ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1992, p. 2439–2443
0066-4804/92/112439-05$02.00/0

Vol. 36, No. 11

Comparison of the Antibacterial Effects of Cefepime and Ceftazidime against *Escherichia coli* In Vitro and In Vivo

HERMAN MATTIE,* BEATE A. RAZAB SEKH, MARC L. VAN OGTROP, AND ELISABETH VAN STRIJEN

Department of Infectious Diseases, University Hospital, 2300 RC Leiden, The Netherlands

Received 10 December 1991/Accepted 7 September 1992

The efficacies of cefepime and ceftazidime in an experimental *Escherichia coli* infection in granulocytopenic mice were related to their in vitro activites and their pharmacokinetic profiles. Cefepime had a higher intrinsic activity in vitro than ceftazidime, and it had a different pharmacokinetic profile, resulting in higher peak concentrations in plasma and a longer elimination half-life. To predict the antibacterial efficacy in vivo on the basis of in vitro activity and pharmacokinetics, we applied a mathematical model in which the in vitro effect is expressed as the difference in growth rate between control cultures and cultures grown in the presence of the antibiotic (E_R), whereas the in vivo effect is given by the difference in the number of CFU between controls and antibiotic-treated animals (E_N). The integral of E_R over time, called $E_R t$, was calculated by using in vivo concentrations. A significant linear relationship was found between E_N and $E_R t$ for different doses at various times up to 4 h after administration, although the slope of this relationship was slightly but significantly less for cefepime (0.44) than for ceftazidime (0.59).

Antibiotics contribute to the cure of patients with infections by inducing a decrease in the number of bacteria at the site of infection, irrespective of whether the drugs act on their own or in combination with host factors. It may therefore be argued that the effect of antibiotics on growth rate is the best in vitro parameter to predict the efficacy in vivo (9). In previous studies (9, 10) the relationship between the effect of antibiotics on the bacterial growth rate in vitro, their pharmacokinetics, and their effect on the number of bacteria in an experimental infection was explored by using a pharmacodynamic-pharmacokinetic model. It was found that a common predictive quantitative parameter for four cephalosporins could be established by using this method; this parameter was based on measurements of the efficacy in vitro and pharmacokinetics in mice. Cefepime is a new and potent cephalosporin which can be used for the treatment of gram-negative infections (3). Because of its potency, it is expected to be clinically effective at lower dosages than those of many cephalosporins already in use.

The present study was undertaken to establish the quantitative relationship between bacterial growth kinetics in vitro and the pharmacokinetics of cefepime in mice and subsequently to use this relationship to predict the effect in an experimental infection. For purposes of comparison, ceftazidime was also studied, since it is a well-established antibiotic for the treatment of gram-negative infections and was also used in the study mentioned above (9). The results of the present study could provide a basis for dosage schedules for humans.

MATERIALS AND METHODS

Antibiotics. Ceftazidime (activity, 84.2%) was obtained from Glaxo, Nieuwegein, The Netherlands, and cefepime (activity, 82.6%) was obtained from Bristol Myers, Brussels, Belgium. Solutions of the drugs were freshly prepared in phosphate-buffered saline (PBS) as recommended by the manufacturers.

* Corresponding author.

Animals. Male specific-pathogen-free Swiss mice (Broekman Institutes, Someren, The Netherlands) were used throughout the study.

Bacteria. A strain of *Escherichia coli* (serotype O-54) (kindly provided by Beecham Research Laboratories, Brockham Park, United Kingdom) was used as the test strain. The MICs of ceftazidime and cefepime for this microorganism, determined by the agar dilution method on Iso-Sensitest agar (Oxoid, Basingstoke, United Kingdom), were 0.125 and 0.032 mg/liter, respectively. The strain was serum resistant.

An overnight culture of the bacterium was prepared in brain heart infusion broth (Oxoid), snap-frozen in liquid nitrogen, and stored at −70°C. Before each experiment, aliquots were rapidly thawed in a water bath at 37°C.

