Efficacy of Ticarcillin-Clavulanic Acid for Treatment of Experimental Staphylococcus aureus Endocarditis in Rats

ELAINE J. CATHERALL,* VALERIE GILLON, SARAH HURN, RAYMOND IRWIN, AND LINDA MIZEN

SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey, RH3 7AJ, United Kingdom

Received 1 July 1991/Accepted 25 October 1991

The efficacy of ticarcillin-clavulanic acid was compared with the efficacies of standard antistaphylococcal agents (fluoxacillin, oxacillin, nafcillin, and vancomycin) and ticarcillin in an experimental model of Staphylococcus aureus endocarditis. Therapy was either initiated soon (8 h) after infection, when numbers of bacteria in aortic valve vegetations were relatively low (approximately 6 to 8 log_{10} CFU/g), or delayed until 24 h after infection, when the vegetations usually contained >9 log_{10} CFU/g. Doses of the antibiotic were selected to produce peak concentrations in rat serum similar to those achievable in humans after administration of parenteral therapeutic doses. Ticarcillin-clavulanic acid was more effective overall than ticarcillin alone against endocarditis caused by \beta-lactamase-producing strains of S. aureus, illustrating the \beta-lactamase-inhibitory activity of clavulanic acid in vivo. Ticarcillin-clavulanic acid was as effective as the standard antistaphylococcal \beta-lactam agents fluoxacillin, oxacillin, and nafcillin in these infections, whereas vancomycin was generally less active. These results illustrate the clinical potential of ticarcillin-clavulanic acid in the prophylaxis or therapy of severe staphylococcal infections.

Staphylococcus aureus remains a major pathogen, and 80% to greater than 90% of clinical isolates of S. aureus are \beta-lactamase producers (10, 17). Clavulanic acid is a potent inhibitor of a wide range of bacterial \beta-lactamas es, including those produced by S. aureus (22). The combination of ticarcillin and clavulanic acid has been shown to extend the spectrum of ticarcillin to include \beta-lactamase-producing strains both in vitro (29) and in vivo (2, 21, 29), and successful treatment of clinical staphylococcal infections with ticarcillin-clavulanic acid has been reported (9, 15).

Experimental Staphylococcus endocarditis represents one of the more severe of the available animal models of staphylococcal infections. It is considered to be a discriminative model closely resembling the human infection in pathology and mortality (10, 25) and is a stringent test of antibiotic efficacy, especially when therapy is delayed until the endocarditis is particularly well established.

The combination of clavulanic acid with amoxicillin has been shown to be effective in rats against early endocarditis in which the vegetations contained approximately 6 to 8 log_{10} CFU of S. aureus per g (3, 5) and against late S. aureus endocarditis in which bacterial counts were 9 to 10 log_{10} CFU/g (5). The activity of a combination of clavulanic acid with ticarcillin against S. aureus endocarditis in rabbits has been reported elsewhere (7, 24).

Accordingly, the efficacy of ticarcillin-clavulanic acid in rats against early and late endocarditis caused by four \beta-lactamase-producing, methicillin-susceptible strains of S. aureus has been assessed. The activity of the combination was compared with that of ticarcillin alone and with the activities of agents commonly employed in the treatment of staphylococcal infections: fluoxacillin (against all four strains), vancomycin (against two strains), and oxacillin and nafcillin (against one strain).

(Materials and methods)

Staphylococcus aureus was isolated from the aortic valve vegetation of each rat. The organisms were propagated in nutrient broth and subcultured to Mueller-Hinton agar (Difco Laboratories). The plates were inoculated with 0.001 ml of an overnight broth culture to yield an inoculum of approximately 6 log_{10} CFU per spot. The plates were incubated for 18 h at 37°C, and the MICs were determined as the lowest concentrations of antibiotic preventing visible growth.

