ANTIBACTERIAL EFFECTS OF CEFROXADINE, CEPHALEXIN AND CEPHRADINE IN A NEW *IN VITRO* PHARMACOKINETIC MODEL

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A pharmacokinetic model has been developed, by means of which all possible time courses of the concentrations of antibiotics in the plasma of treated individuals can be exactly simulated *in vitro* without diluting the test organism and affecting the growth curves. Equieffective concentrations in the system corresponded to the plasma concentrations in man produced by cefroxadine in a single oral dose of 250 mg and cephalexin and cephradine in a single oral dose of 500 mg.

The antibacterial activity of antibiotics *in vitro* is most commonly assessed by determination of the minimum inhibitory concentrations (MIC) in cultures in which the microorganisms are exposed to constant concentrations of the substances for about 18 hours. *In vivo*, however, antibiotic concentrations change according to the pharmacokinetics of the particular drug. Various *in vitro* systems have consequently been devised in an effort to simulate the time course of antibiotic concentrations in human serum. In the first attempts[2~5], the concentrations were altered in a non-continuous fashion. These were followed by improved techniques[6~14] in which the antibiotics were continuously diluted. In most of the published models, however, the bacterial inoculum is diluted at the same rate as the antibiotic. We have developed a new model employing a filter system by means of which the antibiotic concentrations are varied continuously in an exact simulation of the time course of human serum concentrations, without simultaneous dilution of the bacterial inoculum.

Method

The pharmacokinetic model is based on a simple, analogue-computer model for the analysis of the drug-distribution process[1]. The system comprises a reservoir (R) containing the test medium, a compartment (A) containing the antibiotic in the test medium, and a compartment (B) containing the bacteria in the test medium (Fig. 1). A constant flow of the medium through teflon tubing from R to A is maintained by a pump (Labotron LDP-21) and from A to B by the pressure in flask A. The same flow as from R to A and B is maintained by another pump from B into an overflow (C). Compartment B is surrounded by a water-jacket to keep the temperature of the medium constant at 37°C. A magnetic stirrer over the filter (Millipore GS/HA, 0.45 ég) prevents obstruction of the filter by the bacteria. The concentration curves in man were mathematically adapted to the parameters of the *in vitro* model. The slope and intercept of the absorption and elimination curves (ke, E, ka, A) of the plasma profiles in man were determined in a one-compartment model with first-order absorption kinetics (Fig. 2).

The flow rate F in the system is dependent on the volumes in A and B: F=ka·volume A=ke·volume B. The dose D in A is dependent on Co and on volume B in the system, as it is on Co and on the volume of the central compartment in man: D=Co·volume B. If the volume B is arbitrarily fixed at a volume suitable for the capacity of compartment B, the flow rate, the volume A and the dose can be determined: F=ke·volume B, volume A=F/ka, D=Co·volume B.

Fig. 1. Scheme of the kinetic system.

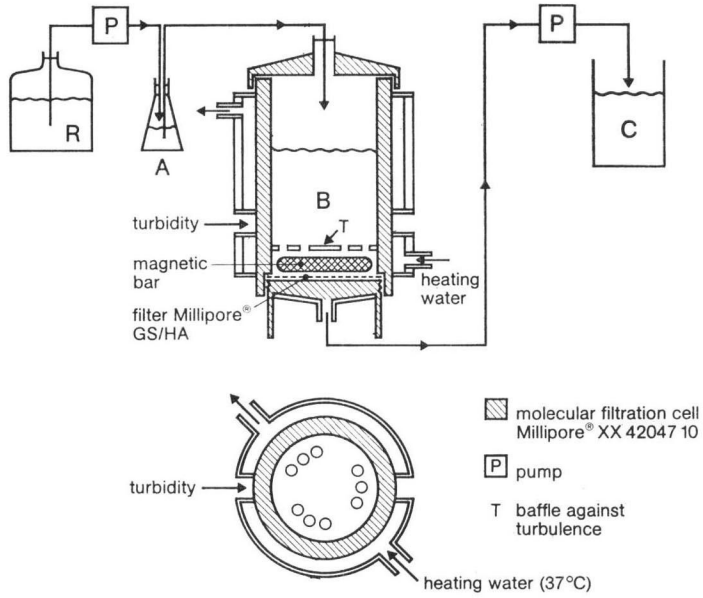
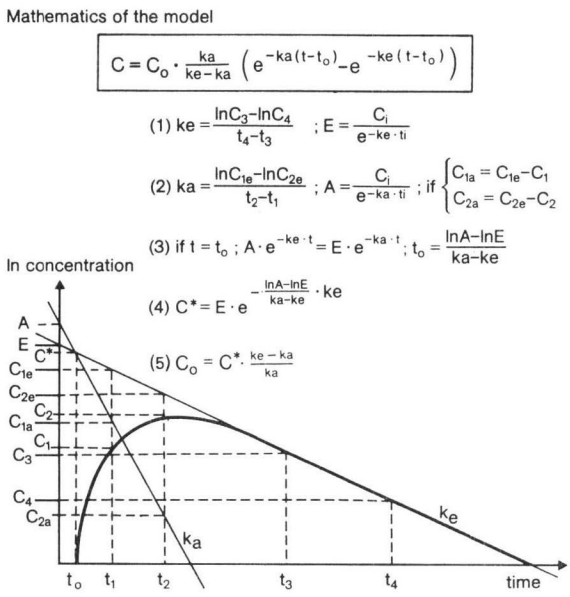


Fig. 2. Mathematics of the model.