Effect of the cephalosporins on the growth rate of *E. coli* in vitro. A 1:3,000 dilution of an overnight culture of *E. coli* in brain heart infusion broth was incubated in a shaking water bath at 37°C for 1 h and then distributed in 20-ml aliquots among 50-ml flasks. Samples were taken at 45-min intervals over a period of 3 h. Appropriate dilutions in PBS were plated onto diagnostic sensitivity test agar (Oxoid) plates and incubated overnight at 30°C; the CFU were then counted. The pharmacodynamic model has been described previously (7, 9). In this model the effect of a given concentration of the cephalosporins on bacterial numbers is expressed as the difference between the logarithms (base 10) of the numbers of CFU in the absence and presence of the antimicrobial agent (E_N). These values of E_N were fitted to the parameters of the following equation by multiple-regression analysis (9):

$$E_N = a_1 t + a_2 t^2 + a_3 e^{-t} + a_4 \quad (1)$$

The net killing rate is defined as the first derivative of E_N. Although the killing curves are not linear, there is some similarity between different concentrations in shape. Therefore, for further calculations the highest value of the net killing rate during the 3 h of exposure (E_R) was used as a quantitative parameter of the effect of a given concentration on bacterial growth. The concentration-effect relationship is given by the Hill equation:

$$E_R = E_{R,max} \times C^s/(EC_{50}{}^s + C^s) \quad (2)$$

where $E_{R,max}$ is the maximum value of E_R estimated from experimental results, C is the concentration of the drug, s is a parameter reflecting the steepness of the concentration-effect relationship, and EC_{50} is the concentration of the drug which yields 50% of the maximum effect. The parameters of the Hill equation were fitted by nonlinear least-squares regression analysis without weighting (NONLIN, SYSTAT 5.0; Systat Inc., Evanston, Ill.).

Effect of the cephalosporins on the number of *E. coli* cells in an experimental thigh muscle infection. An experimental thigh muscle infection model (5) was used to study the therapeutic efficacy of the cephalosporins in vivo. Mice were rendered granulocytopenic by total-body irradiation at 6 Gy (6-MV linear accelerator, SL 75/6; Philips, Eindhoven, The Netherlands). The mice were infected by injection of 8×10^6 to 1×10^7 CFU of *E. coli* into a thigh muscle. The antimicrobial agent was administered subcutaneously at various dosages 1 h later. The dose range studied was 0.5 to 8 mg/kg for ceftazidime and 0.2 to 3.2 mg/kg for cefepime. Control mice received PBS. In the first series of experiments, the mice were divided according to a Latin square. Four hours after administration of the antimicrobial agent, the mice were killed by cervical dislocation and the thigh muscle was excised and homogenized with 5 ml of sterile ice-cold PBS in a tissue homogenizer (Ystral, type X-1020; International Laboratorium Apparate GmbH, Döttingen, Germany). To count the CFU in the homogenate, we processed the samples as described for the in vitro experiments. The potency ratios for cefepime and ceftazidime in vivo were determined by using a parallel-line bioassay procedure and the logarithms of CFU. The parameter of the effect, $E_{N,t}$, is defined as the difference between the log numbers for untreated and antibiotic-treated animals at time t after administration.

In a second series of experiments, mice were sacrificed at 1-h intervals after administration of various doses of the antibiotics. The doses studied were 0.5 and 8 mg/kg for ceftazidime and 0.2 and 3.2 mg/kg for cefepime. Each experiment was performed in duplicate. The thigh muscles were processed as described above.

Pharmacokinetic study of the cephalosporins in mice. Single-dose pharmacokinetic studies of ceftazidime at 5 mg/kg and cefepime at 5 mg/kg were performed. Blood samples were taken by cardiac puncture with heparinized syringes after the animals had been killed by exposure to 100% CO_2. The samples were centrifuged at 1,500 $\times$ g for 10 min at room temperature, the plasma was removed, and the concentration was measured. Results were fitted graphically to a biexponential equation:

$$C_p = C_1 \times e^{-\lambda_1 t} - C_2 \times e^{-\lambda_2 t}$$

where C_p is the concentration and C_1, C_2, λ_1, and λ_2 are constants of the equation.

