Mezlocillin and Ticarcillin Alone and Combined with Gentamicin in the Treatment of Experimental Enterobacter aerogenes Endocarditis

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The efficacies of mezlocillin and ticarcillin, each alone and in combination with gentamicin, in the therapy of experimental left-sided Enterobacter aerogenes endocarditis in rabbits were compared. Each beta-lactam was administered intramuscularly at a dose of 180 mg/kg every 6 h either alone or with gentamicin (1.7 mg/kg intramuscularly every 8 h). Bacterial populations at the start of therapy (7 days after initiation of infection) were 9 to 10 log_{10} CFU/g of vegetation. Ticarcillin produced concentrations in serum that were twice those produced by mezlocillin, but the therapeutic ratios of mezlocillin and ticarcillin (ratio of peak level in serum to MBC) were the same. All of the therapeutic regimens given for either 5 or 10 days were effective in reducing vegetation counts when compared with the untreated controls (P < 0.01 for all comparisons), except mezlocillin alone and ticarcillin alone, which caused insignificant reductions in counts after 5 days of therapy (P > 0.05). After 10 days of therapy, the only regimen that was significantly different from another was that of mezlocillin plus gentamicin, which was significantly better than that of ticarcillin alone (P < 0.01). These studies document that mezlocillin and ticarcillin were both effective in reducing the numbers of E. aerogenes CFU in vegetations in rabbits with experimental endocarditis when the drugs were given over a prolonged course. More rapid and extensive reduction in vegetation counts was achieved with combinations of an aminoglycoside plus mezlocillin or ticarcillin. Mortality was significantly less among rabbits treated with mezlocillin plus gentamicin.

The rabbit endocarditis model is a rigorous test of antibiotic efficacy, owing in part to the large number of bacteria per gram of vegetation, the secluded focus of infection, and the continued presence of a foreign body. Previous data on gram-negative bacillary endocarditis in this model have suggested that antimicrobial agents such as carbenicillin, cefoperazone, and gentamicin, if sufficiently potent, relatively nontoxic, and used in doses which result in high peak bactericidal activity in serum, may be effective (1, 6). Clinically, however, therapy of left-sided endocarditis caused by gram-negative rods with antibiotics such as carbenicillin, ticarcillin, piperacillin, or an aminoglycoside, with or without surgery, has been ineffective (3).

A new group of structurally similar antibiotics, the ureidopenicillins, which includes azlocillin, mezlocillin, and piperacillin, have greater activity against members of the family Enterobacteriaceae and Pseudomonas aeruginosa than does carbenicillin or ticarcillin. However, the activities of the ureidopenicillins have been reported to be influenced by inoculum size, which has been attributed to their greater instability to beta-lactamases. The purpose of this study was to compare the effectiveness of ticarcillin and mezlocillin, each alone and combined with gentamicin, in the treatment of left-sided experimental Enterobacter aerogenes endocarditis in rabbits.

MATERIALS AND METHODS

Organism. A beta-lactamase-positive clinical isolate of E. aerogenes was used (6). The MICs and MBCs of mezlocillin, ticarcillin, and gentamicin for E. aerogenes were determined by a broth dilution method in Mueller-Hinton broth (MHB). The antibiotics were diluted in twofold steps in tubes each containing 0.5 ml of MHB. The bacterial inoculum was added to each tube in 0.5 ml of MHB diluted from an 18-h MHB culture to give a final concentration of 10^{5.5} CFU of E. aerogenes per ml. The MIC was defined as the lowest antibiotic concentration that prevented turbidity after 18 h of incubation at 37°C. The MBC was defined as the lowest antibiotic concentration that reduced the inoculum by $\geq 99.9\%$ within 18 h, as determined by plating portions of the MIC dilutions.

Stock cultures were made by incubating the organism in MHB at 37°C for 24 h and storing 1-ml samples at −20°C. For each experiment, a sample was subcultured into MHB and incubated at 37°C for 18 h.

In vitro studies. The rate of kill of E. aerogenes was studied in MHB containing mezlocillin, ticarcillin, gentamicin, mezlocillin plus gentamicin, or ticarcillin plus gentamicin and in MHB without antibiotics. The inoculum in MHB was added to each flask and incubated at 37°C, and portions were removed at 0, 3, 6, 24, and 48 h. Each portion was serially diluted in 10-fold steps in MHB, and 0.1 ml of each dilution was plated on the surfaces of Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) plates with 5% sheep blood. After 18 h of incubation at 37°C, the numbers of colonies on the plates were counted and the numbers of CFU in the flasks were calculated.

