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Penetration of Cefixime into Fibrin Clots and In Vivo Efficacy against *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*

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The experimental model of infected fibrin clots in rabbits was used to study the penetration and in vivo activity of cefixime against *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*. The respective MICs of cefixime against these strains were 0.25, 2, and 8 μg/ml. The clots were infected with 10^6 to 10^8 CFU/g. Groups of four animals for each strain received an intravenous injection of 100 mg of cefixime per kg over 30 min. High peak levels were observed in serum (146.5 μg/ml) and clots (15.8 μg/g), and the antibiotic was still detectable in the clots (0.6 μg/g) 24 h after administration. The respective serum and clot elimination half-lives were 0.7 and 5.0 h. The mean serum protein binding was 23.8 ± 3.8%. Cefixime was highly bactericidal against *K. pneumoniae* and *E. coli* and reduced, over a 24-h period, their respective colony counts by 7.8 $\log_{10}$ and 6.2 $\log_{10}$ CFU/g of fibrin. It was less effective against *S. aureus* but still reduced the bacterial counts by 2.8 $\log_{10}$ CFU/g of fibrin. The present results demonstrate that cefixime, a new broad-spectrum oral cephalosporin, has a long tissue half-life which ensured, at the dose given here, good in vivo bactericidal activity against both gram-positive and gram-negative bacteria up to 24 h after administration of the antibiotic.

Cefixime (CL 284,635; FK 027) is a new cephalosporin which can be taken orally and which has a wide spectrum of antimicrobial activity against several gram-positive and gram-negative bacteria (10). In humans, this drug has a much longer half-life (3 h) than other oral cephalosporins including cephalexin, cefaclor, cephradine, and cefadroxil (<1.2 h) (4).

In the present investigation, an ex-in-vivo experimental model in which infected fibrin clots are inserted under the skin of rabbits was used to study the tissue penetration of cefixime and its in vivo activity against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Bacterial strains. Three bacterial strains including *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *K. pneumoniae* ATCC 13883 were used for the experiments. The MICs of the antibiotic against the three strains were determined as previously described by a microdilution method in which Mueller-Hinton broth was used as a diluent and the inoculum was 10^6 CFU/ml (9).

Preparation of noninfected and infected fibrin clots. As we have previously described (8), a sterile solution of 3% bovine fibrinogen (Sigma Chemical Co., St. Louis, Mo.) was supplemented with 5% Mueller-Hinton broth (sterile or infected with an inoculum [10^{6-8} CFU/ml] of the strain to be studied and distributed in a 2-ml volume into siliconized test tubes [13 by 100 mm]). A 0.1-ml sample of bovine thrombin (250 U/ml; Parke, Davis & Co., Detroit, Mich.) was added to each tube. The resulting clots were then gently removed, washed in sterile saline solution for 5 min, and immediately inserted subcutaneously in rabbits. In our model, the size, volume, and exchange surface of extravascular sites were identical in all animals. Once under the skin of rabbits, the concentrations of proteins in fibrin clots reached a maximum of 15% of the protein content in serum.

Rabbit model. As described previously (8), New Zealand White female rabbits (2 to 3 kg) were given an intramuscular injection of 20 mg of chlorpromazine per kg. Both flanks were shaved and swabbed with iodine and alcohol. The skin was anesthetized with 2% lidocaine, and a 4-cm incision was made. After blunt dissection of the skin, four to six infected or noninfected clots were placed in each subcutaneous pocket to limit clustering. Autoclips (18 mm) were applied to close the incision. A scalp-vein needle (23 gauge) inserted in the marginal vein of the left ear served for infusion of the antibiotic. A Harvard infusion pump was used to ensure a steady rate of flow. Another scalp-vein needle (21 gauge) placed in the central vein of the right ear was used to collect blood samples.

Antibiotic regimen. A dose of 100 mg of cefixime per kg was infused over 30 min in each rabbit. Four rabbits were used for each bacteria studied and two for the experiments in which sterile clots were inserted. Blood and fibrin clots were removed aseptically before the administration of antibiotics and at 0.5, 1, 2, 4, 6, and 24 h after the beginning of the infusion.

