Continuous-Infusion Ampicillin Therapy of Enterococcal Endocarditis in Rats

CLAUDIE THAUVIN,1,† GEORGE M. ELIOPoulos,1,2* SANDRA WILLEY,1 CHRISTINE WENNERSTEN,1 AND ROBERT C. MOELLERING, JR.1,2

Department of Medicine, New England Deaconess Hospital,1 and Harvard Medical School,2 Boston, Massachusetts 02215

Received 12 May 1986/Accepted 31 October 1986

Interventions administration of ampicillin alone has resulted in high failure rates in previously described animal models of enterococcal endocarditis. We developed a rat model of enterococcal endocarditis which permits comparison of continuous intravenous infusion of ampicillin with intramuscular therapy. Continuous low-dose ampicillin infusion (450 mg/kg [body weight] per day) was compared with the same dose given intramuscularly in three divided doses and with high-dose infusion (4.5 g/kg per day) of the drug. For the infecting strain of Streptococcus faecalis, the MIC and MBC were 1 µg/ml. Mean ampicillin levels in serum were 53.9 ± 4.8 (peak) and 10.7 ± 1.4, and 244 ± 29 µg/ml for intramuscular, low-dose, and high-dose regimens, respectively. Ampicillin infusion therapy significantly increased the survival rate and sterilization of blood cultures. Continuous infusions were superior to intermittent therapy in eradicating bacteremia. After 5 days of treatment, low-dose ampicillin infusion was more effective than intermittent therapy in sterilizing cardiac vegetations (P < 0.01). Continuous-infusion therapy at either dose was significantly more effective than intramuscular injection in reducing bacterial titers in cardiac vegetations (5.4 ± 1.0 log10 CFU/g [low dose], 4.8 ± 0.3 log10 CFU/g [high dose], and 7.7 ± 0.3 log10 CFU/g [intramuscular]). However, no statistically significant advantage was found for high-dose compared with low-dose ampicillin infusion in lowering bacterial titers in vegetations (P > 0.3).

Aside from viridans group streptococci and Staphylococcus aureus, enterococci are the most common pathogens causing bacterial endocarditis, and enterococcal endocarditis represents a major therapeutic challenge (14). Streptococcus faecalis, the most common species of enterococcus to cause human infection, is typically susceptible to ampicillin, but the lack of consistent bactericidal activity of cell-wall-active antibiotics against enterococci usually requires that they be combined with an aminoglycoside to obtain a synergistic bactericidal effect (17). However, under appropriate experimental circumstances, experimental enterococcal endocarditis may be successfully treated with ampicillin alone (21). Studies dealing with the effect of antibiotic administration modes on tissue penetration are limited in number and display contradictory results. However, in some situations, continuous infusion of β-lactam antibiotics may be advantageous (1). The purpose of this study was to evaluate the efficacy of ampicillin administered in a standard fashion by intermittent injection compared with continuous intravenous infusion in experimental enterococcal endocarditis in rats and, further, to compare the effectiveness of low and high doses of ampicillin administered by continuous infusion. These studies assume particular relevance because the recent emergence of S. faecalis isolates containing multiple aminoglycoside-modifying enzymes (16; S. Kathpalia, V. Lorian, R. Levandowski, and G. G. Jackson, Clin. Res. 32:3724, 1984) has created a situation in which penicillin-aminoglycoside therapy is not more effective than penicillin alone and in which the clinician thus has little choice but to use the most active single-drug regimen available when treating endocarditis caused by such organisms.

* Corresponding author.
† Present address: Laboratoire de Bacteriologie, Hotel-Dieu, 76031 Rouen Cedex, France.

MATERIALS AND METHODS

Test organism. S. faecalis 1310 used throughout the study was a clinical blood culture isolate recovered at the Massachusetts General Hospital, Boston, Mass. The MIC and MBC of ampicillin for this strain were 1 µg/ml, determined in dextrose-phosphate broth (GIBCO Diagnostics, Madison, Wis.) by using a macrobroth dilution technique (12).