Production of endocarditis. Endocarditis was produced in rats as previously described (5). Briefly described, under anesthesia, a polyethylene catheter (Portex Ltd., Hythe, Kent, England) (internal diameter, 0.28 mm; external diameter, 0.61 mm) was inserted into the right carotid artery, passed towards the heart so that it crossed the aortic valve, and tied in place. At 24 to 72 h later, each rat received an intravenous injection containing 6 log_{10} CFU of S. aureus from an overnight culture in 0.75 ml of PBS.

The catheter was left in place throughout the studies, resulting in the development of a severe staphylococcal infection. Preliminary studies (results not shown) demonstrated that most animals died from infection in 1 to 4 days in

MATERIALS AND METHODS

Experimental animals. Male CD rats (Charles River, Manton, Kent, England) weighing 250 to 350 g were used in these studies.

Antibiotics. Disodium ticarcillin, potassium clavulanate, fluoxacillin (Floxapen), oxacillin, and nafcillin were supplied by SmithKline Beecham Pharmaceuticals, Worthing, England. Vancomycin was a commercial preparation (Vancom; Eli Lilly, Basingstoke, England). The compounds were dissolved in phosphate-buffered saline (PBS) (0.1 M, pH 7.4).

Test organisms. Four \beta-lactamase-producing, methicillin-susceptible clinical isolates of S. aureus were used in these studies: S. aureus HOP, WB112, G19, and 875. The organisms were maintained on slopes of nutrient agar (Lab-M) at room temperature and cultured into Tryptone Soy Broth (Oxoid) for 18 h for preparation of the infecting inocula.

In vitro susceptibilities of the individual strains to ticarcillin alone, ticarcillin in the presence of 2 \mu g of clavulanic acid per ml, fluoxacillin, oxacillin, nafcillin, and vancomycin were determined by twofold serial dilution in Mueller-Hinton agar (Difco Laboratories). The plates were inoculated with 0.001 ml of an overnight broth culture to yield an inoculum of approximately 6 log_{10} CFU per spot. The plates were incubated for 18 h at 37°C, and the MICs were determined as the lowest concentrations of antibiotic preventing visible growth.

Production of endocarditis. Endocarditis was produced in rats as previously described (5). Briefly described, under anesthesia, a polyethylene catheter (Portex Ltd., Hythe, Kent, England) (internal diameter, 0.28 mm; external diameter, 0.61 mm) was inserted into the right carotid artery, passed towards the heart so that it crossed the aortic valve, and tied in place. At 24 to 72 h later, each rat received an intravenous injection containing 6 log_{10} CFU of S. aureus from an overnight culture in 0.75 ml of PBS.

The catheter was left in place throughout the studies, resulting in the development of a severe staphylococcal infection. Preliminary studies (results not shown) demonstrated that most animals died from infection in 1 to 4 days in

* Corresponding author.
the absence of antibiotic treatment, and bacterial counts in the vegetations of these animals were high (9 to 11 log_{10} CFU/g).

**Therapy.** For early endocarditis, therapy was initiated at 8 h after infection, at which time viable counts of *S. aureus* in the vegetations were between 4 and 8 log_{10} CFU/g, and dosing continued for 3 days. Antibiotics were administered by the subcutaneous route every 8 h. Groups of rats (minimum group size of six animals) were administered ticarcillin-clavulanic acid (500 and 50 mg of ticarcillin and clavulanic acid, respectively, per kg of body weight), ticarcillin (500 mg/kg), or flucloxacillin (400 mg/kg). The activity of ticarcillin-clavulanic acid was also compared with the activities of oxacillin (400 mg/kg) and nafcillin (400 mg/kg) against *S. aureus* HOP.

In the studies in which efficacy against established endocarditis was assessed, therapy began at 24 h after infection, when the vegetations contained 8 to 11 log_{10} CFU/g, and dosing continued every 8 h for 6 days. Ticarcillin-clavulanic acid, ticarcillin, and flucloxacillin were administered as described above.

Vancomycin (160 mg/kg) was included as a comparator agent in the studies with *S. aureus* WB112 (8 and 24 h) and *S. aureus* HOP (8 h only).