Determination of drug concentrations and protein binding. Clots were weighed and homogenized in a 1% solution of trypsin (Difco Laboratories, Detroit, Mich.) in a volume equal to the weight of the clot. Trypsin had no effect on antibiotic activity and did not significantly influence the bacterial count. Dissolved clots and serum samples were bioassayed by a conventional agar diffusion method with *E. coli* MB3804 as the assay organism and a bilayer agar with tryptic soy agar (Difco) as the bottom layer and nutrient agar (Difco) as the top layer. The lower limit of detection was 0.2 μg/ml. Standard solutions were prepared by diluting known

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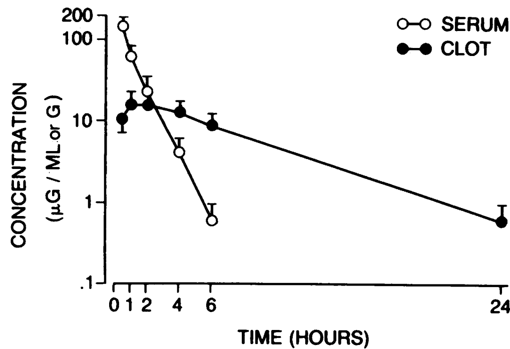

FIG. 1. Mean concentrations of cefixime in serum and clots (pooled infected and noninfected) after a single bolus injection of 100 mg/kg over 30 min. Each point gives the mean value for 16 rabbits, and the vertical bars indicate the standard deviation.

amounts of antibiotic in rabbit serum or in trypsinized fibrin clots.

The degree of protein binding of antibiotic to rabbit serum was determined with four concentrations, 25, 50, 100, and 250 µg/ml. Protein binding was determined by ultrafiltration with the Centrifree micropartition system (Amicon Canada, Mississauga, Ontario) (7).

In vivo efficacy. As previously described (8), the efficacy of the various regimens was evaluated by analysis of the bacterial content of infected clots at each timed interval. Appropriate dilutions of trypsinized clots were inoculated on agar followed by incubation at 37°C for 24 h. Since 0.1 ml of the diluted samples was spread over the entire surface of a 140-mm agar plate, drug carry-over was not a problem.

The rate of bacterial killing in fibrin clots was compared by three mathematical approaches: (i) the variation between bacterial count in fibrin clots at the beginning and the end of therapy ($\log_{10}$ CFU per gram); (ii) the kinetics of bacterial killing over a 24-h period as described by Sande et al. (11), using the slope of a least-squares regression line of surviving microorganisms; (iii) the killing index, which was calculated by dividing the area under the curve of the number of bacteria killed versus time by the number of bacteria at the beginning of therapy and the time of therapy. This percent killing can be expressed mathematically as: (AUC $\log_{10}$ bacteria killed × 100)/($\log_{10}$ bacteria at the beginning × 24 h). This killing index has the advantage of evaluating the overall bactericidal activity over a determined time period after administration of antibiotic.

Pharmacokinetic and statistical analysis. The area under the curve of concentration versus time (AUC) was obtained by the method of successive trapezoidal approximation from time zero to 24 h. The results are presented as the mean ± standard deviation of 14 experiments. Pharmacokinetic analysis was done as previously published (8), using a one-compartment model. Statistics were performed by an analysis of variance with repeated measure and a one-way analysis of variance. Statistics for evaluating efficacy data were performed by correlation tests.

RESULTS

In vitro studies. The respective MICs for *E. coli*, *S. aureus*, and *K. pneumoniae* were 2.0, 8.0, and 0.25 µg/ml. The mean protein binding to rabbit serum in the four concentrations studied was 23.8 ± 3.8%. The percent protein binding was 28.6% at 25 µg/ml, 24.6% at 50 µg/ml, 22.2% at 100 µg/ml, and 19.6% at 250 µg/ml.

Antibiotic levels. Antibiotic levels in serum and fibrin clots are shown in Fig. 1. Peak concentrations were achieved in the serum at the end of the 30-min infusion of the antibiotic in all animals. The mean peak serum level was 146.5 ± 6.2 µg/ml. The concentration at 24 h was 0.3 ± 0.1 µg/ml but was detectable in only 4 of 14 animals, and the concentrations were at the lower level of detection. In clots, the mean peak concentration was 15.8 ± 2.4 µg/ml at 2 h and 0.6 ± 0.1 µg/ml at 24 h and was detectable in all animals. There was no significant difference over the 24-h period in the concentrations of antibiotic observed in either serum or clots between infected and noninfected animals. The respective elimination half-lives in serum and clots were 0.7 ± 0.1 and 5.0 ± 1 h.

In vivo bactericidal activity. The comparative in vivo efficacy of cefixime against the three bacterial strains is shown in Fig. 2. At 24 h, the bacterial count in fibrin clots of untreated control animals was 7.5 $\log_{10}$ CFU/g for *S. aureus*, 8.5 $\log_{10}$ CFU/g for *K. pneumoniae*, and 10 $\log_{10}$ CFU/g for *E. coli*. Cefixime was very effective in reducing titers of all bacteria within the first 6 h of the experiment. Fibrin clots infected with *K. pneumoniae* were sterilized within 24 h after the administration of cefixime. During the first 6 h of the experiments, we observed a rapid decline from 6.3 to 4.1 $\log_{10}$ CFU of *S. aureus* per g; the bacterial count reached a level of 3.5 $\log_{10}$ CFU/g at 24 h. The decrease in the number of *E. coli* within the clot was also very rapid within the first 6 h and almost reached a plateau which was maintained up to 24 h.