Killing curves. Evaluation of the bactericidal activity of ampicillin against the test strain was performed by time-kill curve methods described previously (18). Experiments were performed in dextrose-phosphate broth with an inoculum of 105 CFU/ml and ampicillin concentrations of 1, 5, 10, and 250 µg/ml. Flasks were incubated at 37°C in room air without agitation and sampled at the indicated times.

Production of bacterial endocarditis. Bacterial endocarditis was created by the technique of Santoro and Levison (20) that we modified slightly. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were anesthetized with sodium pentobarbital and ether. The right carotid artery of each rat was cannulated with a polyethylene catheter (PE10 Intramedic tubing) (outside diameter, 0.61 mm; inside diameter, 0.28 mm). The catheter was advanced until resistance and pulsations indicated it had reached the aortic valves and passed into the left ventricle. The catheter was secured and left in place for 30 min. At that time, 1 ml of an overnight culture of S. faecalis 1310 (5 × 107 CFU) in tryptic soy broth (GIBCO Diagnostics) was injected intra-arterially through the catheter. The catheter was then heat sealed, and the skin was closed over...
the catheter with a surgical clip. The catheter was left in position across the aortic valve throughout the experiment. At 24 h after catheter insertion, blood cultures were obtained from the retro-orbital venous plexus of each rat. Only animals with positive blood cultures were included in the study.

**Administration of ampicillin.** (i) **Continuous-infusion treatment.** At 24 h after the bacterial challenge, a sterile Silastic catheter (inside diameter, 0.5 mm; outside diameter, 0.94 mm; Dow Corning Corp., Midland, Mich.) was inserted through the jugular vein into the superior vena cava. The line was tunneled subcutaneously and brought out through a stab wound in the skin of the interscapular region. The external portion of this cannula was connected to a flow-through swivel (Intech Laboratories, Philadelphia, Pa.) which permitted the animals to move in an unrestrained fashion during the experiment. Antibiotics in physiologic saline were delivered by pump (Sage pump 352; Orion Research Inc., Cambridge, Mass.) at a flow rate of 0.8 ml/h. Of the rats receiving continuous-infusion therapy, one-half received a low dose (LD) of ampicillin (450 mg/kg [body weight] per day). The other half was given a 10-fold-higher dose (HD) ampicillin (4,500 mg/kg per day).

(ii) i.m. treatment. One group of animals was treated by intermittent intramuscular (i.m.) injections into the hind limb with the same dose of ampicillin as in the LD continuous-infusion group (450 mg/kg per day). This amount was administered in three doses, one every 8 h.

Treatment with ampicillin (continuous infusion or i.m.) was begun approximately 24 h after injection of enterococci, and therapy was continued until death or for 5 days. Untreated infected rats were used as controls once it had been determined that continuous administration of saline alone did not affect the course of endocarditis.

**Monitoring of therapy and outcome.** Ampicillin levels in serum were determined by an agar well diffusion technique with *Bacillus globigii* as the test organism (19). Standard curves were constructed with known antibiotic concentrations diluted in pooled rat serum. With continuous-infusion therapy, ampicillin levels in serum were obtained 24 h after the beginning of therapy. In the i.m. group, trough and peak ampicillin concentrations in serum were obtained on day 2 or 3 of therapy just before and 30 min after an i.m. injection. Ampicillin concentrations achievable within cardiac vegetations were determined in a separate group of noninfected animals. Sterile vegetations were produced as described above. Ampicillin was given as a continuous infusion of 450 mg/kg per day for 24 h or as 150 mg/kg i.m. every 8 h for three doses. Vegetations were excised immediately after discontinuation of the infusion (five animals) or at 0.5, 2, and 4 h after the last i.m. injection (five animals each). Ampicillin concentrations within vegetations were measured by the microtechnique of Cars and Ogren (6) modified by using rat serum in preparation of standards and *Bacillus subtilis* spores (Difco Laboratories, Detroit, Mich.) as the indicator organism. This assay was capable of detecting ampicillin concentrations as low as 0.4 μg/ml. Results were compared with simultaneously obtained ampicillin levels in serum.