Protein binding of the cephalosporins in murine plasma was determined by equilibrium dialysis at 37°C (8).

HPLC analysis of the cephalosporin concentrations. Acetonitrile, dichloromethane, and sodium acetate were supplied by Merck, Darmstadt, Germany. The high-pressure liquid chromatography (HPLC) apparatus consisted of a constant-flow pump (Analytica BV, Rijswijk, The Netherlands) and a Spectroflow 773 absorbance detector (Kipp & Zonen, Breukelen, The Netherlands), set at 254 nm for ceftazidime and 280 nm for cefepime, connected to a BD40 recorder (Kipp & Zonen). Chromatography was performed on a stainless steel column 10 cm long and 3 mm in internal diameter, packed with 5-μm Hypersil ODS (Shandon SPL, Astmoor, United Kingdom) and fitted with a Rheodyne 7125 (Chrompack, Middelburg, The Netherlands) valve injector with a 20-μl capacity loop.

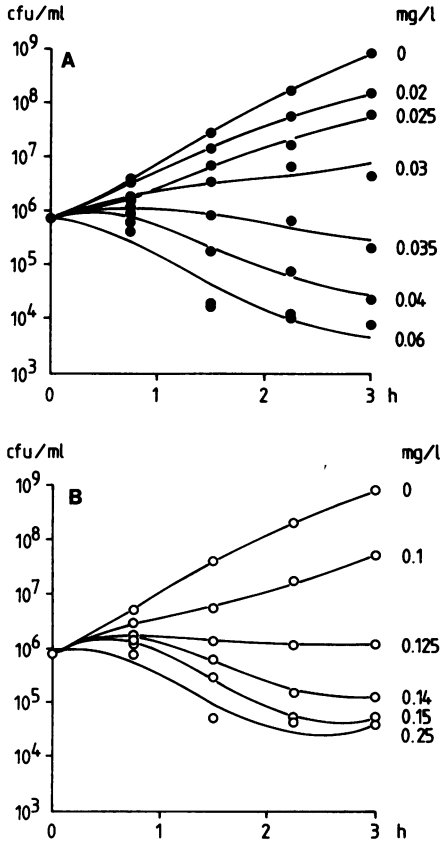

FIG. 1. Number of CFU of *E. coli* in vitro in the presence of various concentrations of cefepime (A) and ceftazidime (B). Curves were fitted according to equation 1 (see text).

The extraction procedure for plasma samples containing ceftazidime was as follows. A 250-μl aliquot of plasma was mixed vigorously in a Vortex mixer for 30 s with an equal volume of acetonitrile. The sample was centrifuged at 1,200 $\times$ g for 5 min; 400 μl of the supernatant was then added to 3 ml of dichloromethane and mixed for 30 s. This was again centrifuged at 1,200 $\times$ g for 5 min. Plasma samples containing cefepime were extracted by the method of Barbhaiya et al. (2).

An aliquot of 20 μl of the supernatant was then injected onto the HPLC column and eluted at a flow rate of 1 ml/min with 0.005 M acetate buffer (pH 5.5) containing 0.7% (vol/vol) acetonitrile. A calibration line was obtained by assaying standards prepared by adding known amounts of the drugs to pooled murine plasma. This was linear between 0.2 and 100 mg of ceftazidime per liter and between 0.5 and 100 mg of cefepime per liter. The coefficient of variation at the lowest concentration was 2% for ceftazidime and 3% for cefepime. At concentrations of 50%, the variation coefficients were similar.

