Paradoxical Activity of β-Lactam Antibiotics against *Proteus vulgaris* in Experimental Infection in Mice

YASUSHI IKEDA,1* YOSHIKAZU FUKUOKA,1 KEIKO MOTOMURA,1 TAKASHI YASUDA,1 AND TAKESHI NISHINO2

Research Laboratory, Toyama Chemical Co., Ltd., 2-4-1, Shimookui, Toyama 930,1 and Department of Microbiology, Kyoto Pharmaceutical University, Yamashina, Kyoto 607,2 Japan

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In previous papers (Y. Ikeda and T. Nishino, Antimicrob. Agents Chemother. 32:1073–1077, 1988; Y. Ikeda, T. Nishino, and T. Tanino, Antimicrob. Agents Chemother. 31:865–869, 1987), we reported that many of the 7-aminothiazolyl cephalosporins, such as cefmenoxime, showed paradoxically reduced activity against *Proteus vulgaris* at higher concentrations, whereas these paradoxical effects were not observed for other types of cephalosporins, such as cefbuperazone and cefoperazone. In this study, we compare the therapeutic effect of cefmenoxime with that of cefbuperazone and explore the in vivo paradoxical effect of cefmenoxime by using an experimental infection model in mice. In an intraperitoneal infection with *P. vulgaris* 11, the survival rate with cefmenoxime was increased to 43% at 3.13 mg/kg but was lower at higher doses. On the other hand, cefbuperazone did not show such a paradoxical therapeutic effect. In mice infected with *P. vulgaris* 11, cefmenoxime levels in both serum and peritoneal washings were rapidly reduced and β-lactamase activities in the peritoneal cavity were increased at higher cefmenoxime doses. These findings suggested that high levels of cefmenoxime at the infection site induced increased production of β-lactamase, which then rapidly inactivated the antibiotic. We conclude that the paradoxical therapeutic effect of cefmenoxime against *P. vulgaris* occurs by the same mechanisms as the in vitro effect and that the high β-lactamase inducibility and low β-lactamase stability may account for the paradoxical therapeutic effect of cefmenoxime against *P. vulgaris*.

The antibacterial activity of an antibiotic is generally proportional to its concentration. However, several exceptions to this trend have been reported (3, 9, 13). In previous papers (4, 5), we reported that some 7-aminothiazolyl-type cephalosporins showed paradoxically reduced activities against *Proteus vulgaris* when they were present at high concentrations. Indeed, it has been difficult to demonstrate such paradoxical antibacterial activity clinically, but it appears that the in vivo paradoxical effect can be confirmed by using an experimental infection model. Eagle et al. (2), using an animal model of *Staphylococcus aureus* infection, found that the paradoxical antibacterial effect holds true in vivo. However, the mechanism of this effect was not explained sufficiently. In this study, we confirm that cefmenoxime has a paradoxical therapeutic effect in an experimental intraperitoneal model of *P. vulgaris* infection in mice. We also investigate the mechanism involved.

**MATERIALS AND METHODS**

**Antibiotics.** Cefmenoxime, prepared by Takeda Chemical Industries, Co., Ltd., Osaka, Japan, and cefbuperazone, prepared by Toyama Chemical, Co., Ltd., Tokyo, Japan, were used as the test antibiotics.

**Bacterial strains.** *P. vulgaris* 11 and *P. vulgaris* 11-S, a β-lactamase-noninducing mutant of *P. vulgaris* 11 (4), were used in this study.

**Intraperitoneal infection.** Male ICR strain mice (Shizuoka Agricultural Cooperative Associations for Laboratory Animals, Shizuoka, Japan) weighing 18 to 19 g were used in this study. Bacterial cells from an overnight culture on heart infusion agar (Eiken Chemical, Tokyo, Japan) were suspended in physiological saline solution supplemented with 5% gastric mucin (Nakarai Chemicals, Kyoto, Japan) to give an inoculum of 1.0 × 10⁷ cells per ml. A 0.5-ml portion of cell suspension was inoculated into each mouse by the intraperitoneal route (five times the 50% lethal dose). Three hours after infection, 0.2-ml portions of doubling dilutions of cefmenoxime or cefbuperazone in physiological saline solution were injected subcutaneously at the back of the neck. The numbers of mice surviving for 7 days after infection were observed. The 50% lethal doses of *P. vulgaris* 11 and *P. vulgaris* 11-S in mice were identical.

