In Vitro Activity of Temocillin, a New β-Lactamase-Stable Penicillin Active Against Enterobacteria

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The activity of temocillin was investigated in vitro against 523 clinical isolates of enterobacteria and Pseudomonas aeruginosa. The minimum inhibitory concentration of the new compound for all ampicillin-susceptible enterobacteria and for 90% of ampicillin-resistant enterobacteria was 16 μg/ml or less, a concentration readily achieved in plasma. P. aeruginosa strains were uniformly resistant to temocillin. All but 3 of a separate group of 48 enterobacteria exhibiting resistance to the combination of clavulanic acid and amoxicillin were found to be inhibited by 16 μg or less of temocillin per ml. The new compound also displayed good activity against a group of laboratory stock cultures selected on the basis of differential resistance to presently available β-lactam agents. Two of these strains were cefotaxime resistant.

Temocillin is a new penicillin derivative developed by Beecham Research Laboratories. The compound is structurally unique among presently available penicillins in that it has a methoxy substituent on the β-lactam ring and an unusual spectrum of activity. It is inactive against the common gram-positive pathogens, but exhibits a broad spectrum of activity among gram-negative bacteria, including most enterobacteria, Haemophilus influenzae, and Neisseria gonorrhoeae (6).

We investigated the activity of temocillin against a wide variety of enterobacteria freshly isolated from clinical specimens, against clinical isolates of Pseudomonas aeruginosa, and against organisms selected to represent various patterns of resistance to presently available β-lactam agents, including a group resistant to the combination of amoxicillin and clavulanic acid.

MATERIALS AND METHODS

Bacterial strains. A total of 472 strains of enterobacteria and 31 strains of P. aeruginosa were unselected isolates obtained from clinical specimens received in the diagnostic microbiology laboratory of the hospital.

In addition, we tested 48 strains of enterobacteria found to be resistant to Augmentin (amoxicillin plus clavulanic acid), as determined by the absence of a zone of inhibition around a disk containing 20 μg of amoxicillin and 10 μg of clavulanic acid. These strains were obtained from rectal swabs examined in the course of a study of the use of Augmentin in geriatric inpatients.

The activity of temocillin was also investigated against 11 laboratory stock cultures of enterobacteria (5 Escherichia coli, 3 Klebsiella aerogenes, 2 Hafnia alvei, and 1 Proteus mirabilis) representing various types of susceptibility to β-lactam agents as described elsewhere (1, 2, 4).

Antibiotics. Temocillin, amoxicillin, and clavulanic acid were all provided by Beecham Pharmaceuticals Research Division. Suitable concentrations of antibiotic were freshly prepared, as required.

Antibiotic titrations. Minimum inhibitory concentrations (MICs) of antibiotic for the 523 fresh clinical isolates were estimated by the agar dilution method. Serial doubling dilutions of antibiotic were freshly prepared in Oxoid DST agar. Bacteria were spotted inoculated onto the surface of the plates with an automatic multipoint inoculator (Denley Instruments) which delivered approximately 1,000 colony-forming units from overnight broth cultures of bacteria diluted 1:104.

For the 48 Augmentin-resistant strains, two series of plates were used containing serial dilutions of amoxicillin in the presence and absence of 8 μg of clavulanic acid per ml.

MICs of strains used in the turbidimetric studies were estimated in “complete” broth with an inoculum of ca. 106 organisms per ml.

Turbidimetric studies. Tubes with 20-ml volumes of complete broth (3) containing antibiotic at the required concentration were inoculated with approximately 108 bacteria per ml from overnight broth cultures. The tubes were then incubated in a modified version of the bacterial growth monitoring device described by Mackintosh et al. (5), in which the turbidity of 12 independent bacterial cultures can be continuously monitored. In certain experiments, growth in the turbidimeter was allowed to proceed until the opacity of the culture reached a level of 30% of maximum (equivalent to a viable count of ca. 109 bacteria per ml) before antibiotic was added.