Blood cultures were obtained 24 h after the beginning of treatment and on day 5 of therapy as described above. After death or sacrifice of the rat, the heart was excised. Cardiac vegetations were aseptically removed, weighed, suspended in 1 ml of sterile saline, and homogenized in a tissue grinder. Serial 10-fold dilutions of the homogenate were plated onto blood agar plates and incubated at 37°C for 24 h. Bacterial titers were recorded as CFU per gram of vegetation. Only results from animals that survived to receive 5 days of therapy were included in calculations of bacterial titers in vegetations. Animals which died spontaneously were included only in determination of whether sterilization of the vegetation had occurred.

Animals that received continuous-infusion therapy were sacrificed within a few minutes after termination of the antibiotic infusion; those treated with i.m. ampicillin were sacrificed 8 h after the last injection. The following experiment was performed to determine whether residual ampicillin within vegetations falsely reduced bacterial titers measured in animals treated with continuous infusions. Endocarditis was established in six animals, and ampicillin (450 mg/kg per day) was administered for 48 h. Immediately upon termination of therapy, vegetations were excised and processed as described above, except that samples were prepared in duplicate. One sample was plated on Mueller-Hinton agar containing penicillinase (65,000 U/ml; Difco Laboratories), and the other sample was plated on plain agar. Colony counts determined by the two methods were compared.

A 1:2,000 dilution of an overnight culture of *S. faecalis* 1310 was prepared in dextrose-phosphate broth and incubated for 2 h to a cell density of ca. 3 × 10^9 CFU/ml to study the effect of transient ampicillin exposure on subsequent growth of the test organism (postantibiotic effect). Ampicillin was added to a final concentration of 20 μg/ml, and incubation was done for 1 h more. At this time, the cells were pelleted by centrifugation, the antibiotic-containing supernatant was removed, and the cells were suspended in an equal volume of fresh prewarmed broth. Samples of 0.5 ml were removed every 20 min for determination of colony counts.

**Statistical evaluation.** The chi-square test with Yates' correction was used for examination of discrete variables. Differences in bacterial titers among treatment groups were examined by analysis of variance followed by application of the Scheffe multiple comparison method (11).

**RESULTS**

**Bactericidal activity of ampicillin.** The in vitro bactericidal activity of ampicillin against *S. faecalis* 1310 determined by time-kill methods is shown in Fig. 1. The drug demonstrated 24-h bactericidal activity (>99.9% killing) at concentrations comparable to mean levels achieved in serum with both LD and HD ampicillin regimens (see below).

**In vivo endocarditis model.** The levels of ampicillin in serum that were achieved with the various treatment regimens are shown in Table 1. Trough levels of ampicillin determined 8 h after administration of an i.m. dose were undetectable (<1 μg/ml) in all rats in the i.m. group. In two rats in which serial ampicillin levels were measured at six time points over the first 90 min after i.m. dosing, levels averaged 1.8 μg/ml at 2 h and were undetectable by 4 h. Serum levels in blood were obtained at 30 min and 2 h in five additional rats treated with i.m. ampicillin. From these curves of the ampicillin level in serum, an elimination half-life of ca. 0.3 h was estimated, which is consistent with previously observed values of 15 to 28 min for the half-life of ampicillin in serum of rats (10, 22).

Ampicillin concentrations achievable within sterile cardiac vegetations were studied in a group of four uninfected rats that received continuous-infusion LD ampicillin. The mean (± standard error of the mean) concentration of
ampicillin in serum was 9.1 ± 1.8 μg/ml, whereas ampicillin concentrations within vegetations averaged 4.1 ± 2.1 μg/ml. Three groups of five rats each were sacrificed at 30 min, 2 h, and 4 h after the last of three i.m. injections of 150 mg/kg administered every 8 h. In this group of uninfected rats, the 30-min peak ampicillin concentration (75 μg/ml) in serum was higher than that in the original group of infected rats treated with this regimen, but the level in serum fell to a mean of 1.8 μg/ml by 2 h. Ampicillin concentrations within vegetations averaged 50.4 ± 14.6, 71.2 ± 19.5, and 12.7 ± 1.9 μg/ml at 0.5, 2, and 4 h, respectively, giving a crude estimate of an elimination half-life of ca. 0.88 h from the vegetations. By extrapolation, ampicillin concentrations within the vegetations would have exceeded concentrations achieved by LD continuous infusion for ca. 4.25 h of the interdose interval.

