methicillin and gentamicin for S. aureus E2371 were determined twice for this sterile-filtered aspirate, and the results differed by <1 dilution from the results obtained with beef broth supplemented with human serum.

**Quantitation of intracellular bacteria.** Table 3 shows the

![FIG. 1. Time course for log CFU per milliliter (median and range for three to six mice) in mice with an intraperitoneal catheter segment after challenge (time zero) with S. aureus E2371. B, blood culture; P, peritoneal washings; C, catheter washings.](image-url)
results of microscopic examination of methylene blue-stained smears. The median number of high-power fields counted was four (range, two to seven), and the median number of leukocytes counted per smear was 106 (range, 75 to 126). The percentage of intracellular bacteria was significantly increased in all treatment groups, to 60 to 75% compared with 20 to 30% in the control group, while the number of intracellular bacteria did not differ (Table 3).

**DISCUSSION**

The foreign body infection model described here is easy to perform and inexpensive and permits short study periods. Therefore, the model is well-suited for large-scale studies. The model is highly reproducible, as all the control mice were infected when an inoculum of approximately $10^8$ CFU was used (Table 2). Normally, mice are difficult to infect with *S. aureus* by the intraperitoneal route because of the great phagocytic capacity of the peritoneal macrophages, and this normal defense has to be inhibited by an additive, such as human serum, plasma, or fibrinogen, that blocks the phagocytic activity (1, 21, 31). In this study, the control mice also cleared the inoculum within a few days, and it was again demonstrated that the pathogenicity of *S. aureus* increases in the presence of a foreign body (18, 44). The model also shows other characteristics common for foreign body infections. First, the infection became localized to the catheter, while the distant spread was limited (Fig. 1). Second, the infection became chronic with only a slow increase in the bacterial count (Fig. 1). A potential limitation of the model is that the foreign body has no function. The usefulness of the model demonstrated in this study is its capacity to allow evaluation of different treatment and prophylaxis regimens, as well as studies of local defense mechanisms, and evaluation of the intracellular effect of antibiotics in vivo (Table 3).

Our model shares some of the potential found for the tissue cage model, which was initially developed with rabbits and guinea pigs and recently also rats (9, 26, 34, 44, 45). Both models are highly reproducible, and it is possible to induce an infection with a low inoculum (Table 1) (34). The tissue cage allows repeated punctures from the focus with the possibility of monitoring the antibiotic concentrations and other local factors in the same animal (34, 43, 44). In our model, repeated punctures are not possible, but aspiration from the abscess allows the same events to be monitored in groups of animals. Thus, the same potential exists for the investigation of local defense mechanisms (43, 44) and local development of antibiotic resistance (34). The major difference between the models is the time between insertion of the foreign body and infection, which is long in the tissue cage model, while in our model the infection is induced with the installation of the catheter. Also, the amount of fluid that can be obtained is greater in the tissue cage model, while the model described here clearly has an advantage in cost.

The poor effect of treatment of foreign body infection in the clinical setting (5, 8, 32) may depend on a range of factors, such as the antibiotic pharmacokinetics at the site of infection (7, 26), inhibition of the antibiotic effect by local factors in the milieu (4), the protective effect of the biofilm (12, 41), development of bacterial resistance (34, 42), changes in the metabolism of the bacteria resulting in, e.g., longer generation periods or adaptation to a dormant state (28), and yet-unknown factors. The present finding of an only marginal effect of treatment (Table 2) in experimental foreign body infections fits well with the findings of other groups (22, 34, 42).

In this study, we found that the local concentrations of antibiotics, achieved by clearly higher dosages than used systemically in humans, were greater than the MIC for methicillin for at least 72 h and for gentamicin gave nine peaks of $>13$ μg/ml during the 3 days (Fig. 2). The high dosage of especially methicillin and the dosing regimen were adjusted after preliminary experiments to secure both a high concentration in the abscess and a concentration in serum greater than the MIC for the bacteria for at least 3 days. The effects in vitro of methicillin and gentamicin correlate with the time above the MIC and the peak value, respectively (23–25, 38), and the local antibiotic concentrations measured here should predict a substantial effect. We are not able to explain the concentrations of both drugs in serum before dosing the second day and on the following days. We can only speculate that the very high concentrations lead to trapping of the drugs in the abscess and possibly other tissues, explaining why the
drug elimination does not follow usual first-order kinetics. This merits further investigation.