**Antibiotic concentrations in serum and peritoneal washings.** A group of uninfected mice and a group of infected mice were given a single subcutaneous injection of cefmenoxime or cefbuperazone at 3 h after induction of infection with *P. vulgaris* 11. The mice were killed (eight animals at a time) at intervals during the 4-h period after drug administration, and serum and peritoneal washings were obtained. The peritoneal washings were collected by the following methods (1). A 0.5-ml portion of 0.05 M phosphate buffer (pH 7.0) was injected into the peritoneal cavity of the mouse, and the abdomen was gently massaged to ensure adequate mixing. Then, a small incision was made through the skin and peritoneal wall, and a sample of washings was collected with a Pasteur pipette. To this sample was added the same volume of methanol to inactivate the β-lactamase. All samples were stored at −20°C until bioassay. The concentrations of antibiotics were determined by a previously described bioassay method (5).

**β-Lactamase induction in the peritoneal cavity.** Doses of 50, 12.5, or 3.13 mg of cefmenoxime or cefbuperazone per kg were subcutaneously injected into the mice at 3 h after infection. At 1 h after drug administration, a 0.5-ml sample of the peritoneal washings was collected by the method described above. The peritoneal washings were centrifuged at 8,000 × g for 5 min at 4°C, and the cells were harvested. After two successive washings, the cells were suspended in
0.05 M phosphate buffer and treated with an ultrasonic disrupter (Tomy Seiko, model UR-200P) for 2 min in an ice bath. The disrupted cell suspension was centrifuged at 15,000 × g for 30 min at 4°C, and the supernatant was recovered as a crude β-lactamase preparation. β-Lactamase activity was determined by the spectrophotometric method of Samuni (11), with cephaloridine as the substrate. The amount of enzyme that hydrolyzed 1 μmol of the substrate in 1 min at 30°C in 0.05 M phosphate buffer (pH 7.0) was defined as 1 U of enzyme activity. The protein concentration of β-lactamase was determined by the method of Lowry et al. (7), with bovine serum albumin as the standard. For each dose, five mice were used in this study.

RESULTS

Therapeutic effect of the antibiotics in intraperitoneal infection. Figure 1 shows the therapeutic effects of cefmenoxime and cefbuperazone against intraperitoneal infections caused by P. vulgaris 11 and P. vulgaris 11-S in mice. Cefmenoxime showed a paradoxical therapeutic effect in P. vulgaris 11-infected mice. The survival rate of the mice increased with the dose of antibiotic up to 3.13 mg/kg, the peak effective dose (survival rate, 43%), but then decreased to 33 and 20% at 12.5 and 50 mg/kg, respectively. However, this paradoxical therapeutic effect of cefmenoxime was not observed in the mice infected with P. vulgaris 11-S, which is a β-lactamase-noninducing mutant of P. vulgaris 11.

On the other hand, the therapeutic effect of cefbuperazone in both the P. vulgaris 11-infected and P. vulgaris 11-S-infected mice was proportional to the dose. Cefbuperazone had a dose-dependent therapeutic effect on both of P. vulgaris 11 and P. vulgaris 11-S infections, with 50% lethal doses almost equal to those of cefmenoxime in P. vulgaris 11-S-infected mice.

Levels in serum and peritoneal washings. The serum concentration-time curves for cefmenoxime and cefbuperazone in control mice and mice infected with P. vulgaris 11 are shown in Fig. 2 and 3, respectively. The mean concentration of cefmenoxime in serum in mice given 50 mg/kg was higher than that in mice given 3.13 mg/kg at 15, 30, and 60 min, whether the mice were infected or uninfected. However, either dose-independent or irregular concentrations in serum were observed at 120 and 180 min in P. vulgaris 11-infected mice. The half-life in the P. vulgaris 11-infected mice given 50 mg/kg was remarkably short (about 20 min) compared with that in the other group of animals (about 30 min). On the other hand, cefbuperazone did not give dose-independent serum concentration-time curves in any cases. The half-lives of cefbuperazone in serum in the same four groups were nearly uniform (about 20 min).

The concentration-time curves for peritoneal washings from mice given cefmenoxime and cefbuperazone are shown...
96 peritoneal washings were used. Infected mg/kg (infected mice); ○, 50 mg/kg (noninfected mice); △, 3.13 mg/kg (infected mice); Δ, 3.13 mg/kg (noninfected mice).