Quantitative comparison of in vivo and in vitro efficacy. The antibiotic effect on numbers of CFU in vivo ($E_{N,t}$) was postulated to be proportional to the integral of the in vitro effect on growth rate over time, as represented by the following equation:

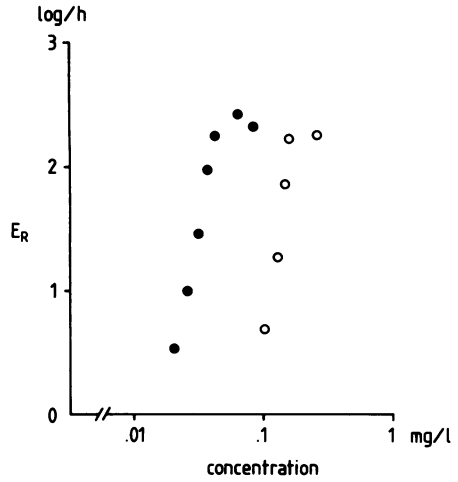

FIG. 2. Calculated values of the effect (E_R) of cefepime (●) and ceftazidime (○) on the in vitro growth rate of *E. coli*.

$$E_{N,t} = p\int_0^t E_R dt \quad (3)$$

in which $E_{N,t}$ is the difference between the logarithms of the numbers of CFU in untreated and antibiotic-treated animals and p is a proportionality factor which equals the ratio between the predicted and observed effects (9). The integral in equation 3, called $E_R t$, was computed by using Simpson's rule (4).

RESULTS

Antibacterial effect in vitro. Representative results of the short-term growth experiments are shown in Fig. 1. The exponential growth rate (base 10) of *E. coli* in brain heart infusion broth in the absence of antibiotics, as determined by linear regression analysis, was 0.93 h^{-1}, corresponding to a doubling time of 19 min. The concentration-effect relationships for the two cephalosporins in vitro, established from equation 2, are shown in Fig. 2; the values of the parameters of the Hill equation were calculated by assuming the same value of $E_{R,max}$ for both antibiotics. This value was 2.4 h^{-1}. The values of s were 5.40 and 4.53 and those of EC_{50} were

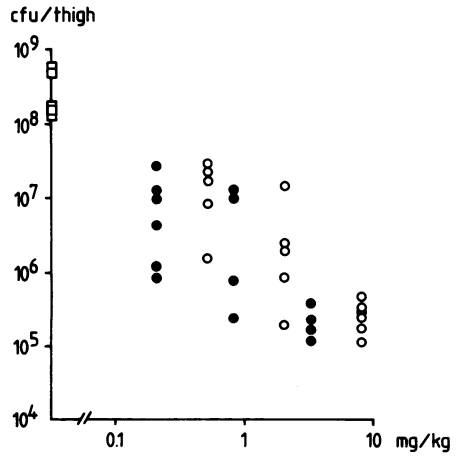

FIG. 3. Number of CFU of *E. coli* in an experimental thigh infection in irradiated mice 5 h after infection with 2×10^7 *E. coli* cells and 4 h after subcutaneous administration of cefepime (●), ceftazidime (○), or PBS (□).