The effect of transient antibiotic exposure on cell growth is illustrated by the following results by averaging data obtained from two separate experiments. Colony counts (log₁₀ CFU per milliliter) at 20-min intervals after a 1-h exposure to 20 μg of ampicillin per ml were the following: (time zero after ampicillin, 6.0), 6.1, 6.0, 6.0, 6.0, 6.0, 6.0, 6.1, 6.2, 6.5, 6.6, and 6.8. Colony counts exceeded the starting inoculum by 0.5 log₁₀ CFU/ml only after 3 h.

Five-day survival among rats treated with either continuous-infusion regimen was significantly more frequent than among controls (P < 0.01), but the groups did not differ from each other in this respect. Although survival among animals in the i.m. group (61%) exceeded that in the control group (31%), this result was not statistically significant. Both LD and HD regimens were effective in quenching bacteremia (P < 0.01). Although more animals had sterile vegetations after LD therapy than after HD ampicillin, these results were not statistically significant.

Reduction in bacterial titers within cardiac vegetations was comparable in both infusion groups (P > 0.3). Further, both LD and HD regimens were significantly more effective than i.m. therapy in reducing bacterial titers (P < 0.05 and P < 0.01, respectively). Because of the small size of the cardiac vegetations and dilution factors involved, antibiotic carry-over could not have influenced the determination of bacterial counts within vegetations. This was confirmed by the observation that in a sample of six animals treated with ampicillin for 48 h, colony counts determined on plates containing penicillinase (6.31 log₁₀ CFU/ml ± 0.7 standard error of the mean) did not differ from those obtained on plates made without the enzyme (6.43 log₁₀ CFU/ml ± 0.7 standard error of the mean).

Because of the rapid elimination of ampicillin in treated rats (elimination half-life, ca. 0.3 h), eight additional animals were treated with the i.m. regimen plus probenecid (50 mg/kg per day) subcutaneously. The addition of probenecid prolonged the elimination half-life to ca. 1 h (determined in two rats) but failed to increase the 5-day survival (50%) or to significantly affect the bacterial titer within vegetations (mean, 7.07 log₁₀ CFU/g).

**DISCUSSION**

The relative value of intermittent versus continuous administration of antibiotics during therapy of serious infections such as endocarditis has been debated for years. Although continuous infusions of ampicillin (often with an aminoglycoside) have been used clinically with good success in the treatment of streptococcal endocarditis (8, 15, 23), some researchers have described better results with and, hence, a preference for intermittent dosage schedules (7, 9). Support for the latter mode of administration has emerged from animal experiments such as those demonstrating higher antibiotic levels in subcutaneously implanted fibrin clots when ampicillin is administered by bolus injection rather than by continuous infusion (2). Recently, Lavoie and Bergeron (13) demonstrated that antibiotic penetration into fibrin clots during ampicillin infusion is slow and results in poor killing of bacteria. However, at equilibrium, continuous

**TABLE 1. Comparison of ampicillin therapy by continuous infusion and i.m. injection in rats with enterococcal endocarditis**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of animals</th>
<th>Ampicillin level in serum (mean ± SD) (μg/ml)</th>
<th>No. (%) of survivors to day 5</th>
<th>No. (%) with sterile blood culture/no. alive</th>
<th>No. (%) with sterile vegetations</th>
<th>Bacterial titer in vegetations (mean ± SD) (log₁₀ CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>5 (31)</td>
<td>0/15(0)</td>
<td>0 (0)</td>
<td>9.8 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin i.m.</td>
<td>18</td>
<td>53.9 ± 4.8‡</td>
<td>11 (61)</td>
<td>3/10 (30)</td>
<td>7.7 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>LD</td>
<td>19</td>
<td>8.7 ± 1.4</td>
<td>16 (84)</td>
<td>15/16 (94)</td>
<td>5.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>HD</td>
<td>15</td>
<td>244.0 ± 29</td>
<td>13 (87)</td>
<td>13/13 (100)</td>
<td>4.8 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

*‡ Number alive at time of blood culture.

