COMPARATIVE BACTERICIDAL EFFECT OF CEFORANIDE (BL-S 786) AND FIVE OTHER CEPHALOSPORINS IN AN *IN VITRO* PHARMACOKINETIC MODEL*

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The bactericidal activity of ceforanide was compared, in an *in vitro* kinetic model, with that of five other cephalosporins: cephalothin, cefazolin, cefamandole, cefuroxime, and cefoxitin. Cultures of various pathogens in 95% human serum were incubated for 12 hours in the presence of the cephalosporins whose concentrations were modified periodically—by addition of a concentrated solution of drug or dilution with unmedicated serum—in order to simulate the variation of antibiotic concentration in human blood after a one-gram intramuscular dose. One Gram-positive strain and six Gram-negative strains were used. Bactericidal activity was assessed by monitoring changes in the number of colony-forming units.

Tests showed that against *Klebsiella pneumoniae*, ceforanide was the most active of the six cephalosporins. *Proteus mirabilis* was more susceptible to ceforanide and cefuroxime than to the other compounds; *Enterobacter cloacae* to ceforanide, cefuroxime, and cefamandole; *Escherichia coli* to ceforanide, cefuroxime, cefamandole, and cefazolin. The number of viable cells of *Staphylococcus aureus* was reduced below detectable levels by all cephalosporins except cefoxitin. On the other hand, *Providencia stuartii* was virtually unaffected by all of the cephalosporins except cefoxitin.

Ceforanide, formerly known as BL-S 786, is a new semi-synthetic cephalosporin with a broad spectrum of antibacterial activity[1~4]. One of its outstanding characteristics is high and prolonged blood levels after intramuscular administration to humans[5] as well as laboratory animals[3]. This favorable pharmacokinetic profile undoubtedly contributes to its efficacy as a therapeutic agent in the treatment of experimental mouse infections[3,4].

A major disadvantage of standard assay procedures for testing antibiotic activity is that their results do not reflect the putative effect of pharmacokinetic properties on activity. Consequently, the full antimicrobial potential of a compound with a superior pharmacokinetic profile may be overlooked. With this in mind, we have studied the activity of ceforanide in an experimental model designed to consider the effects of such properties.

The model is an adaptation of one recently described by NISHIDA *et al.* for determining bactericidal activity[6]. In this pharmacokinetic model, the concentration of antibiotic is modified periodically by addition of a concentrated solution of drug, or by dilution with unmedicated medium, in order to simulate the variation of antibiotic concentration in the blood stream. In adapting NISHIDA's method, we have made one significant change. In order to simulate physiological conditions more closely, our studies were conducted in 95% human serum instead of broth.

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This paper contains results of experiments using the pharmacokinetic model to compare the bactericidal activity of ceforanide with that of five other cephalosporins: cephalothin, cefazolin, cefamandole, cefoxitin, and cefuroxime.

Materials and Methods

Cephalosporins

Ceforanide was synthesized by members of the Product Development Department, Bristol Laboratories[7]. The other cephalosporins were supplied by Eli Lilly and Company (Na cephalothin, cefamandole nafate), Merck, Sharpe & Dohme (Na cefoxitin), Glaxo Research Ltd. (Na cefuroxime), and Bristol-Europe, S.p.A. (Na cefazolin). All compounds were adjusted for chemical purity.

Bacteria

The organisms were of clinical origin and typical in their sensitivity to the cephalosporins used in this study, as determined by standard assay procedures for growth inhibitory activity. They were as follows: *Klebsiella pneumoniae* A15130, *Proteus mirabilis* A20121, *Escherichia coli* A15119, *E. coli* A20108, *Enterobacter cloacae* A20464, *Providencia stuartii* A20692, and *Staphylococcus aureus* A20405. The susceptibility patterns of these organisms to the six cephalosporins under standard assay conditions in MUELLER-HINTON medium are given in Table 1.

Table 1. Susceptibility of various bacterial strains to six cephalosporins in MUELLER-HINTON medium under standard assay conditions*

Organism		Minimum inhibitory concentration (μg/ml)					
		Ceforanide	Cephalothin	Cefazolin	Cefamandole	Cefoxitin	Cefuroxime
K. pneumoniae	A 15130	0.5	4	1	1	2	4
P. mirabilis	A 20121	0.25	8	4	1	4	2
E. coli	A 15119	0.25	8	1	0.5	4	4
E. coli	A 20108	0.25	4	1	0.25	4	4
E. cloacae	A 20464	16	>125	>125	16	>125	16
P. stuartii	A 20692	1	125	32	2	1	8
S. aureus	A 20405	2	0.25	0.5	0.5	2	1

