Exudate Levels and Bactericidal Activity of Cefazolin in a New Local Infection System Using Rat Granuloma Pouches

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An experimental local infection system has been developed in which exudates are induced with croton oil in granuloma pouches of rats. This system provided a suitable model for the evaluation of the therapeutic effect of two antibiotics, cefazolin and cephalothin. Exudate levels of cefazolin were found to be higher than those of cephalothin, and these levels correlated with the higher serum level of cefazolin. The therapeutic effect of cefazolin, after intramuscular injection of 20 mg of each antibiotic per kg, was superior to that of cephalothin in eradicating both Staphylococcus aureus and Escherichia coli.

Evaluation of a new antibiotic usually includes measurements of the levels of its agent in the blood, urine, and tissue homogenates and in vivo estimates of activity against systemic infections in experimental animals. Such studies provide an overall assessment of efficiency and pharmacokinetic characteristics. Their results may or may not be pertinent to the levels of the antimicrobial agent attained at the site of infection and the activity of the drug against the local lesions.

In the present study, we investigated the penetration of cefazolin and cephalothin into exudates in granuloma pouches induced by croton oil in rats. Exudate levels and bactericidal activities of the test antibiotics after parenteral administration were studied to assess the activity of these drugs against local lesions.

MATERIALS AND METHODS

Drugs. The following drugs were used: cefazolin (Fujisawa Pharmaceutical Co., Ltd.), cephalothin (Eli Lilly and Co.), and other reagents of guaranteed grade.

Animals. Sprague-Dawley strain rats aged 5 to 6 weeks were used.

Organisms. A standard strain of Staphylococcus aureus 209P JC-1 (minimum inhibitory concentration [MIC]—cefazolin, 0.1 µg/ml; cephalothin, 0.05 µg/ml) and a clinical isolate of Escherichia coli 312 (MIC—cefazolin, 1.56 µg/ml; cephalothin, 12.5 µg/ml) were used.

Procedure for formation of granuloma pouches and determination of antibiotic levels. Granuloma pouches were induced in rats by the following procedure: 25 ml of air was injected deep into the loose connective tissue between the shoulders with a 27-gauge needle. This was immediately followed by injection of 1 ml of 1% croton oil solution in olive oil into the resulting air space with the same needle (7). Seven days after formation of the granuloma pouch, with or without infection induced by the test organisms, rats were given a single intramuscular (i.m.) injection of cefazolin or cephalothin (20 mg/kg), and levels of the two antibiotics were measured in the sera and exudates. Analytical values of the exudates were obtained with a Technicon Autoanalyzer (Table 1). The antibiotic levels were assayed by the disk method using Bacillus subtilis ATCC 6633 as the test organism (5). Standard solutions for bioassay were prepared with the serum for serum levels and with the exudate for exudate levels.

Inoculation of test organisms into the granuloma pouch. The test organisms were cultured overnight at 37°C on heart infusion agar slants. A cell suspension (about 10⁴ to 10⁶ colony-forming units [CFU]/ml) in 0.9% saline or 5% mucin solution was prepared from the culture, and 0.5 ml of the cell suspension was injected into the granuloma pouch with a 27-gauge needle. At regular intervals, 0.5 ml of the exudate was withdrawn from the granuloma pouch to count viable cells. The test groups consisted of four or five rats, and the results are presented as the mean value of each group.

Determination of viable cells. Viable organisms in the exudates or medium were enumerated by performing colony counts: 10-fold dilutions in 0.9% saline were made. For S. aureus, 0.05 ml of each dilution was spread over the surface of staphylococcal agar medium no. 110 (Nissui), and for E. coli 1 ml of each dilution was mixed with melted deoxycholate agar medium (about 40°C) in a petri dish; the agar media were incubated for 18 to 24 h at 37°C before colony counting.

RESULTS

Cefazolin and cephalothin levels in exudates and sera of rats with granuloma pouches. Cefazolin and cephalothin levels in the exudates of the rat granuloma pouches after a single i.m. injection of 20 mg/kg were compared with those in the sera. Exudate levels of the two antibiotics were also investigated in the rat granuloma pouches.
pouches without infection and in those with infection due to *S. aureus* or *E. coli*.

Serum levels of cefazolin in rats after i.m. injection of 20 mg/kg peaked at 68.9 µg/ml at 15 min and were 33.0 µg/ml at 1 h and 6.4 µg/ml at 2 h (Fig. 1a). Exudate levels of cefazolin in the granuloma pouches without infection were 4.9 µg/ml at 2 h, 5.7 µg/ml at 4 h, and 3.1 µg/ml at 8 h. Exudate levels of cefazolin did not increase as much as serum levels, but persisted long after disappearing from the blood. No significant differences in exudate levels were observed between the granuloma pouches without infection and those with infection due to *S. aureus* when cefazolin was given i.m. in a dose of 20 mg/kg 30 min after challenge. Exudate levels of cefazolin in the granuloma pouches with infection, however, were about one-half those without infection when cefazolin was given i.m. 24 h after infection with *S. aureus* 209P. Figure 1b shows the exudate levels of cefazolin in the granuloma pouches infected with *E. coli* 312. The patterns of the exudate levels were almost the same as those obtained from the granuloma pouches infected with *S. aureus* 209P.

