Comparison of Amoxicillin and Ampicillin Activities in a Continuous Culture Model of the Human Urinary Bladder

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Sixteen isolates of Escherichia coli were obtained from women with significant acute urinary tract infections who were subsequently treated with amoxicillin. The activities of amoxicillin and ampicillin against these organisms were compared in urine in a continuous culture apparatus which partly simulated the milieu of the human urinary bladder. After introduction of amoxicillin into the bladder model, mean viable counts for 14 susceptible strains (minimum inhibitory concentration < 32 μg/ml) at 10, 20, 30, and 45 min fell to 34, 8, 0.4, and 0.2% of the original. Corresponding figures for ampicillin were 56, 24, 11, and 2.4%. Viable counts obtained at seven timed intervals up to 2 h were significantly (P = 0.025) lower with amoxicillin than ampicillin. Both antibiotics had a similar activity in conventional disk susceptibility and surface plate minimum inhibitory concentration tests. The realism of the model was confirmed by comparing response to amoxicillin in vivo and in vitro. A serious discrepancy was seen in only one of the 16 cases.

Amoxicillin and ampicillin appear to have similar activity in conventional laboratory tests (11, 17). Unfortunately such tests seldom indicate differences in killing rates which may be important in some circumstances, e.g., in the single-dose treatment of urinary infections, where effective antibiotic concentrations are transient. Many features of the pathogenesis and treatment of acute urinary infection may be explained in terms of a balance between bacterial growth rates and the flushing effect of urine (2, 5, 8, 9, 12, 13). Antibiotic activity is usually concentration-dependent and may be affected by growth conditions and medium (14). We have developed a laboratory model of the lower human urinary tract which employs realistic concentrations of antibiotic in urine. This apparatus also permits study of interactions between bacterial growth and death and urine washout. Our model is a development of that of Greenwood and O'Grady (8, 13). It differs in that urine was used as medium instead of broth, antibiotic was added continuously rather than as a pulse, and populations were monitored by following changes in viable counts rather than by nephelometry. As in the early stages of treatment, progressively rising urinary antibiotic concentrations were used in this apparatus. It did not however simulate the cyclical changes in urinary antibiotic concentrations found in patients taking repeated doses of antibiotic.

Organisms for in vitro studies were obtained from patients with significant urinary infections who were subsequently treated with amoxicillin (1). The realism of the apparatus was evaluated by comparing responses in vivo and in vitro.

MATERIALS AND METHODS

Organisms. A selection of 16 isolates of Escherichia coli was obtained before treatment in a trial which compared the efficacy of two regimens of amoxicillin for treating acute urinary infections in women. The majority of cases have been described in a preliminary publication (1). Patients exhibited symptoms of urinary infection, with pyuria (>100 leukocytes per mm³) and significant bacilluria (>10⁵ organisms per ml in a single specimen of urine). Organisms were identified by the API 20E system (API Laboratory Products Ltd., St. Laurent, Quebec, Canada).

A further 167 hospital isolates of Enterobacteriaceae were obtained from patients with a variety of infections and used to compare response to ampicillin and amoxicillin in the disk susceptibility test.

Urine. Pooled midstream urine specimens from healthy males and females were sterilized by filtration (2). Urine samples were obtained from three to five individuals for each experiment, and pooled specimens had a pH of between 5.7 and 6.1; the osmolality of specimens was not determined.

Antibiotics. Amoxicillin trihydrate (gift of Beecham Laboratories, Montreal, Quebec) and ampicillin sodium (Penbritin injection) were dissolved in urine (250 μg/ml as antibiotic base). No detectable loss of antibiotic activity occurred during storage in a reservoir at 4°C during bladder model experiments. Minimum inhibitory concentration (MIC) determinations were carried out with assay standard antibiotics (Beecham Laboratories).