Therapeutic experiments. Female white New Zealand rabbits (Ace Animals, Inc., Boyertown, Pa.), each weighing 2 to 2.7 kg, were anesthetized, and the right carotid artery of each was cannulated as previously described (6). At 24 h after the placement of the catheter, each rabbit was inoculated in an ear vein with 1 ml of normal saline containing 3 × 10^6 CFU of E. aerogenes washed in saline. This inoculum produced endocarditis in all 188 rabbits injected, of which 118 survived for 7 days. At this time, the rabbits were randomly divided into five groups and treated in one of the following manners: 19 rabbits were given mezlocillin, 16 were given mezlocillin plus gentamicin, 23 were given ticar-
cillin, 22 were given ticarcillin plus gentamicin, and 20 were
given gentamicin. A sixth group of 18 rabbits was left
untreated. Antibiotics were administered intramuscularly
to a dose of either 180 mg of mezlocillin or ticarcillin per kg
of body weight every 6 h (7 a.m., 12 noon, 6 p.m., and 12
midnight) or 1.7 mg of gentamicin per kg every 8 h (8 a.m.,
4 p.m., and 12 midnight).

After 5 or 10 days of therapy, the surviving rabbits were
sacrificed by the intravenous injection of sodium pentobarbi-
tal 12 h after the last injection of antibiotic. The chest
was opened, and all aortic valve vegetations from the rabbit
were excised, pooled, and weighed. The vegetations from
each rabbit weighed a total of 0.01 to 0.7 g. The vegetations from
each rabbit were suspended in 10 times the weight (by
volume) of MHB and homogenized, following which the
numbers of CFU in the homogenates were determined by
previously described serial dilution and plating techniques
(6). In “sterile” vegetations, the number of CFU was
recorded as 2 log₁₀ CFU/g, since the largest weight of
vegetation plated was 0.01 g. The catheter was left in place
throughout the experiment.

Drug concentrations in serum. Blood was taken from the
ear veins of uninfected rabbits at 0.25, 0.5, 1, 2, and 4 h after
the first injection of each antibiotic. These rabbits were then
discarded from the experiments. Serum was separated and
stored at −20°C until assay for antibiotic levels in serum.
Concentrations of mezlocillin, ticarcillin, and gentamicin in
serum were determined by an agar diffusion method with
paper disks (3). In a group of infected rabbits, creatinine
and gentamicin concentrations in serum were measured initially
and after 10 days of antibiotic therapy.

Statistical analysis. The half-lives of the antibiotics in
serum were calculated by the method of least squares (3).
Two-factor analysis of variance, followed by the Tukey A
post hoc procedure with the harmonic mean cell size, was
used to determine significant differences among bacterial
counts in vegetation. The independent variables were therapeu-
tic agents and duration of therapy. The dependent variable
was CFU per gram of vegetation. Statistical differences
in mortality and sterilization of vegetations were analyzed by
the chi-square test.

RESULTS

In vitro studies. The MICs and MBCs of ticarcillin, mezlo-
cillin, and gentamicin for E. aerogenes were both 31.3, 15.6,
and 3.1 μg/ml, respectively.

Figure 1 shows the rate of decrease in numbers of E.
aerogenes CFU in broth containing 100 μg of mezlocillin,
100 μg of ticarcillin, and 3 μg of gentamicin, alone and in
combinations, per ml. The inoculum was 10⁸.⁵/ml, which is
similar to bacterial populations in vegetations. Mezlocillin
alone caused slow and incomplete killing by 48 h of incuba-
tion. Ticarcillin alone caused an initial fall in numbers of
CFU, after which regrowth occurred. Only the combinations
of either mezlocillin or ticarcillin plus gentamicin reduced
populations rapidly and completely. Bioassay of each broth
containing the beta-lactams alone indicated that inactivation
of the beta-lactam had not occurred. Also, the MIC and
MBC for the surviving E. aerogenes population had not
changed.

Antibiotic levels in serum. Table 1 shows the mean concen-
trations and half-lives of mezlocillin, ticarcillin, and genta-
micin in serum. The half-lives of mezlocillin and ticarcillin in
serum were both 0.8 h, and the half-life of gentamicin
was 1.3 h. For evaluation of the possibility of nephrotoxicity
from gentamicin, concentrations of creatinine and gentami-
ic in the sera of 10 infected rabbits at 1 h after dosing, were
determined after 10 days of therapy with gentamicin alone or
in combination with either mezlocillin or ticarcillin. Creati-
nine concentrations in serum ranged from 0.7 to 1.5 mg/dl, a
level which is normal for rabbits (7). Gentamicin concentra-
tions in serum at 1 h after dosing ranged from 2.9 to 3.2 μg/ml
in rabbits on day 10 of therapy, in comparison with a mean
concentration of 5.4 μg/ml in serum at 1 h after the first dose.