*§ After 5 days of therapy.

*Peak level in serum.
infusion produced antibiotic levels in fibrin clots that were similar to those achieved when the same daily dose was given intermittently in four portions. Bergeron et al. (4) also found combinations of penicillin with gentamicin to be less effective in reducing titers of S. faecalis within fibrin clots when given by continuous infusion rather than by intermittent bolus injection. Nevertheless, the present study demonstrated the definite superiority of continuous-infusion ampicillin therapy over an intermittent bolus regimen incorporating the same total daily dose. An advantage for the former mode of administration was apparent in the reduction not only of bacterial titers within vegetations but also of mortality and number of animals with positive blood cultures. Another recent study demonstrated an advantage of continuous rather than intermittent penicillin dosage in the treatment of pneumococcal infections in complement-depleted rats (1).

In large part, the superiority of the continuous-infusion method of administration can be attributed to the rapid elimination of ampicillin in rats. Nevertheless, prolongation of the elimination half-life of this drug with probenecid to approximately 1 h failed to substantially enhance the effectiveness of the i.m. regimen. After i.m. injection, ampicillin concentrations within cardiac vegetations achieved a level of approximately 90% of the peak level in serum and diminished more slowly than did concentrations in serum. Furthermore, we observed a lag time of approximately 3 h before regrowth of the test strain after exposure to ampicillin concentrations achievable within vegetations, a result consistent with the previously reported postantibiotic effect of ampicillin (2 to 4 h) on other streptococci (5). In light of these facts, it is somewhat surprising that the i.m. regimen used here was so poorly effective. Although we cannot exclude the possibility that circulating antibiotic resulted in spurious high rates of apparent blood culture sterilization in animals receiving continuous-infusion therapy, our results show conclusively that antibiotic carry-over could not have affected the determination of bacterial titers within cardiac vegetations.

The ability to reliably deliver this β-lactam antibiotic at a continuous rate in this animal model permitted assessment of the relative effectiveness of HD versus LD ampicillin therapy. Despite an apparent advantage of the higher antibiotic concentration by in vitro time-kill study, there was little evidence for enhanced efficacy with the HD regimen in our animal model.

Although treatment of serious enterococcal infections such as endocarditis is generally thought to require combination therapy consisting of a cell-wall-active antibiotic plus an aminoglycoside to achieve a synergistic bactericidal effect, it is conceivable that such therapy is not always necessary or possible (3, 21). Indeed, against strains of S. faecalis which produce multiple aminoglycoside-modifying enzymes and, therefore, which are not synergistically killed by ampicillin-aminoglycoside combinations, β-lactam monotherapy may be the only therapeutic option (16; Kathpalia et al., Clin. Res., 1985). The present study suggests that if this were the case, it might be preferable to administer ampicillin by continuous infusion, or at least at very short dosage intervals, to maximize the chance of therapeutic response. However, in view of the fact that only a single test strain was examined in this study and in light of the dangers inherent in generalization from an animal model, such extrapolation to a clinical setting must be done with extreme caution. This is particularly true because there is already a report of apparent failure of ampicillin to cure endocarditis in a patient infected with a strain of S. faecalis resistant to multiple aminoglycosides (Kathpalia et al., Clin. Res., 1985). However, the mode of ampicillin administration was not specified in that report. Indeed, some enterococcal strains may show a greater degree of in vitro tolerance to the bactericidal activity of ampicillin than did the strain selected for the present study.

The model described here provides a convenient and relatively inexpensive means to corroborate in vitro test results and to compare various therapeutic regimens. Because treatment courses lasting longer than 5 days are associated with a high rate of failure of line patency, this model may be inappropriate for assessing cures for infections such as endocarditis, which generally require a longer duration of therapy.

ACKNOWLEDGMENT

This work was supported in part by a research fellowship grant from the Centre National de la Recherche Scientifique (Ministere de l’Industrie et de la Recherche), Paris, France, to C.T.

LITERATURE CITED