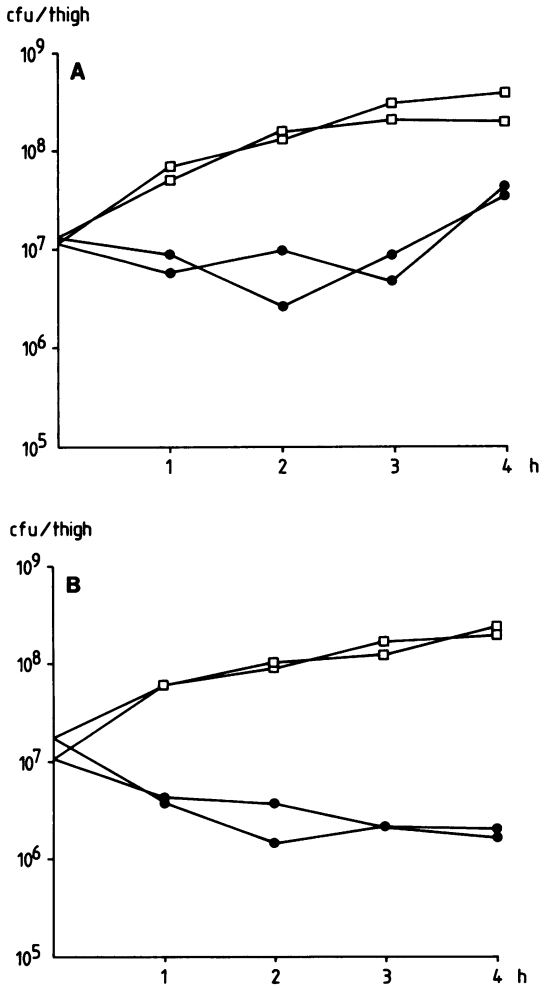

FIG. 4. Number of CFU of *E. coli* in thigh muscles of irradiated mice treated with cefepime (●) or PBS (□) at 0.2 (A) or 3.2 (B) mg/kg. Two experiments were performed at each dose.

0.030 and 0.122 mg/liter for cefepime and ceftazidime, respectively.

Antibacterial effect in vivo. The results of the first series of in vivo experiments are shown in Fig. 3. According to the standard bioassay procedure, there was a significant dose-effect relationship for the two antibiotics ($P < 0.001$) and the slopes were not significantly different ($P > 0.40$). The potency ratio was 3.73 (95% confidence limits, 1.98 and 7.04) in favor of cefepime.

A representative example of the results of the second series of experiments is shown in Fig. 4.

Pharmacokinetics. The concentrations of ceftazidime and cefepime in plasma after a dose of 5 mg/kg are shown in Fig. 5. Essentially, ceftazidime and cefepime did not bind to murine plasma (less than 5% was bound).

Quantitative comparison of in vivo and in vitro efficacies. As an example, the observed values of the antibiotic effect in vivo (E_N) and the values predicted on the basis of the effect in vitro and the pharmacokinetics ($E_R t$) for a dose of 3.2 mg of cefepime per kg are shown in Fig. 6. A declining value of E_N after 3 h was sometimes seen; this was due to a decline in growth rate in the control animals (compare with Fig. 4). Still, these values were included in further analysis. The

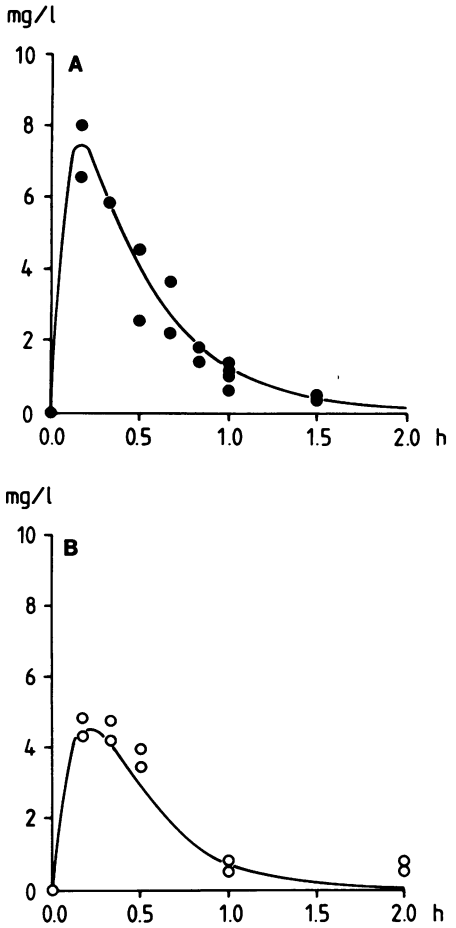

FIG. 5. Concentrations of two cephalosporins in plasma in mice after administration of a single subcutaneous dose of cefepime (A) or ceftazidime (B) at 5 mg/kg. The corresponding equations for the concentrations of free cephalosporins in plasma are $C_p = D \times 2.7 \times (e^{-2.41t} - e^{-13t})$ for cefepime and $C_p = D \times 3.6 \times (e^{-3.24t} - e^{-6.5t})$ for ceftazidime.