The doses administered were selected to produce peak concentrations of the antibiotics in rat serum of the same order as those observed in humans after parenteral dosage for serious infections. In each study, control rats were killed at the time of starting treatment to determine the magnitude of valvular infection and incidence of positive blood cultures.

**Assessment of therapy.** The surviving rats in the treated groups were killed approximately 16 h after the last dose, by which time antibiotic concentrations in the sera were undetectable. Blood samples were taken, the hearts were removed aseptically, and aortic valve vegetations were excised, weighed, and homogenized in 1 ml of PBS. Bacterial numbers in the homogenates and blood samples on nutrient agar were determined by using a spiral plater (Don Whitney Scientific Ltd., Shipley, United Kingdom) and the spread plate technique. Plates were read after incubation at 37°C for 24 to 48 h. Results were expressed as CFU per gram of vegetation (this method permitted the detection of 2 log_{10} CFU/g) and CFU per milliliter of blood. The bacterial counts in the vegetations of any rats that died during therapy were also assessed when the rats had received at least 5 doses of antibiotic.

**Measurement of antibiotic concentrations in serum.** Blood samples were removed from the tail veins of normal rats at intervals after subcutaneous administration of ticarcillin-clavulanic acid (500 and 50 mg/kg, respectively), flucloxacillin (400 mg/kg), oxacillin (400 mg/kg), nafcillin (400 mg/kg), or vancomycin (160 mg/kg), and the serum was separated. Concentrations were determined by using a large-plate agar diffusion microbiological assay, with *Sarcina lutea* ATCC 9341 as the assay organism for flucloxacillin, oxacillin, nafcillin, and vancomycin and *Pseudomonas aeruginosa* ATCC 29336 as the assay organism for ticarcillin. Clavulanic acid was assayed by a β-lactamase-inhibition assay with *Klebsiella pneumoniae* ATCC 29665 (18). The plates were incubated for 18 h at 37°C, and antibiotic concentrations were derived from standard lines prepared from standard solutions in diluted pooled rat serum.

**Statistical evaluation.** Bacterial counts in the vegetations were analyzed for each strain by the Kruskal-Wallis nonparametric one-way analysis of variance (26) for early and established endocarditis. Within each analysis, ticarcillin-clavulanic acid was compared with each of the other treatments in turn. This was achieved by pooling data for the group in question with data for the ticarcillin-clavulanic acid group and comparing the resulting chi-square statistic with that for the analysis using the original groupings. Statistical significance was assessed by using Bonferroni's adjustment for multiple comparisons (28).

**RESULTS**

**Antibiotic concentrations in serum.** The antibiotic concentrations in rat serum following subcutaneous administration of ticarcillin-clavulanic acid, flucloxacillin, oxacillin, nafcillin, and vancomycin are shown in Fig. 1. The peak concentrations observed in the rats were of the same order as those measured in humans after parenteral dosing for serious infections.

**MIC determinations.** The MICs of ticarcillin were 32, 16, 64, and 128 μg/ml for *S. aureus* WB112, HOP, G19, and 875, respectively. The activity of ticarcillin against these β-lactamase-producing strains of *S. aureus* was notably enhanced (2 μg/ml for *S. aureus* WB112 and HOP and 4 μg/ml for *S. aureus* G19 and 875) in the presence of 2 μg of clavulanic
Ticarcillin-clavulanic acid

Ticarcillin

(flucloxacillin and nafcillin were similar in efficacy against S. aureus WB112, and 25% of the vegetations were sterilized by both treatments. In contrast, ticarcillin administered alone was not active against this strain, and no vegetations in this group were sterilized.

Blood samples from the treated groups in all 8-h studies were sterile, except for those from one rat administered vancomycin in the S. aureus WB112 study and one rat administered ticarcillin in the S. aureus HOP study; both these animals had very high counts in the vegetations.