* As described by BUCK & PRICE[8]

Medium

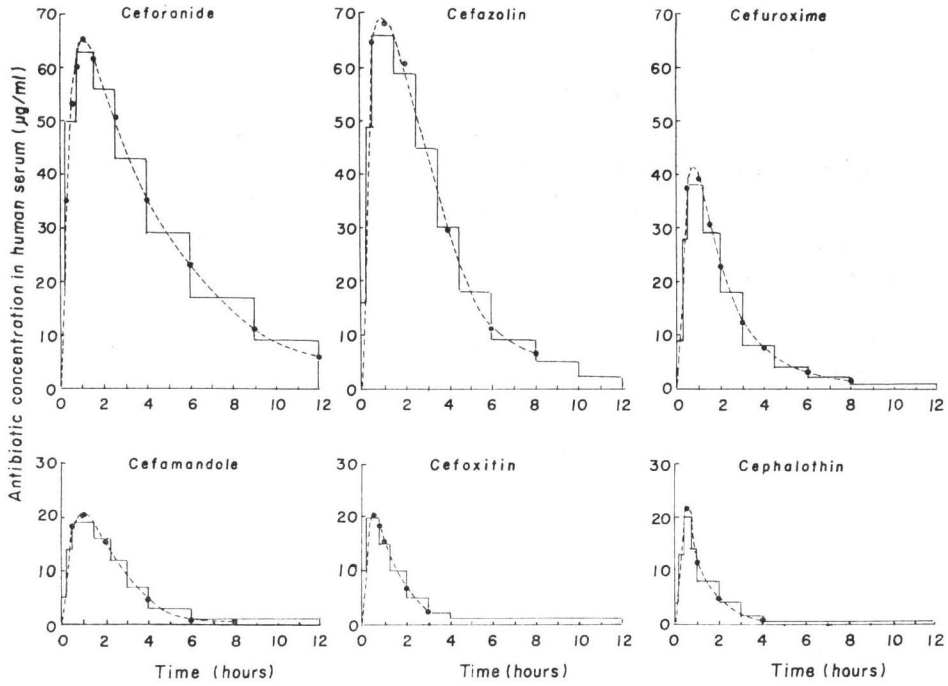
Studies were conducted in 95% pooled human serum with 5% MUELLER-HINTON Broth (Difco). The latter was of 10-fold normal strength for *P. mirabilis* and *P. stuartii*. These two organisms, as well as *E. coli* A20108, also required serum inactivation (heating at 56~60°C for 30 minutes). The medium was adjusted to pH 7.4 with HCl and sterilized with Nalgene filters (0.45 μm). Unless otherwise specified, the same medium was used for all stages of culturing and diluting throughout these studies.

Bactericidal activity by the kinetic model

The desired concentration adjustments for each antibiotic were determined by drawing a stepwise simulated curve based on the actual average serum levels obtained in man after a 1-g dose given intramuscularly (Fig. 1). For all compounds, the areas under the simulated and actual serum level curves were approximately the same. Intervals between adjustments were a function of the slope of the actual curves, which were constructed from data supplied by the respective manufacturers of ceforanide (Tolerance study #103 - Bristol Laboratories), cephalothin[9], cefazolin (Data Brochure: Ancef-Cefazolin Sodium, Smith Kline & French Laboratories-1973), cefamandole[10], cefoxitin[11], and cefuroxime[12].

The inoculum was prepared by transferring bacterial cells from a bead preparation[13] to a tube containing the appropriate serum-supplement medium and incubating overnight at 37°C. The culture

Fig. 1. Actual and simulated serum levels of six cephalosporins after an intramuscular dose of 1 g to humans.



was then adjusted to a cell-density of about 5×10^5 cells/ml. Aliquots of approximately 10 ml were transferred to test tubes (25×200 mm), one for a growth control and one for each antibiotic to be tested. The tubes were placed in a water bath at 37°C and the test was begun by adding 0.1 ml of antibiotic stock solution, made in the same medium as the inoculum. Antibiotic concentrations were adjusted according to the simulated pattern of the blood level curve by adding concentrated stock solution or unmedicated medium (See Table 2 for a typical schedule of adjustments). Control cultures were not diluted. The test was terminated after 12 hours of incubation.

At selected times (0, 1.5, 2.5, 3.5, 4.5, 6, 8, 10, 12 hours), cultures were vortexed and samples removed, diluted in saline, and plated in triplicate for viable cell count determinations. β-Lactamase (Broad Spectrum Mixture, Whatman Biochemicals Limited) was added when necessary before plating, to hydrolyze any residual antibiotic. A kill-curve was constructed on semi-log paper, plotting cell concentration against time. Each point was corrected by a dilution factor to compensate for volume changes due to the adjustments with medicated and unmedicated medium.