Tissue penetration and efficacy parameters. Parameters for the evaluation of tissue penetration and efficacy are presented in Table 1. Each tissue penetration variable was paired with efficacy parameters by means of a correlation test. The best correlation was observed between time above the MIC in fibrin clots and the killing index ($r = 0.998$; $P < 0.001$). While there was no correlation between the ratio of

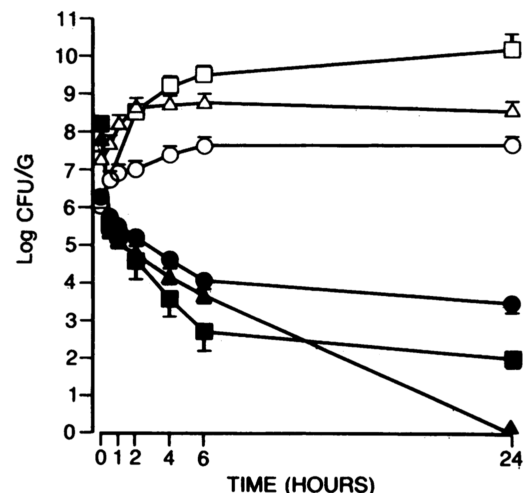

FIG. 2. Comparative in vivo efficacy of cefixime against *E. coli*, *K. pneumoniae*, and *S. aureus* inserted in fibrin clots. Each point gives the mean value for four rabbits, and vertical bars indicate the standard deviation. Symbols: ○, control *S. aureus*; ●, *S. aureus* plus cefixime; □, control *E. coli*; ■, *E. coli* plus cefixime; △, control *K. pneumoniae*; ▲, *K. pneumoniae* plus cefixime.

TABLE 1. Parameters for the evaluation of penetration and efficacy of cefixime in infected fibrin clots

Microorganisms	AUC_{0-24} (clots/serum)[a]	Peak level (clots/serum)[a]	Time (h) above MIC[b]	Log_{10} CFU/g at 24 h	Slope of killing curve	Killing index (%)[c]
Klebsiella pneumoniae	0.781 ± 0.1	0.134 ± 0.02	24	0	−0.33	67
Escherichia coli	1.12 ± 0.08	0.116 ± 0.02	22	2.0	−0.26	65
Staphylococcus aureus	1.0 ± 0.22	0.134 ± 0.02	7.5	3.5	−0.12	35

[a] Mean ± standard deviation of four replicate experiments.
[b] MIC of cefixime for *K. pneumoniae* was 0.25 μg/ml, for *E. coli*, 2.0 μg/ml, and for *S. aureus*, 8.0 μg/ml.
[c] Killing index was calculated by determining the AUC of bacteria killed versus time.

peak levels in fibrin clots and serum and the killing index ($r = -0.45$), a poor correlation between the ratio of the AUC in fibrin clots to the AUC in serum and efficacy was observed ($r = -0.21$). There was a good correlation between MIC and killing index ($r = -0.99$; $P < 0.01$).

DISCUSSION

There are very limited data on the pharmacology of cefixime and its tissue penetration. In this study, we examined simultaneously the interaction between the concentration of antibiotic in serum and fibrin clots and its efficacy in vivo. We showed that a single large injection of cefixime resulted in high peak levels and AUC in both serum and fibrin clots. The elimination half-life of cefixime in fibrin clots was longer than the serum half-life. These data are consistent with our previous observations with other antibiotics (1, 3, 8). The serum half-life of 0.7 h for cefixime was shorter than that observed in humans (4), but at 24 h, four animals still had detectable levels of cefixime which were above the MIC for *K. pneumoniae*. Fibrin is extremely hard to penetrate (3), but a single injection gave very high concentrations of cefixime within the clots and resulted in rapid killing of the sensitive bacteria. The limited protein binding to rabbit serum (23.8%) did not greatly influence the penetration and efficacy of the drug. Of interest is the observation of decreasing percent protein binding with increasing cefixime concentration.