β-Lactamase. Each ampoule of lyophilized enzyme
(Whatman Biochemicals Ltd., Maidstone, Kent, England) was dissolved in phosphate buffer (50 ml, pH 7.4) and stored at -20°C for up to 1 week. Antibiotic present in urine samples was destroyed before viable count determinations by incubation (37°C) with β-lactamase solution (1 + 9, vol/vol) for 5 min. Preliminary work showed that antibiotic was effectively destroyed within 1 min.

**Human urinary bladder model.** A full description and details of the operation of this apparatus have been described previously (2). For these experiments facilities were provided to pump urine or urinary solutions of antibiotic from a reservoir maintained at 4°C. Principal features of the apparatus (outline only) are as follows.

A cylindrical glass vessel (volume 500 ml) served as a culture chamber. Cultures were aerated and mixed by passing air through the urine. The apparatus was maintained at 37°C by heating lamps controlled by a thermostat in the base of the apparatus. Sterile urine with or without antibiotic was pumped into the bladder with a peristaltic metering pump (60 ml/h). The culture vessel was automatically emptied every 4 h to leave a residual volume of 4 ml. Culture samples for viable count determinations were withdrawn with a hypodermic syringe and needle via a rubber cap in the base of the apparatus.

**Operation of the bladder model.** The apparatus was inoculated with organisms from overnight broth cultures suspended in urine (20 ml; mean viable count of 32 experiments, 2.8 ± 3.1 × 10^8/ml). The mean viable count rose (1.0 ± 2.1 × 10^8/ml) after incubation for 5 h. At 5 h when the model contained 64 ml of culture, urine containing antibiotic was substituted for antibiotic-free urine, and incubation continued for 2 h in all cases and for 24 h for a selection of eight organisms (see Results).

Urine was withdrawn from the apparatus at the intervals shown in Table 1 for determination of viable counts by inoculation of serial dilutions onto agar plates (after destruction of antibiotic with β-lactamase).

Side-by-side antibiotic-free controls were not possible because only one piece of apparatus was available. In sequential experiments without antibiotic, bacterial populations at 24 h were between 2 and 3 times higher than those at 5 h. Changes in bacterial populations after introduction of antibiotics (Table 1) were therefore expressed as a percentage of the viable count in each experiment immediately before introduction of antibiotic at 5 h.

**Determination of MICs of organisms.** An overnight broth culture of organisms was subcultured to fresh broth and incubated at 37°C to a turbidity corresponding to MacFarland standard no. 1. These actively growing organisms were then diluted 10^−4 and portions (0.005 ml, containing approximately 10^2 organisms) were inoculated onto freshly prepared plates of Mueller-Hinton medium, and onto medium containing a range of doubling dilutions of antibiotic from 0.5 to 1,024 μg/ml. Inhibitory concentrations were determined by examining the plates after incubation at 37°C for 18 h.

**Disk susceptibility tests.** Susceptibility to ampicillin and amoxicillin was determined on Mueller-Hinton medium with disks containing 10 μg of antibiotic (10). A break point of 11 mm was used for both antibiotics.

### RESULTS

All organisms with an amoxicillin MIC of 32 μg/ml or less were regarded as susceptible to urinary antibiotic concentrations and were rapidly killed by amoxicillin in the bladder model (Table 1). Amoxicillin is clearly more rapidly bactericidal than ampicillin for susceptible organisms in this test system. A three-factor variance was carried out on the survival counts of susceptible organisms up to 120 min. These factors were 14 organisms, the seven times at which data were obtained, the two antibiotics. Survival of organisms was significantly different in ampicillin and amoxicillin (P = 0.025). The experiments were continued for 24 h with both of the resistant organisms and a sample of six susceptible organisms. The resistant organisms all continued to grow, and the susceptible organisms, as expected, yielded no growth at 24 h.