Microscopy. Morphological changes in bacteria exposed to temocillin for 1 h were observed in untreated “wet” preparations by interference contrast microscopy.
TABLE 1. Antibacterial activity of temocillin against 523 strains of enterobacteria and P. aeruginosa

<table>
<thead>
<tr>
<th>Organism*</th>
<th>No. of strains</th>
<th>MIC (µg/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mode</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>E. coli (ampicillin-S)</td>
<td>118</td>
<td>0.5-16</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>E. coli (ampicillin-R)</td>
<td>50</td>
<td>1-32</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>109</td>
<td>1-&gt;128</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>74</td>
<td>1-16</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Proteus spp. (ampicillin-R)</td>
<td>51</td>
<td>0.5-8</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Other coliforms (ampicillin-S)</td>
<td>20</td>
<td>1-8</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Other coliforms (ampicillin-R)</td>
<td>50</td>
<td>1-&gt;128</td>
<td>4</td>
<td>&gt;128</td>
</tr>
<tr>
<td>P. aeruginosa (carbenicillin-S)</td>
<td>37</td>
<td>64-512</td>
<td>128</td>
<td>512</td>
</tr>
<tr>
<td>P. aeruginosa (carbenicillin-R)</td>
<td>14</td>
<td>256-&gt;512</td>
<td>256</td>
<td>512</td>
</tr>
</tbody>
</table>

* S, Susceptible; R, resistant.
* MIC<sub>90</sub>, Concentration inhibiting 90% of strains.
* Citrobacter spp. (16), Acinetobacter spp. (2), and Salmonella spp. (2).
* Enterobacter spp. (47), Citrobacter spp. (2), and Serratia marcescens (1).

RESULTS

MICs. The MICs of temocillin for 472 strains of enterobacteria and 51 strains of P. aeruginosa are shown in Table 1. All ampicillin-susceptible strains were inhibited by 16 µg or less of temocillin per ml. Ampicillin-resistant enterobacteria exhibited a wider range of MICs. Overall, 91% of ampicillin-resistant enterobacteria and 95% of all enterobacterial isolates were inhibited by the new compound at a concentration of 16 µg/ml, a concentration readily achieved in plasma by an intramuscular injection of 250 mg of temocillin (6).

In contrast, all P. aeruginosa strains tested required 64 µg or more of temocillin per ml for inhibition, and well over half the strains displayed MICs of 256 µg or more per ml.

Of the 48 Augmentin-resistant strains tested, only 3 (2 Proteus spp. and 1 Klebsiella sp.) exhibited MICs of temocillin of greater than 16 µg/ml (Table 2). Among these strains, clavulanic acid, at a concentration of 8 µg/ml, was able to reduce the MICs of amoxicillin for 17 of 22 klebsiellae by a factor of four or more; with the other species tested, little or no improvement in the activity of amoxicillin was provided by clavulanic acid.

Turbidimetric studies. When temocillin was added to dense cultures of E. coli ECSA 1 (MIC of temocillin, 8 µg/ml) in the logarithmic growth phase (30% opacity; see above), bacterial growth was not prevented by drug concentrations up to and including 128 µg/ml. However, microscopy of these cultures revealed inhibition of division, causing the bacteria to grow as long filaments at drug concentrations of 1 µg/ml and above. No spheroplast formation was detected over the concentration range tested.

Since residual bacterial growth (in the form of filaments) allowed the culture to enter the stationary phase of growth before any antibacterial effect was detectable turbidimetrically when a dense bacterial culture was used, the remainder of the experiments were carried out with a lower bacterial inoculum of ca. 10<sup>6</sup> bacteria per ml.

In these experiments, the minimum antibacterial concentration (the concentration causing a deviation from the normal growth curve in the turbidimetric system) of temocillin was found to be 2 µg/ml, and the MIC (the concentration inhibiting growth overnight) was 16 µg/ml (Fig. 1). The culture growing in 8 µg of temocillin per ml in this experiment was used to provide the inoculum for a fresh series of antibiotic-containing tubes of broth. In this case, bacteria exposed to 8 µg/ml grew as freely as an antibiotic-free control, and 64 µg/ml was required to inhibit growth overnight.