The MICs of cefixime against the three microorganisms studied were compatible with previously published data (10). The levels of cefixime were maintained above the MIC for the *K. pneumoniae* imbedded in fibrin for 24 h and sterilized the clots. Against *E. coli*, cefixime was also very effective but could not sterilize the clots. This was most likely due to the MIC of cefixime against *E. coli*, which was almost 10 times higher than that for *K. pneumoniae*. As observed in bacterial endocarditis (5) and in pyelonephritis (2), a high ratio between tissue concentration and MIC may be necessary to neutralize bacteria in a protected environment such as vegetations or the medulla of the kidney where host defenses are not necessarily operating in conjunction with antibiotics to cure the infection. In the fibrin clot model bacteria are also exposed to the antibiotics without the help of host defenses. As demonstrated before, there is a progressive gradient in the concentration of drugs toward the core of infected tissues or abscesses (6). Although the antibiotic level in fibrin was above the MIC for long periods after the administration of the antibiotic, bacteria might have been protected in the core of the fibrin clots where concentrations could have been lower than on the surface. This could explain why at 6 h *E. coli* and *S. aureus* counts reached a plateau and were maintained almost at that level up to the end of the experiments. Moreover, the subinhibitory concentrations still detectable in fibrin clots up to 24 h could have prevented the regrowth of bacteria. We cannot eliminate the possibility of an in vivo postantibiotic effect which could have prevented regrowth of bacteria (11). Against *S. aureus*, which in general is more resistant to cefixime or other broad-spectrum cephalosporins, cefixime exhibited some activity.

Cefixime is one of the few oral broad-spectrum cephalosporins that are now under investigation. It has a long half-life, good in vitro activity, limited protein binding, and excellent fibrin penetration and in vivo efficacy against both gram-positive and gram-negative bacteria. The present results demonstrate that the long tissue half-life of cefixime provided, at the dose given here, good in vivo bactericidal activity up to 24 h after administration of the antibiotic.

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LITERATURE CITED

1. **Bergeron, M. G., D. Beauchamp, A. Poirier, and A. Bastille.** 1981. Continuous vs. intermittent administration of antimicrobial agents; tissue penetration and efficacy in vivo. Rev. Infect. Dis. **3**:84–97.
2. **Bergeron, M. G., and Y. Marois.** 1986. Therapeutic benefit from intrarenal levels of gentamicin when compared with ampicillin, cephalothin, and trimethoprim in the treatment of *E. coli* pyelonephritis. Kidney Int. **30**:481–487.
3. **Bergeron, M. G., B. M. Nguyan, S. Trottier, and L. Gauvreau.** 1977. Penetration of cefamandole, cephalothin, and desacetylcephalothin into fibrin clots. Antimicrob. Agents Chemother. **12**:682–687.
4. **Brittain, D. C., B. F. Scully, H. Toshiham, and H. C. Neu.** 1985. the pharmacokinetic and bactericidal characteristics of oral cefixime. Clin. Pharmacol. Ther. **38**:590–594.
5. **Eagle, H., R. Fleischman, and M. Levy.** 1953. "Continuous" vs. "discontinuous" therapy with penicillin. The effect of the interval between injections on therapeutic efficacy. N. Engl. J. Med. **248**:481–488.
6. **Hansson, E., A. Hanugren, and S. Ullberg.** 1966. Autoradiographic studies with labelled penicillin, streptomycin, tetracycline, and cyclosporin, p. 156–164. *In* M. Herald and Z. Gabriel (ed.), Antibiotics, advances in research, production and clinical use. Butterworths, London.
7. **Kurz, H., H. Trunk, and B. Weitz.** 1977. Evaluation of methods to determine protein binding of drugs. Equilibrium dialysis, ultrafiltration, ultracentrifugation, and gel filtration. Arzneim. Forsch. **27**:1373–1380.
8. **Lavoie, G. Y., and M. G. Bergeron.** 1985. Influence of four modes of administration or penetration of aztreonam, cefuroxime, and ampicillin into interstitial fluid and fibrin clots, and on in vivo efficacy against *Haemophilus influenzae*. Antimicrob. Agents Chemother. **28**:404–412.
9. **National Committee for Clinical Laboratory Standards.** 1983.

Tentative standard: methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.

10. **Neu, H. C., N. X. Chin, and P. Labthavikul.** 1984. Comparative in vitro activity and beta-lactamase stability of FR 17027, a new orally active cephalosporin. Antimicrob. Agents Chemother. **26**:174–180.
11. **Sande, M. A., G. M. Korzeniowski, G. M. Allegro, R. O. Brennan, O. Zak, and M. Sheld.** 1981. Intermittent or continuous therapy of experimental meningitis due to *Stretococcus pneumoniae* in rabbits. Preliminary observations on the post antibiotic effect in vivo. Rev. Infect. Dis. **3**:98–109.