Therapeutic experiments. Results of experiments in treated
and untreated rabbits after 5 and 10 days are shown in Table
2. The analysis of variance among vegetation bacterial
counts revealed a significant main effect of both antibiotics
(P < 0.001) and duration of treatment, i.e., 5 versus 10 days
(P < 0.001); the two-factor interaction was also significant (P
= 0.011). The Tukey A post hoc procedure revealed (Table
3) that all of the therapeutic regimens given for either 5 or 10
days were effective in reducing vegetation counts when
compared with the untreated controls (P < 0.01 for all
comparisons), except mezlocillin alone and ticarcillin alone,
with which insignificant reductions in counts were observed
after 5 days of therapy (P > 0.05). However, these latter two
regimens resulted in significant reductions (P < 0.01) after 10
days of therapy (5.4 and 3.8 log₁₀, respectively). Mezlocillin
alone did not differ significantly from ticarcillin alone after 5
or 10 days of therapy (P > 0.05). The activity of mezlocillin
plus gentamicin was not significantly different from that of
ticarcillin plus gentamicin (P > 0.05) but was significantly
better than those of gentamicin, ticarcillin, and mezlocillin
each alone (P < 0.01) after 5 days of therapy. In contrast,
although the combination of ticarcillin plus gentamicin was
more effective than either ticarcillin or mezlocillin alone (P
< 0.01), it was not more effective than gentamicin alone (P
> 0.05) after 5 days of therapy. After 10 days of therapy, the
only regimen that was significantly different from another

FIG. 1. Rate of decrease of numbers of E. aerogenes CFU in
broth containing 100 μg of ticarcillin (T), 100 μg of mezlocillin (M),
or 3 μg of gentamicin (G) per ml alone and in combinations (M+G,
T+G).
was that of mezlocillin plus gentamicin, which was significantly better than that of ticarcillin alone (P < 0.01).

Mortality was 36% (70 of 188 rabbits died) in the 7 days before initiation of therapy. Subsequently, 3 of 18 rabbits left untreated for 5 to 10 days died, 2 of 20 rabbits treated with gentamicin alone died, and 10 of 45 rabbits treated with ticarcillin with or without gentamicin died, but 0 of 35 rabbits treated with mezlocillin with or without gentamicin died (χ² = 8.9, P > 0.05 at 3 df for 4 × 2 table comparison of mortality versus rabbits treated with mezlocillin with or without gentamicin, ticarcillin with or without gentamicin, and gentamicin alone and untreated controls). To find which groups contributed to the significance in the 4 × 2 comparison, we performed 2 × 2 chi-square analyses at the original 3 df to control for possible erroneous inferences as a consequence of making comparisons suggested by the data (4). The following groups were analyzed: control versus gentamicin alone (P > 0.05); ticarcillin with or without gentamicin versus control and gentamicin alone (P > 0.05), mezlocillin with or without gentamicin versus control and gentamicin alone (P > 0.05), and mezlocillin with or without gentamicin versus ticarcillin with or without gentamicin (χ² = 8.9, P < 0.05 at 3 df).

After 5 and 10 days, 10 of 17 and 8 of 16 rabbits, respectively, treated with either beta-lactam plus gentamicin had sterile vegetation groups. When rabbits were treated with single agents, however, i.e., mezlocillin, ticarcillin, and gentamicin each alone, only 1 of 32 and 4 of 23 rabbits had sterile vegetations after 5 and 10 days of therapy, respectively. (χ² = 26.9, P < 0.01 after 5 days and χ² = 12.8, P < 0.05 after 10 days, each at 5 df for 6 × 2 table comparison of all six groups. For the comparison of groups treated with single agents versus those treated with combined therapy, χ² = 25.7, P < 0.01 at 5 df after 5 days and χ² = 4.9, P > 0.05 at 5 df after 10 days.)

**DISCUSSION**

In the present study, we compared ticarcillin alone with mezlocillin alone and each in combination with gentamicin in doses which would result in antibiotic concentrations in serum similar to those achieved in humans. These regimens were shown to be effective in the treatment of *E. aerogenes* endocarditis in rabbits.