Bactericidal activity at fixed antibiotic concentrations

Standard kill-curve studies were conducted for ceforanide and cefamandole against the *S. aureus* strain. Conditions for this test were identical to those of the kinetic model, except that the antibiotic concentration was kept constant throughout the test. Cephalosporin concentrations ranged from $0.5 \sim 32$ μg/ml. In duplicate tests the initial cell concentration was $1 \sim 2 \times 10^5$ cells/ml. Samples were removed for cell count determinations over a 12-hour incubation period, at the same intervals as in the kinetic model.

Stability in serum

Stability in the test medium was determined at 37°C over a period of 24 hours. The initial antibiotic concentration was 50 μg/ml for ceforanide, cefoxitin, cefazolin, and cefuroxime. For cephalothin and cefamandole, 20 μg/ml were used. Residual antibiotic activity was determined periodically by a turbidimetric assay procedure (*S. aureus* ATCC 6538P) or by an antibiotic diffusion technique (*Bacillus subtilis* ATCC 6633).

Results

1. Bactericidal Activity by the Kinetic Model

All seven strains were tested under conditions simulating antibiotic concentrations in serum following a one-gram intramuscular (i.m.) dose to humans. In some cases the medicated culture overgrew the control once drug concentration was below inhibitory levels; the control, not being diluted, was limited by the medium. Data are based on an average of two or more experiments.

K. pneumoniae A15130. As shown in Fig. 2, ceforanide was the most active cephalosporin against this strain. In the presence of all other compounds, the cultures resumed growth at varying times and at different rates, while in the presence of ceforanide they failed to do so within the 12-hour

Fig. 2. Bactericidal activity of six cephalosporins against *Klebsiella pneumoniae* and *Proteus mirabilis* determined by the kinetic model.

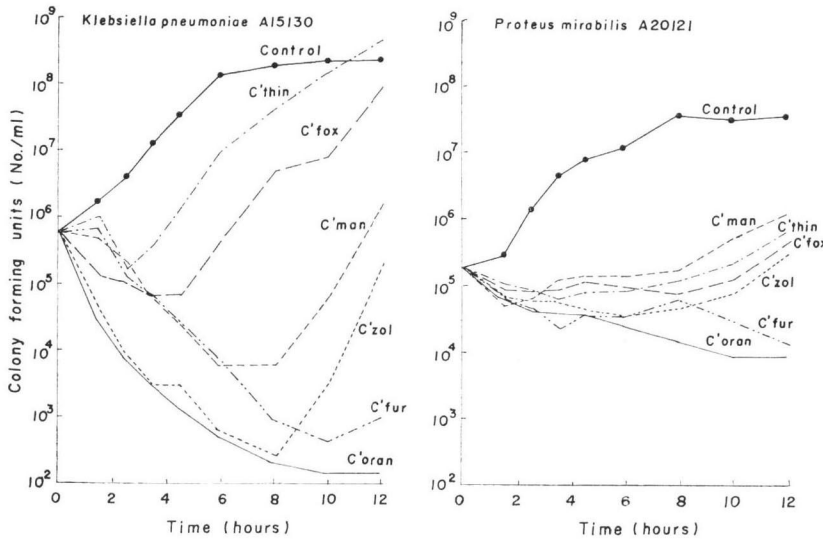


Fig. 3. Bactericidal activity of six cephalosporins against two strains of *Escherichia coli* determined by the kinetic model.