**Therapy: established endocarditis.** The median viable counts in the vegetations of the control rats killed at 24 h after infection exceeded 9 log_{10} CFU/g (Table 2), and bacteria (3 to 6 log_{10} CFU/ml) were detected in all the blood cultures. Against the established infection of S. aureus WB112 (Table 2), ticarcillin-clavulanic acid was as effective as flucloxacillin, and both treatments sterilized 50% of the vegetations. Ticarcillin-clavulanic acid was significantly more effective than ticarcillin alone (P ≤ 0.05), and no vegetations were sterilized in this group. Vancomycin sterilized 22% of the vegetations in this study. No rats in the ticarcillin-clavulanic acid groups died, whereas 70% of the rats administered ticarcillin alone died, and 38 and 33% in the groups that received flucloxacillin or vancomycin died, respectively. Blood samples from all surviving animals administered ticarcillin-clavulanic acid, flucloxacillin, or vancomycin were sterile, but blood samples of only 66% of rats given ticarcillin alone were sterile.

The treatments were less effective against the fully established infection with S. aureus HOP (Table 2) than against S. aureus WB112. Flucloxacillin reduced the mean viable count in the vegetations compared with that of the controls, and

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>S. aureus WB112</th>
<th>S. aureus HOP</th>
<th>S. aureus G19</th>
<th>S. aureus 875</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats</td>
<td>% of vegetations sterile</td>
<td>Median CFU/g of vegetation (range)</td>
<td>No. of rats</td>
</tr>
<tr>
<td>None (control)</td>
<td>4</td>
<td>0</td>
<td>10.7** (6.2-11.4)</td>
<td>4</td>
</tr>
<tr>
<td>Ticarcillin-clavulanic acid</td>
<td>8</td>
<td>50</td>
<td>2.6 (&lt;2-8.4)</td>
<td>10</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>10</td>
<td>0</td>
<td>9.7* (4.0-10.6)</td>
<td>9</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>8</td>
<td>50</td>
<td>2.1 (&lt;2-4.6)</td>
<td>11</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>9</td>
<td>22</td>
<td>4.7 (&lt;2-10.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* a **, significant at P ≤ 0.05, compared with results for ticarcillin-clavulanic acid.

**b**, significant at P ≤ 0.01, compared with results for ticarcillin-clavulanic acid.
27% of the vegetations were sterile. Mean bacterial numbers in the vegetations were reduced marginally by therapy with ticarcillin-clavulanic acid, and the vegetations were sterilized in 10% of the animals. In contrast, ticarcillin alone was ineffective. Death rates were 70% in the ticarcillin-clavulanic acid group, 55% in the flucloxacillin group, and 89% in the group given ticarcillin alone. Blood samples from the surviving rats from the ticarcillin-clavulanic acid and flucloxacillin groups but not from the one rat from the ticarcillin group that survived, were sterile.

Ticarcillin-clavulanic acid caused a significant reduction in bacterial numbers in the vegetations (P ≤ 0.05 compared with infected controls) against S. aureus G19 (Table 2), sterilizing 33% of the vegetations. The combination was significantly more effective than ticarcillin alone (P ≤ 0.01), and no vegetations in the ticarcillin group were sterilized. Flucloxacillin also reduced bacterial numbers in the vegetations, but in contrast to the results with ticarcillin-clavulanic acid, no vegetations were sterilized. Death rates in the groups were 33% for ticarcillin-clavulanic acid, 40% for flucloxacillin, and 56% for ticarcillin. Blood samples from surviving animals in all the treated groups in this study were sterile.

DISCUSSION

The data presented here demonstrate that in the presence of clavulanic acid, ticarcillin was active against β-lactamase-producing strains of S. aureus in the rat endocarditis model. Although ticarcillin alone did show some activity against two of the strains when therapy was initiated early and bacterial numbers in the vegetations were relatively low, it was ineffective against all strains tested when treatment was delayed, in contrast to ticarcillin-clavulanic acid, demonstrating the β-lactamase-inhibitory effects of clavulanic acid in vivo.