Although mezlocillin was twofold more active in vitro than ticarcillin at an inoculum size of 10⁵.⁵ CFU/ml, mezlocillin peak concentrations of ticarcillin in serum were twice those of mezlocillin. Lower peak levels of the ureidopenicillins, including mezlocillin, as compared with ticarcillin and carbencillin, has been also noted in human serum and is attributed to a larger volume of distribution for the ureidopenicillins (2, 8). As the half-lives and protein binding of these agents in serum are similar, the therapeutic ratios (ratio in concentration in serum to MBC) of the two agents would be expected to be equivalent. In addition, no accumulation would be expected to occur with either beta-lactam during the course of therapy, as the interval between dosing (6 h) was more than five times the half-life in serum for both mezlocillin and ticarcillin and there was no evidence of gentamicin-induced nephrotoxicity (i.e., normal creatinine levels and stable peak gentamicin concentrations in serum) in animals treated with the combination of a beta-lactam plus gentamicin.

In the in vitro time-kill studies, an inoculum of 10⁸.⁵/ml, which is similar to bacterial counts in vegetations, was used. Concentrations of antibiotic used in the in vitro time-kill studies were similar to those present in the sera of rabbits between 60 and 120 min after injection. These in vitro studies demonstrated rapid bactericidal activity for both beta-lactam antibiotics when each was combined with gentamicin. However, each beta-lactam alone against these high inocula either failed to kill or allowed regrowth of organisms, observations which could not be attributed to the emergence of resistant subpopulations or to inactivation of antibiotic. Nevertheless, in the rabbit *E. aerogenes* endocarditis model, these studies document that both mezlocillin and ticarcillin alone, when given over a prolonged course, were effective in reducing the number of *E. aerogenes* CFU in vegetations in rabbits with severe experimental endocarditis. More rapid and extensive reduction in vegetation counts was achieved with combinations of an aminoglycoside plus mezlocillin or ticarcillin. Mortality in rabbits treated with mezlocillin with or without gentamicin was significantly lower than that in rabbits treated with ticarcillin with or without gentamicin.

**TABLE 1. Concentration and half-life of antibiotics in serum after first dose**

<table>
<thead>
<tr>
<th>Antibiotic*</th>
<th>Mean ± SD concn (µg/ml) in serum at following time (min) after injection:</th>
<th>Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Mezlocillin</td>
<td>169 ± 80</td>
<td>179 ± 59</td>
</tr>
<tr>
<td>Ticarcillin</td>
<td>291 ± 60</td>
<td>348 ± 111</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>7.4 ± 0.9</td>
<td>6.0 ± 0.2</td>
</tr>
</tbody>
</table>

* Each treatment group comprised four rabbits.

**TABLE 2. Comparison of gentamicin alone or ticarcillin and mezlocillin each alone and combined with gentamicin administered intramuscularly to rabbits with *E. aerogenes* endocarditis**

<table>
<thead>
<tr>
<th>Regimen*</th>
<th>No. of survivors/total</th>
<th>No. of survivors with sterile vegetation</th>
<th>Log₁₀ CFU/g in survivors (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 days*</td>
<td>10 days</td>
<td>5 days</td>
</tr>
<tr>
<td>T</td>
<td>10/13</td>
<td>8/10</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>11/11</td>
<td>8/8</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>11/11</td>
<td>7/9</td>
<td>1</td>
</tr>
<tr>
<td>T + G</td>
<td>8/9</td>
<td>9/13</td>
<td>4</td>
</tr>
<tr>
<td>M + G</td>
<td>9/9</td>
<td>7/7</td>
<td>6</td>
</tr>
<tr>
<td>No treatment</td>
<td>12/15</td>
<td>3/3</td>
<td>0</td>
</tr>
</tbody>
</table>

* Dosages (in milligrams per kilogram of body weight) were as follows: ticarcillin (T), 180; mezlocillin (M), 180; gentamicin (G), 1.7.

* Length of treatment period.
rabbits treated with ticarcillin with or without gentamicin. Autopsy did not disclose a cause for the excess mortality in the ticarcillin-treated groups.

Delays in instituting antibiotic therapy for experimental bacterial endocarditis have been noted to result in a diminishing effect on the numbers of bacteria per gram of vegetation. It is remarkable that 5 days of therapy with antimicrobial combinations of either beta-lactam with gentamicin was capable of sterilizing more than half of the vegetations in rabbits infected 7 days before the initiation of therapy.

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LITERATURE CITED