Figure 2a and b shows the serum and exudate levels of cephalothin in rats after i.m. injection of 20 mg/kg. Serum levels of cephalothin in rats after i.m. injection were 14.1 µg/ml at 15 min, 6.4 µg/ml at 30 min, 1.6 µg/ml at 1 h, and not more than 0.5 µg/ml at 2 h. Exudate levels of cephalothin in the granuloma pouches without infection were 1.8 µg/ml at 2 h and 0.79 µg/ml at 6 h after administration and were lower than those of cefazolin. Exudate levels of cephalothin in the granuloma pouches with infection were almost the same as those without infection when the drug was given i.m. 30 min after infection with *S. aureus* 209P, but were lower than those without infection when the drug was given i.m. 24 h after infection (Fig. 2a). Exudate levels of cephalothin in the granuloma pouches with infection due to *E. coli* 312 were almost the same as those with infection due to *S. aureus* (Fig. 2b).

### Table 1. Analytical values of exudates in granuloma pouches

<table>
<thead>
<tr>
<th>Exudate no.</th>
<th>Erythrocytes (× 10³/mm³)</th>
<th>Leukocytes (× 10⁹/mm³)</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Glucose (mg/dl)</th>
<th>AI-P (mU/ml)</th>
<th>sGPT (mU/ml)</th>
<th>sGOT (mU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>1.1</td>
<td>3.7</td>
<td>2.2</td>
<td>46</td>
<td>130</td>
<td>35</td>
<td>126</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>1.2</td>
<td>4.0</td>
<td>2.5</td>
<td>40</td>
<td>190</td>
<td>32</td>
<td>215</td>
</tr>
</tbody>
</table>

* a Pouches were analyzed on day 7 after forming. AI-P, Alkaline phosphatase; sGPT, serum glutamic pyruvic transaminase; sGOT, serum glutamic oxaloacetic transaminase.

* Specific gravity for exudate no. 1, 1.035; for no. 2, 1.037.

Bactericidal effect of cefazolin and cephalothin on *S. aureus* 209P in rat granuloma pouches. (i) Establishment of a local infection system. Figure 3 shows the influence of 5% mucin on the growth of *S. aureus* 209P in rat granuloma pouches. On day 7 after forming, the granuloma pouches were injected with 0.5-ml saline suspensions containing the test organisms, *S. aureus* 209P, in concentrations of 10⁴, 10⁵, and 10⁶ CFU/ml. The exudates in the pouches were examined after 24 and 48 h for viable bacteria. Viable bacteria decreased with all inoculum sizes. However, when 5% mucin was inoculated into the pouches containing 10⁶ and 10⁷ CFU/ml, there was no decrease in viability at 24 and 48 h. To further examine these in vivo findings, pouch exudates and rat sera were inoculated with *S. aureus* 209P to investigate the effect of mucin in vitro. The results of this study are shown in Fig. 4. When the exudates without mucin were inoculated with *S. aureus* 209P at the initial count of 5× 10⁶ CFU/ml, the viable bacterial count did not change for 8 h after inoculation. The bacteria in serum without mucin were killed rapidly under the same conditions.

When 3 ml of 5% mucin was added to 7 ml of exudate, the viable bacterial counts increased during the 8 h after inoculation. The bactericidal effect of the serum was significantly inhibited by the addition of mucin, with proliferation of the organisms from 4 to 8 h after inoculation. In heart infusion broth the growth of *S. aureus* 209P was not influenced by the addition of 5% mucin. These results show that mucin reduced the bactericidal activity of sera and exudates.

The above results confirmed that a local infection utilizing granuloma pouches in rats was suitable for evaluating these antibiotics. (ii) Bactericidal effect. Rat pouches were inoculated with *S. aureus* 209P in 3 ml of 5% mucin in a final concentration of 10⁶ CFU/ml. Thirty minutes after inoculation the animals were given 20 mg of either cefazolin or cephalothin per kg i.m., and the viable bacteria in the exudates were determined. In the control group without cephalosporins, the viable bacterial count in the exudates was maintained at 10⁶ CFU/ml for 48 h (Fig. 5). In both antibiotic
groups the count decreased to approximately $10^4$ CFU/ml at 5 h and approximately $10^6$ CFU/ml at 24 h. In the cefazolin group, however, the viable bacterial count decreased further to $10^2$ CFU/ml at 48 h, whereas in the cephalothin group the count returned to the 5-h level of $10^4$ CFU/ml and thereafter tended to increase.

Bactericidal effect of cefazolin and cepha-

**Fig. 1.** Cefazolin levels in exudate and serum after i.m. injection of 20 mg/kg in rats with granuloma pouch (day 7 after forming).

**Fig. 2.** Cephalothin levels in exudate and serum after i.m. injection of 20 mg/kg in rats with granuloma pouch (day 7 after forming).
EXUDATE LEVELS OF CEFAZOLIN

Fig. 3. Effect of inoculum size on growth of S. aureus 209P in the granuloma pouch (day 7 after forming). Inoculum size: (left) 10^4 cells/ml; (middle) 10^6 cells/ml; (right) 10^8 cells/ml. Bars: (stippled) without mucin (control); (hatched) with mucin.