When dosing began early (8 h after infection), ticarcillin-clavulanic acid was highly effective and sterilizing the majority of the aortic valve vegetations in the studies with three of the four strains. Ticarcillin-clavulanic acid was comparable in efficacy to flucloxacillin, oxacillin, and nafcillin. In contrast, the activity of ticarcillin alone in these studies was variable. Against two strains (S. aureus WB112 and G19), ticarcillin was almost as effective as ticarcillin-clavulanic acid, whereas against S. aureus HOP and 875, ticarcillin was less effective. Since in terms of MICs S. aureus G19 and 875 were the strains most resistant to ticarcillin, this suggests that factors other than the in vitro susceptibilities were responsible for these differences in vivo activity, and differences in strain virulence may be one of the factors.

The bacterial numbers in the vegetations and blood samples of rats 24 h after infection were higher than those in rats 8 h after infection, reflecting the greater severity of the endocarditis at the later time. Consequently, although therapy was continued for 6 days in the 24-h studies, all treatments were less effective in sterilizing the vegetations, more blood cultures were positive, and a greater number of rats died during therapy than in the studies in which dosing began at 8 h after infection. The rats that died during therapy and/or contained bacteria in their blood tended to be the animals with the highest numbers of bacteria in the vegetations (usually >8 log10 CFU/g).

Ticarcillin-clavulanic acid and flucloxacillin were similarly effective against the established S. aureus WB112 and G19 infections; however, all the delayed treatments showed poor activity against S. aureus HOP, regardless of MICs. This variation in response among the strains may be a further indication of differences in virulence. Ticarcillin administration was considerably less effective than ticarcillin-clavulanic acid against the fully established endocarditis with all the strains tested, in contrast to results from the 8-h studies, presumably because local levels of β-lactamase were higher at the later time, when the vegetations contained greater numbers of bacteria more closely packed together and overlaid with fibrin (11).

The mortality rates in these established endocarditis studies were similar to those of around 30 to 50% that have been reported to occur in both animals (10, 25) and humans (10, 20), despite appropriate therapy of S. aureus endocarditis, or in serious staphylococcal infections in general (12, 17). These experimental studies are, therefore, in keeping with published clinical findings which stress the need for early diagnosis and prompt antibiotic treatment of staphylococcal endocarditis (12, 14).

Vancomycin was generally less effective than ticarcillin-clavulanic acid or flucloxacillin, although concentrations of vancomycin in rat serum were above the MIC for the duration of the dosing period. This relatively poor activity of vancomycin has been demonstrated in other S. aureus endocarditis studies, both in rats (3–5) and in rabbits (6, 8). It has also been reported that in vitro vancomycin may kill S. aureus more slowly than β-lactam antibiotics (27) and that in experimental endocarditis in both rats and rabbits, the efficacy of vancomycin was improved by extending the dosing period (1, 4, 25). It is of interest that failures in the treatment of severe clinical staphylococcal infections with vancomycin as a single agent have been reported (13, 16, 19, 27), and vancomycin is often combined with another antibiotic in these situations (13, 30).

In summary, the results described here clearly demonstrate the efficacy of ticarcillin-clavulanic acid against severe staphylococcal infection in rats. When administered in combination with clavulanic acid, ticarcillin was at least as effective as flucloxacillin, oxacillin, and nafcillin, agents with proven clinical efficacy in the treatment of severe staphylococcal infections. Ticarcillin and clavulanic acid are less serum bound than the isoxazolyl penicillins and nafcillin in humans (23), and this may indicate a greater clinical potential for the combination. The experimental model employed is generally considered to be predictive for the clinical situation, and thus these results are encouraging in supporting the use of ticarcillin-clavulanic acid for the prophylaxis and treatment of staphylococcal infections, particularly those caused by β-lactamase-producing, methicillin-susceptible S. aureus.

ACKNOWLEDGMENTS

We thank Ian Macpherson and Caroline Wong for carrying out the statistical evaluation and Joyce Broullit for typing the manuscript.

REFERENCES


In vivo and in vitro observations. JAMA 236:1604-1606.