Ten other strains of enterobacteria, represent-

TABLE 2. Antibacterial activity of temocillin against 48 strains of enterobacteria resistant to the amoxicillin-clavulanic acid combination

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of strains</th>
<th>MIC range (µg/ml)</th>
<th>Amoxicillin</th>
<th>Amoxicillin in presence of clavulanic acid*</th>
<th>Temocillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>6</td>
<td>512-&gt;512</td>
<td>32-256</td>
<td>2-8</td>
<td>1-8</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>22</td>
<td>256-&gt;512</td>
<td>8-&gt;512</td>
<td>(1 strain, 256)</td>
<td>1-16</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>11</td>
<td>32-512</td>
<td>32-512</td>
<td>(1 strain, 64; and 1 strain, 256)</td>
<td>2-8</td>
</tr>
<tr>
<td>Other coliforms</td>
<td>9</td>
<td>64-&gt;512</td>
<td>32-&gt;512</td>
<td>2-8</td>
<td></td>
</tr>
</tbody>
</table>

* Clavulanic acid at 8 µg/ml.
Discriminating six distinct types of susceptibility to \( \beta \)-lactam antibiotics (4), were tested in the turbidometric system with an inoculum of \( 10^6 \) bacteria per ml. These strains included two which were resistant to ampicillin by an intrinsic (nonenzymic) mechanism and two \( H. \) alvei strains previously found to be resistant to cefotaxime and ceftriaxone (1). The response of these strains to temocillin in the turbidometric system was very uniform: the minimum antibacterial concentration of temocillin for all 10 strains was in the range of 2 to 8 \( \mu \)g/ml, and the MIC was in the range of 16 to 64 \( \mu \)g/ml. In most cases, the MIC was one dilution higher than that found in conventional broth dilution MIC titrations in which an inoculum of ca. \( 10^4 \) bacteria per ml was used.

**DISCUSSION**

Temocillin exhibits the novel feature of an \( \alpha \)-methoxy substituent on the \( \beta \)-lactam ring. This feature, also possessed by cephemycins such as cefoxitin, appears to stabilize the \( \beta \)-lactam ring, making the molecule as a whole insensitive to the majority of \( \beta \)-lactamases. Apart from the \( \alpha \)-methoxy grouping, temocillin is structurally identical to ticarcillin. However, unlike ticarcillin, the new compound has poor activity against \( P. \) aeruginosa and no useful activity against gram-positive cocci (6). These marked differences in spectrum highlight once more the profound changes that can be brought about by minor modifications in the structure of \( \beta \)-lactam antibiotics.

The \( \beta \)-lactamase stability of temocillin, as judged by its range of activity against ampicillin-resistant enterobacteria, is impressive: well over 90% of unselected clinical isolates of enterobacteria examined in the present study were susceptible to the new compound at a concentration of 8 \( \mu \)g/ml, and only 3 of 48 strains found to be nonsusceptible to the amoxicillin-clavalanic acid combination were also resistant to temocillin. Furthermore, no strain from a group of enterobacteria selected on the basis of differential resistance to other \( \beta \)-lactam antibiotics proved nonsusceptible to the new agent. These included two \( Hafnia \) strains previously shown to be resistant to cefotaxime and ceftriaxone.

The morphological response of \( E. \) coli to temocillin, i.e., filamentation, suggests that the new compound is not rapidly bactericidal to susceptible gram-negative bacilli, nor does the new compound exhibit the outstanding intrinsic activity of the new-generation cephalosporins, as judged by MICs. Furthermore, the observation that bacteria recovering during the overnight exposure period (Fig. 1) display enhanced resistance to temocillin is worrying. A stepwise increase in resistance to all \( \beta \)-lactam antibiotics can be induced in the laboratory by sequential subculture, but such resistance does not usually occur as readily as we have observed with temocillin.

Nevertheless, this new agent, the first penicillin to display stability to a wide range of enterobacterial \( \beta \)-lactamases, appears to possess good intrinsic activity against most enterobacteria, including some which are resistant to all presently available \( \beta \)-lactam antibiotics.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