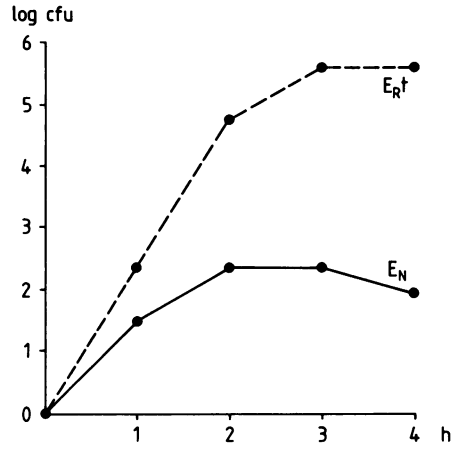

FIG. 6. Observed effect (E_N) and predicted effect ($E_R t$) of a dose of 3.2 mg of cefepime per kg on the $\log_{10}$ CFU in a thigh infection in mice. The effect is defined as the difference between the log CFU in treated and untreated animals.

relationship between all observed values of E_N and the values of $E_R t$ is shown in Fig. 7.

The relationships between the predicted and observed values are about the same for the two drugs. There is a small but significant ($P = 0.003$) nonlinear component of the regression of E_N on $E_R t$. This may also be due to the decreased inclusion of the observations at later time points, in particular at 4 h. The intersect with the abscissa (0.13) was not significantly different from zero ($P = 0.10$). The slopes through zero, representing the proportionality factor p in equation 3, were 0.44 (standard error of the mean [SEM], 0.05) for cefepime and 0.59 (SEM, 0.05) for ceftazidime, and the difference was statistically significant ($P < 0.001$).

DISCUSSION

The results of our study were used to calculate a quantitative parameter that relates the efficacies of cefepime and ceftazidime against *E. coli* in vivo to those in vivo. This parameter, called p, is the ratio between the observed effect in vivo and the effect predicted on the basis of a killing rate in vivo that is equal to the maximal killing rate in vitro. The value of p is about the same for the two antibiotics, i.e., 0.44 and 0.59 for cefepime and ceftazidime, respectively, but the difference is significant.

The value of p for ceftazidime was also very similar to that found in an earlier study, i.e., 0.65 (9). In that study a fixed time point was chosen to observe the effect in vivo, i.e., 4 h after administration of the antibiotic, but in the present study it could be shown that the value of p is independent not only of the dose but also of the time after antibiotic administration. Apparently the delay of onset of killing as seen in vitro is no longer significant in vivo 1 h after administration of the antibiotic, although it may contribute to the value lower than unity of the relationship between observation and prediction. Moreover, in that earlier study, similar values were also found for cefamandole and cefuroxime against *E. coli* as well as *Klebsiella pneumoniae*; for ceftriaxone it was slightly less. Since the results of the two studies are so consistent, a general conclusion can be formulated: that the effect of a single dose of a cephalosporin in vivo has about 50% of the effect predicted on the basis of the maximal killing rate in vitro and on the basis of the pharmacokinetics.

In the present study the pharmacokinetics in mice were fairly similar for the two cephalosporins. This explains why the potency ratio for each of the two drugs in vivo (i.e., 3.73) was also quite similar to that obtained with the bioassay procedure in vitro (i.e., 4.1). This kind of agreement, how-

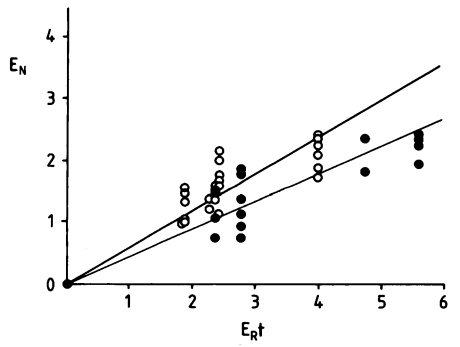

FIG. 7. Observed antibacterial effect (E_N) in a thigh infection in mice versus the predicted effect ($E_R t$) for cefepime (●) and ceftazidime (○) in vivo at all doses and all time points.

ever, is not found when there are important differences in pharmacokinetics between the drugs used (5). The calculation of p eliminates discrepancies between in vitro and in vivo potency ratios that are due to pharmacokinetic differences. It should be noted that the prediction of the in vivo effect is based on concentrations in plasma and not on those in tissue, since it was shown that in the infection model used, the unbound concentrations in tissue follow those in plasma very closely (6). Moreover, linear pharmacokinetics were assumed, since the sensitivity of the assay did not allow the measurement of concentrations in plasma at the doses used in the infection experiments.