The poor result of treatment could, at least for methicillin, be influenced by an “Eagle effect” (16). With the Eagle effect the rate, but not the effect, of bacterial killing is reduced (16). Furthermore, no paradoxical killing effect has been reported for the aminoglycosides. In this study, the effect was measured after 4 days, i.e., 1 day after the last dose. It is therefore unlikely that an Eagle effect plays any significant role in the results.

Local parameters may inhibit the effect of antibiotics, e.g., pH and divalent cations for gentamicin, protein concentration for methicillin, and so-called abscess factors (4, 13). We did not investigate the concentration of divalent cations but found a pH in the range of 7.5 to 8.0 and a protein concentration in the range of 32 to 72 g/liter, which should inhibit neither methicillin nor gentamicin. Furthermore, the antibiotic concentration in the abscess fluid was measured by means of a biological assay. This finding, as well as the finding that sterile-filtered abscess fluid showed no inhibition of either antibiotic in vitro, indicates that both methicillin and gentamicin are biologically active in the abscess.

We could not find any signs of development of resistance against methicillin and gentamicin in bacteria isolated after treatment, as has been found for rifampin in the tissue cage model (34). The experiments comparing the effects of antibiotics on catheters infected in vitro and in vivo gave results that were not easy to interpret (Fig. 3). The influence of agitation, for example, is obvious for catheters infected in vivo and may be a result of disintegration of the biofilm (Fig. 3E and F). However, no similar effect was found when catheters were infected in vitro (Fig. 3C and D). It seems that the localization of the bacteria on the catheters to some extent inhibits the antibiotic effect, as found by others (3, 41), but the relative impact on the poor antibiotic response cannot be assessed.

During treatment of the foreign body infection with methicillin and gentamicin, which both are distributed largely extracellularly, we found that the proportion of intracellular bacteria increased (Table 3). It is well known that S. aureus has the potential for intracellular survival (19, 37), and our finding may
TABLE 3. Number of bacteria per high-power field in smears from aspirates of mice treated for 3 days

<table>
<thead>
<tr>
<th>Treatment (no. of mice)</th>
<th>Median no. of bacteria (range)</th>
<th>Median % intracellular (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Extracellular</td>
</tr>
<tr>
<td>None (6)</td>
<td>1,699 (1,563–3,559)</td>
<td>1,236 (1,189–2,853)</td>
</tr>
<tr>
<td>Methicillin (5)</td>
<td>1,422 (712–2,562)</td>
<td>493 (226–842)</td>
</tr>
<tr>
<td>Gentamicin (6)</td>
<td>1,368 (918–1,866)</td>
<td>376 (287–486)</td>
</tr>
<tr>
<td>Methicillin and gentamicin (6)</td>
<td>795 (156–1,364)</td>
<td>233 (39–344)</td>
</tr>
</tbody>
</table>

* Significantly higher ($P < 0.05$) than value for no treatment.

FIG. 3. Time-kill curves (means ± standard deviations for three to five experiments) for methicillin (M), gentamicin (G), or both (M + G) at concentrations two times the MIC for S. aureus E2371. (A and B) Free bacteria in suspension. (C and D) Catheter segments infected in vitro. (E and F) Catheter segments after infection in mice. Experiments were performed without (A, C, and D) and with (B, D, and F) agitation. For further details, see Materials and Methods. O, control.

imply that intracellular survival influences the outcome of treatment at least in certain foreign body infections. Killing of intracellular bacteria may also partly explain the successful treatment of foreign body infection in the tissue cage model, with the use of a combination of fleroxacin and rifampin (intracellular distribution) and vancomycin (extracellular distribution) (11). However, whether intracellular survival plays a role in clinical foreign body infection remains to be demonstrated. Also, the influence of bacterial metabolism and/or generation time for the bacteria needs to be studied both in experimental models and in clinical studies.

In conclusion, the mouse model of foreign body infection is a simple and reproducible model that can be used to simulate clinical infection. The effect of treatment with methicillin and/or gentamicin was poor, and the location of the bacteria on the catheter and their intracellular survival both seemed to influence the response.

ACKNOWLEDGMENT

This work was supported by the Danish Medical Research Council (grant 12-9510).

REFERENCES

TREATMENT OF S. AUREUS FOREIGN BODY INFECTION