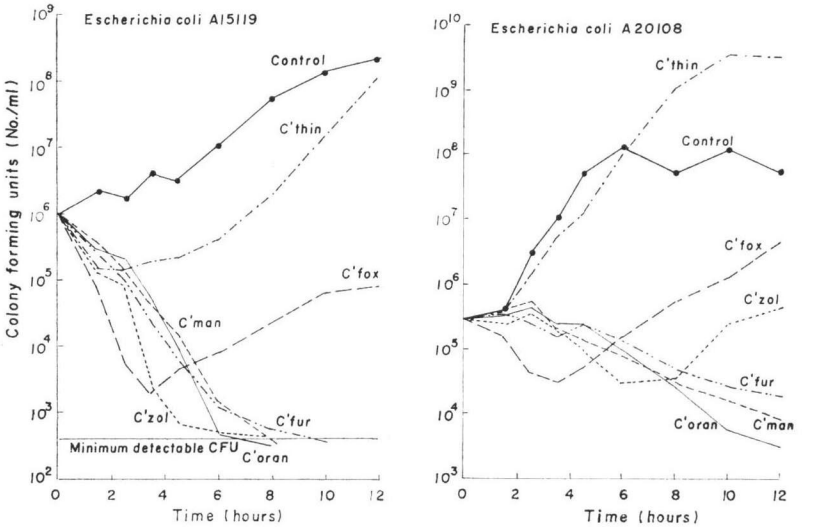
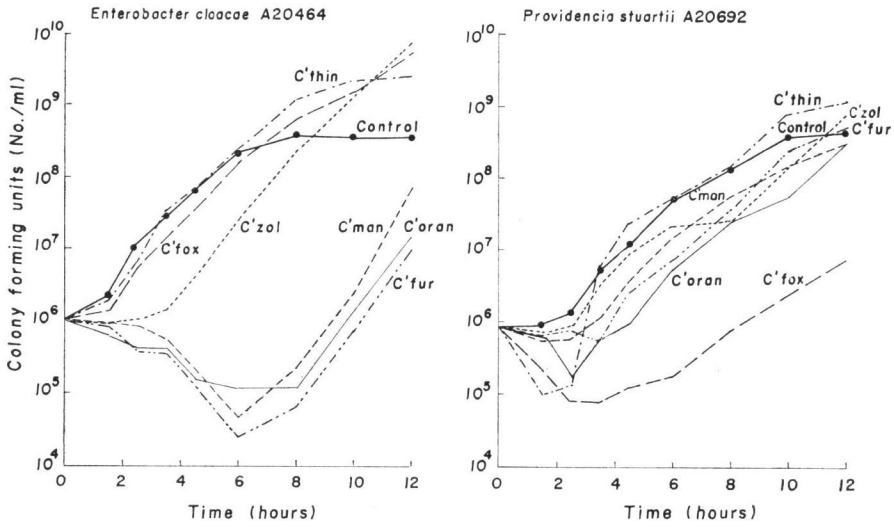


Fig. 4. Bactericidal activity of six cephalosporins against *Enterobacter cloacae* and *Providencia stuartii* determined by the kinetic model.

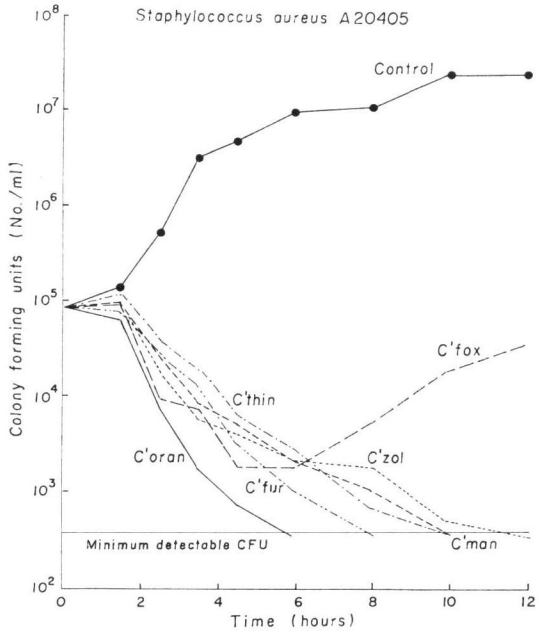


test period. The cidal effect of cefazolin was quite similar to that of ceforanide but only through 8 hours, when cells resumed growth.

P. mirabilis A20121. Ceforanide was slightly more effective than cefuroxime, with neither compound permitting cell regrowth within 12 hours (Fig. 2). The other four cephalosporins had some effect in that there was no net change in the number of colony-forming units within the first 8 hours of incubation. However, there were indications of subsequent regrowth of cells incubated in the presence of these compounds.

E. coli A15119. Ceforanide, cefazolin, and cefamandole reduced the number of colony-forming units to below detectable levels by 8 hours, and cefuroxime by 10 hours (Fig. 3). None of these compounds allowed regrowth of cells through 12 hours. On the other hand, cefoxitin decreased the number of colony-forming units by almost three logs within 3.5 hours but regrowth occurred shortly thereafter.

Fig. 5. Bactericidal activity of six cephalosporins against *Staphylococcus aureus* determined by the kinetic model.



E. coli A20108. This organism was more susceptible to ceforanide, cefamandole, and cefuroxime than to the other antibiotics, as there was no regrowth of cells through 12 hours (Fig. 3). Cefazolin was similarly effective but only up to 8 hours, when cells resumed growth.