Our results can be applied to the human situation to predict an optimal dosage schedule for cefepime, taking into account human pharmacokinetics. Assuming a volume of distribution in humans of about 20 liters, a half-life in plasma of 2 h, 20% protein binding (1), and a microorganism with a sensitivity in vitro similar to that of the *E. coli* strain used in our study, a dosage schedule of 80 mg twice a day would yield 99.9% maximal efficacy. This is much lower than the dosages that have been used in clinical trials (11). Dosage schedules for the initial clinical trials are often established arbitrarily; however, once they have been shown to be effective, they tend to be used in subsequent trials. The pharmacodynamic-pharmacokinetic model discussed here could provide a more rational basis for the choice of dosage schedules for initial clinical trials.

ACKNOWLEDGMENTS

We thank A. Boon, Department of Clinical Oncology, for performing the irradiation of the mice; Glaxo for providing ceftazidime; and Bristol-Myers for providing cefepime.

This study was supported financially by Bristol-Myers, Brussels, Belgium.

REFERENCES

1. **Barbhaiya, R. H., S. T. Forgue, C. R. Gleason, C. A. Knupp, K. A. Pittman, D. J. Weidler, and R. R. Martin.** 1990. Safety, tolerance, and pharmacokinetic evaluation of cefepime after administration of single intravenous doses. Antimicrob. Agents Chemother. **34**:1118–1122.
2. **Barbhaiya, R. H., S. T. Forgue, W. C. Shyu, E. A. Papp, and K. A. Pittman.** 1987. High-pressure liquid chromatography analysis of BMY-28142 in plasma and urine. Antimicrob. Agents Chemother. **31**:55–59.
3. **Bodey, G. P., D. H. Ho, and B. LeBlanc.** 1985. In vitro studies of BMY-28142, a new broad-spectrum cephalosporin. Antimicrob. Agents Chemother. **27**:265–269.
4. **Flanders, H.** 1985. Calculus. W. H. Freeman, New York.
5. **Kunst, M. W., and H. Mattie.** 1978. Cefazolin and cephradine: relationship between antibacterial activity in vitro and in mice experimentally infected with Escherichia coli. J. Infect. Dis. **137**:391–402.
6. **Mattie, H., J. J. Hoogeterp, and J. Hermans.** 1987. The relation between plasma and tissue concentrations of antibiotics. Description of a method. J. Pharmacokinet. Biopharm. **15**:191–202.
7. **Mattie, H., and J. Vaishnav.** 1983. The effect of antibacterial drugs on the growth kinetics of Bacteroides in vitro. Curr. Microbiol. **8**:191–194.
8. **Mattie, H., and G. B. van der Voet.** 1981. The relative potency of amoxycillin and ampicillin in vitro and in vivo. Scand. J. Infect. Dis. **13**:291–296.
9. **Mattie, H., A. M. van Dokkum, L. Brus-weijer, A. M. Krul, and E. van Strijen.** 1990. Antibacterial activity of four cephalosporins in an experimental infection in relation to in vitro effect and pharmacokinetics. J. Infect. Dis. **162**:717–722.
10. **Peetermans, W. E., J. J. Hoogeterp, A. M. Hazekamp-van Dokkum, P. J. van den Broek, and H. Mattie.** 1990. Antistaphylococcal activities of teicoplanin and vancomycin in vitro and in an experimental infection. Antimicrob. Agents Chemother. **34**: 1869–1874.
11. **Rivera-Vazquez, C., N. Zapata, S. Saavedra, and C. Ramirez-Ronda.** 1990. Cefepime versus ceftazidime in severe soft tissue infections: a randomized comparative study. Clin. Res. **38**: 560A.