These organisms had been isolated from patients before treatment either with a single 1-g dose of amoxicillin or with a course of 250 mg

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>14 susceptible organisms (MIC ≤ 32 μg/ml)</th>
<th>2 resistant organisms (MIC ≥ 1,024 μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Ampicillin</td>
<td>Amoxicillin</td>
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<tr>
<td>10</td>
<td>56 ± 27</td>
<td>34 ± 38</td>
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<tr>
<td>20</td>
<td>24 ± 34</td>
<td>8 ± 18</td>
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<tr>
<td>30</td>
<td>11 ± 26</td>
<td>0.4 ± 0.6</td>
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<td>45</td>
<td>2.4 ± 8.0</td>
<td>0.2 ± 0.3</td>
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<tr>
<td>60</td>
<td>0.5 ± 1.2</td>
<td>0.08 ± 0.1</td>
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<td>80</td>
<td>0.2 ± 0.5</td>
<td>0.06 ± 0.1</td>
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<tr>
<td>100</td>
<td>0.09 ± 0.2</td>
<td>0.09 ± 0.2</td>
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<tr>
<td>120</td>
<td>0.19 ± 0.53</td>
<td>0.03 ± 0.04</td>
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</table>

* All values are means with standard deviation.

* Viable count expressed as a percentage of that at the start of addition of antibiotic.
every 8 h for 7 days (1). There was a good correlation between the response in vivo and in vitro (Table 2). For the purposes of Table 2, success was defined as clearance of symptoms and bacilluria within 3 days of treatment. Patients were followed up for 42 days from the start of treatment (1). A major discrepancy was seen only with isolate 16 where an apparently resistant organism was cleared from the patient by day 2 of treatment.

Conventional tests failed to distinguish between the activity of ampicillin and amoxicillin. Mean zone sizes in the disk susceptibility test for these 16 isolates of E. coli were not significantly ($P > 0.05$) different for the two antibiotics. Support for this view was obtained in a larger unrelated study of 167 isolates of Enterobacteriaceae from hospital patients with a variety of infections, in which mean zone sizes were again not significantly different (ampicillin 12.40 ± 6.28 mm; amoxicillin 12.65 ± 6.82 mm; $P = 0.7$). MICs of amoxicillin for the 16 E. coli isolates by a surface plate method are illustrated in Table 2, column 2. The MIC of amoxicillin was twofold higher for one organism, twofold lower for another, and identical for the remaining 14 isolates.

**DISCUSSION**

Urine flow characteristics through this model simulated those of the human lower urinary tract. However, a residual volume of 4 ml was chosen because women prone to infection frequently have a higher than normal ($<1$ ml) residual volume (15). Urinary antibiotic concentrations were chosen for this model because we wished to assess its value in simulating acute infection where deep parenchymal tissue involvement is unlikely and urine antibiotic activity predominates. Mean urinary concentrations of amoxicillin during 6 h after a single 1-g dose varied from 880 to 6,400 µg/ml (4). Urinary concentrations in two volunteers on day 3 of a course of 250 mg thrice daily were 370 and 330 µg/ml (3). In each experiment addition of antibiotic commenced 1 h after emptying when the bladder model contained 64 ml of urine. Assuming no degradation, calculated urinary antibiotic concentrations (micrograms per milliliter) in the model would be: 18 at 5 min, 121 at 1 h, 163 at 2 h, and 246 at 4 h. Antibiotic concentrations in the bladder model, although well above MICs (Table 2) in most instances, are thus modest compared with those found in the urine of patients. Ampicillin is less well absorbed than amoxicillin and should be given in higher dosage (6, 7, 16). Both amoxicillin and ampicillin cause lysis of E. coli at concentrations above the MIC. Lysis has been suggested to be the primary cause of death, or at least to coincide with loss of viability (14).

The bladder model, the antibiotic disk susceptibility test, and a surface plate MIC test all failed to predict the response of organism 16 to treatment or to explain the recurrence of infection occurring with organisms 5 and 11 (Table 2). It would be naive to claim that such a simple model could reproduce all host factors influencing acute urinary tract infections. However, as with studies involving shake culture in urine (3) and with lower antibiotic concentrations in broth (14), amoxicillin has exhibited a more rapid bactericidal action than ampicillin, in this model. This fact, compounded with its superior absorption, may give amoxicillin a potential advantage in the single-dose treatment of urinary infection.

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**LITERATURE CITED**