E. cloacae A20464. This strain was equally susceptible to ceforanide, cefuroxime, and cefamandole (Fig. 4). However, by 8 hours cells in the presence of each of these compounds resumed logarith-

mic growth.

P. stuartii A20692. This strain was virtually unaffected by any of the cephalosporins except cefoxitin (Fig. 4). Even this effect was slight, causing only a one-log decrease in cell count, with regrowth occurring after 4~6 hours.

S. aureus A20405. All cephalosporins except cefoxitin reduced the number of viable cells to below detectable levels (Fig. 5). Ceforanide showed the best cidal effect with over 99.5% loss of viability within 6 hours of incubation. Cefuroxime exhibited the same effect after 8 hours, cefamandole and cephalothin after 10 hours, and cefazolin after 12 hours of incubation.

2. Bactericidal Activity at Fixed Antibiotic Concentrations

A standard kill-curve study was conducted with ceforanide and cefamandole against *S. aureus* in order to gain greater insight as to the role of pharmacokinetic properties in the kinetic model. When concentrations of these cephalosporins were kept constant throughout the test, 4 μg/ml of cefamandole were required to kill 99.9% of the initial population versus 32 μg/ml of ceforanide. This was in good agreement with the order of activity obtained by conventional assay procedures for bacteriostatic activity (Table 1), but contrasted with that obtained in the kinetic model.

Table 2. Schedule of concentration adjustments for cephalothin in the kinetic model

Time	Type*	Change in volume (ml)	Volume (ml)	Antibiotic concentration (μg/ml)
0	A	0.1	9.8	4
10 min.	A	0.23	10.1	13
25 min.	A	0.18	10.3	20
45 min.	B	4.1	14.4	14
1 hr.	B	10.8	25.2	8
1.5 hrs.	C	−15.2	10.0	8
2 hrs.	B	10	20.0	4
2.5 hrs.	C	−10	10.0	4
3 hrs.	B	15	25.0	1.6
3.5 hrs.	C	−20	5.0	1.6
4 hrs.	B	15.1	20.1	0.4

* A: Addition of antibiotic stock solution (400 μg/ml).
B: Addition of unmedicated serum broth.
C: Removal of aliquots for cell count determinations.

3. Stability in Serum

In vitro, all cephalosporins except cephalothin were relatively stable in human serum, having half-lives in excess of 24 hours; cephalothin had a half-life of 5 hours. It is unlikely that the relative instability of cephalothin is a primary determinant of its behavior in the kinetic model, since within 3 hours its blood levels fall below minimal inhibitory concentrations for all organisms except *S. aureus*. Values we obtained for cephalothin and cefazolin are consistent with those reported by PITKIN *et al.*[14].

Discussion

In this kinetic model study, the bactericidal activity of ceforanide was equal to or greater than that of the other five cephalosporins against six of the seven strains tested. Only against *P. stuartii* did any other cephalosporin exhibit a better antimicrobial effect. Results from additional studies with *K. pneumoniae* A15130 and *E. coli* A20108, simulating serum levels after a 500-mg dose, were consistent with these data.

This investigation was undertaken with the expectation that results in the kinetic model would reflect the influence of pharmacokinetic parameters on activity. There are indications this was true since, in some cases, the order of activity of the cephalosporins could not have been predicted on the basis of conventional assay methods (Table 1). For example, when tested against *S. aureus*, ceforanide was 4-fold less active than cefamandole by the conventional method but more active than the latter in the kinetic model.

Although pharmacokinetic parameters were assumed to be responsible for the contrasting results, other test methods were needed to support this hypothesis. This was necessary because of the uncontrolled variables between the kinetic model and conventional assay methods, namely, (1) type of activity measured (bactericidal *vs.* bacteriostatic), (2) medium (serum *vs.* broth), (3) incubation period (12 hours *vs.* 16~20 hours), and (4) concentration state (fluctuating *vs.* fixed). The latter was critical because of its close association with the principle behind the kinetic model. To pursue this objective, a standard kill-curve study was conducted under conditions identical to those of the kinetic model except for usage of fixed, instead of fluctuating, drug concentrations. Consequently, three of the variables were harnessed, leaving only the fourth (concentration state) to explain the differences in activity patterns between the kinetic model and standard MIC techniques.

Since results from our standard kill-curve test were consistent with MIC data but contrasted with those of the kinetic model, it is logical to assume that pharmacokinetic parameters have been, at least to some extent, responsible for such differences. Regardless of the specific role of pharmacokinetics, the kinetic model does allow one to measure antibiotic activity under conditions simulating those in blood more closely than do standard methods.

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