Fig. 4. Effect of mucin on growth of S. aureus 209P in heart infusion (HI) broth, exudate, and serum. Symbols: (●) HI broth + saline; (○) HI broth + mucin; (□) exudate + mucin; (△) serum + mucin; (■) exudate + saline; (▲) serum + saline.

lothin on E. coli 312 in granuloma pouches in rats. (i) Experimental conditions. Rat pouches were inoculated with various inoculum sizes of E. coli 312, and the viable cells in the exudates were counted. The results of this experiment are shown in Fig. 6. Although the viable bacterial counts of E. coli 312 in the exudates were lower than those of S. aureus at the time of inoculation, there was an increase to 10^8 CFU/ml or greater at 24 h for all inoculum sizes. Mucin was not necessary for the growth of E. coli 312, although it was needed for the growth of S. aureus 209P.

(ii) Bactericidal effect. Rat pouches were inoculated with E. coli 312 in a concentration of approximately 10^5 CFU/ml. Thirty minutes later the animals were given 20 mg of either cefazolin or cephalothin per kg i.m., and the pouch exudates were examined for viable bacteria at 5 and 24 h. Viable bacterial counts of E. coli 312 increased in the pouch exudates of the control group untreated with antibiotics and of the cephalothin group at 5 h, but decreased in the cefazolin group at 5 h (Fig. 7). At 24 h viable bacterial counts of 10^8 CFU/ml or greater were found in both drug groups. No satisfactory inhibitory effect of either cephalosporin was obtained under these conditions.

Figure 8 shows the results of two i.m. injections of cefazolin (20 mg/kg) given 30 min and 5 h after inoculation with E. coli 312. In the untreated control group, viable bacterial counts in the exudates increased to greater than 10^8 CFU/ml at 24 h, whereas in the cefazolin group viable bacterial counts decreased to less than 10^5 CFU/ml. In the cephalothin group, how-
ever, no bactericidal effect was obtained after two injections of the test drug.

**DISCUSSION**

In the laboratory evaluation of an antibiotic, in vivo activity is usually assessed by studying the protective effect in systemic infection in mice. The therapeutic effect, however, should be evaluated on the basis of efficacy in the treatment of established infection. In local infection systems, the effect of an antibiotic depends upon the drug concentrations that reach the site of infection. Therefore, the therapeutic effect in experimental local infections should be assessed on the basis of both in vitro activity and the antibiotic concentration achieved at the infection site. For this reason, we have modified the local infection system of Smith and Wood (9) in granuloma pouches in rats and have compared the therapeutic effect of cefazolin and cephalothin against *S. aureus* 209P and *E. coli* 312 in this system. The therapeutic effect of cefazolin was found to be greater than that of cephalothin, which correlated with the higher exudate levels obtained with cefazolin. Cefazolin is known to be characterized by higher serum levels and higher protein binding in humans and other animals (2, 5, 8). It is generally believed that the high serum levels are due to the high serum protein binding of this antibiotic. It is also conjectured that the high protein binding may inhibit the distribution of this drug into tissue or tissue fluids (3, 7, 10).
The tissue distribution of cefazolin in healthy animals, however, is not lower than that of other cephalosporins, as has been reported in a previous paper (5). Likewise, in the present study, exudate levels of cefazolin in granuloma pouches were higher than those of cephalothin. Monti et al. (4) have also reported that cephalosporin concentrations in peritoneal and subcutaneous fluids are unaffected by the degree of protein binding but are better correlated with blood levels achieved and the duration of such levels.

In the local infection system provided in this study, viable cell counts of S. aureus 209P, which has a low MIC of 0.1 \( \mu g/ml \), markedly decreased with a single i.m. injection of 20 mg of cefazolin per kg. In the case of E. coli 312, for which the MIC is 1.56 \( \mu g/ml \), however, two i.m. injections of 20 mg/kg each were required to reduce the viable cell count, even though the

**FIG. 7.** Bactericidal effect of cefazolin and cephalothin against E. coli 312 in granuloma pouch (day 7 after forming) in rats after a single i.m. injection. Dose: 20 mg/kg 0.5 h after inoculation. Bars: (stippled) control; (dotted) cefazolin; (hatched) cephalothin.

**FIG. 8.** Bactericidal effect of cefazolin and cephalothin against E. coli 312 in granuloma pouch (day 7 after forming) in rats after two i.m. injections. Dose: 20 mg/kg each 0.5 and 5 h after inoculation. Bars: (stippled) control; (dotted) cefazolin; (hatched) cephalothin.
peak concentration of cefazolin in the exudate exceeded the MIC. These results suggest that the duration of antibiotic levels in an exudate and the bacterial species studied are important factors in determining the therapeutic effect of an antibiotic. For the relation between the duration of levels and bactericidal activity of antibiotics, Eagle et al. (1) reported the importance of the aggregate time, when penicillin remains at effective bactericidal levels. These relationships will be evaluated in future studies.

LITERATURE CITED