FIG. 3. Serum concentration-time curves for cefbuperazone in mice infected with P. vulgaris 11. For each time period, eight mice were used. Data are means and standard errors. Symbols: ●, 50 mg/kg (infected mice); ○, 50 mg/kg (noninfected mice); △, 3.13 mg/kg (infected mice); Δ, 3.13 mg/kg (noninfected mice).

in Fig. 4 and 5, respectively. Cefmenoxime concentrations in peritoneal washings were dose independent or irregular at 120 and 180 min in the mice infected with P. vulgaris 11. At 180 min, the mean concentration in peritoneal washings in the infected mice given 50 mg of cefmenoxime per kg was significantly lower than that in the infected mice given 3.13 mg/kg (P < 0.01). Moreover, the half-life of cefmenoxime in the infected mice given 50 mg/kg (about 17 min) was shorter than in any of the other groups (about 50 to 60 min). The dose-independent and irregular peritoneal wash concentration-time curve and irregular half-life were not observed in mice given cefbuperazone.

Induction of β-lactamase in the peritoneal cavity. The β-lactamase-inducing activities of cefmenoxime and cefbuperazone in the peritoneal cavities of mice infected with P. vulgaris 11 are shown in Table 1. Cefmenoxime and cefbuperazone both had high inducer activity for β-lactamase production in the peritoneal cavity. However, induction by cefmenoxime was dose dependent, whereas that by cefbuperazone was not. At high concentrations, cefbuperazone showed suppressed β-lactamase-inducing activity.

DISCUSSION

We previously demonstrated the paradoxical effect of cefmenoxime and other aminothiazolyl cephalosporins against P. vulgaris and explored the mechanism involved. We conjectured that this paradoxical effect reflected the relationship between the β-lactamase inducibility of bacteria and their stability against β-lactamases (4). In the present study, cefmenoxime showed a paradoxical therapeutic effect in intraperitoneal infection of mice by P. vulgaris 11. This paradoxical therapeutic effect has also been confirmed in an urinary tract infection model in mice (data not shown).

It is very likely that the therapeutic effect of an antibiotic depends on two factors: its antibacterial activity and its concentration at the infection site.

Most pharmacokinetic studies of infected animals have been done for only one dose rather than after repeated doses. In many cases, the concentrations of antibiotics at the infection site are greatly influenced by whether the infecting...
bacteria are β-lactamase positive or β-lactamase negative (1, 6). Moreover, it is well known that the β-lactamase inducibility of bacteria depends markedly on the β-lactam antibiotic used and its concentration in vitro (8, 10).

In this study, we have shown that this phenomenon could also take place in vivo. At high doses, cefmenoxime induced large amounts of β-lactamase, but was then rapidly hydrolyzed by its own β-lactamase; this caused its therapeutic effect to be reduced. This explains why cefmenoxime showed the paradoxical therapeutic effect in the experimental infection in mice. On the other hand, cefmenoxime showed a strong and proportional therapeutic effect in mice infected with *P. vulgaris* 11-S, a β-lactamase-noninducing mutant of *P. vulgaris* 11. The concentration profiles of cefmenoxime in the serum and peritoneal washings of *P. vulgaris* 11-S-infected mice were similar to those in uninfected mice, except that the concentrations themselves were higher in the infected mice. This is probably responsible for the dose-dependent and proportional therapeutic effect of cefmenoxime in *P. vulgaris* 11-S infections.

It is very difficult to verify whether a higher dose will result in diminished therapeutic efficacy in patients. However, our results show that the in vitro phenomenon may well be reproduced in vivo. Considering this fact, it is possible that there may be a reduced therapeutic effect with higher dosages of drug in clinical infection caused by β-lactamase-inducing bacteria. We hope that more attention will be paid to the dosage, especially in patients supposedly infected with β-lactamase-inducing bacteria.

### TABLE 1. β-Lactamase induction by cefmenoxime and cefbuperazone in the peritoneal cavities of mice infected with *P. vulgaris* 11

<table>
<thead>
<tr>
<th>Injection dose (mg/kg)</th>
<th>Mean β-lactamase activity ± SE (U/mg of protein) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cefmenoxime</td>
</tr>
<tr>
<td>50</td>
<td>1.39 ± 0.55</td>
</tr>
<tr>
<td>12.5</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>3.13</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td>0 (control)</td>
<td>&lt;0.001</td>
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### LITERATURE CITED