Any biexponential concentration curve can be simulated in this system on the principle of exponential dilution of the drug in A, transfer of a constant volume of the diluted drug from A to B and exponential dilution of the drug in B by the constant flow from B into the overflow.

The tests were performed with DST broth Oxoid as test medium. Cefroxadine, synthesized in the Ciba-Geigy Laboratories, and cephalexin and cephradine, purchased from commercial sources, were dissolved in volume A of the medium. The turbidity of the suspensions in compartment B was monitored continuously with a biophotometer (Bonet Maury et Jouan) and plotted as % transmission.

Samples were drawn from B at various times for viable-cell counts and determinations of the drug concentration. The colony forming units (cfu) were counted by plating out 0.05 ml aliquots of appropriate dilutions on agar. The concentrations were determined by the agar well diffusion test with Antibiotic Medium No. 1 Oxoid and *B. subtilis* ATCC 6633 as test strain. DST broth was used for the standard curves and the inhibition zones were measured in a Quantimet 720 P (Cambridge Instruments).

The pharmacokinetic parameters were determined on the basis of data on serum profiles of antibiotics taken from BERGAN[15], HIRTZ *et al.*[16] and PFEFFER *et al.*[17].

Results

Drug Concentrations

The concentrations of the drugs in the system correlated well with the data from man (Figs. 3~5). No discrepancies were found between the calculated human plasma concentration curve and the periodically determined concentrations in the system.

Fig. 3. Concentrations of cefroxadine after a single oral dose of 250 mg.

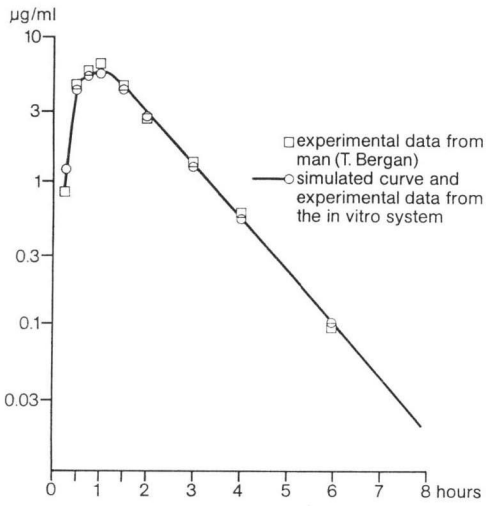
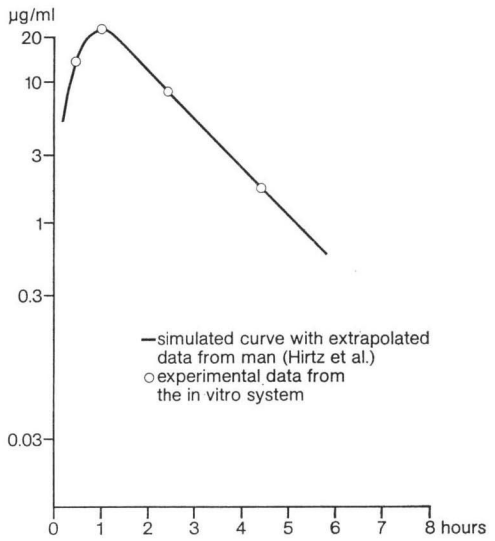


Fig. 4. Concentrations of cephalexin after a single oral dose of 500 mg.



Control Culture

No influence of the system on the growth of *S. typhimurium* 277 was seen, by comparison with a control culture grown without dilution of the test medium (Fig. 6).

Antibacterial Effects of the Drugs

The growth of *E. coli* 205 was inhibited by cefroxadine for more than two hours longer than by cephalexin after exposure to concentrations corresponding to the plasma concentration - time curve produced by a single oral dose of 250 mg in man (Fig. 7). If the concentrations of cephalexin were increased to levels corresponding to a single oral dose of 500 mg, the antibacterial effects were similar to those of cefroxadine, whether determined turbidimetrically or by viable-cell counts (Fig. 8). Replicate experiments with *S. aureus* 14 Smith, *P. mirabilis* 564 and E 30 and *S. typhimurium* 277 confirmed the equieffectiveness of cefroxadine at concentrations corresponding to those after a single oral dose of 250 mg and cephalexin at concentrations twice as high (500 mg) (Figs. 9~12).

Fig. 5. Concentrations of cephradine after a single oral dose of 250 and 500 mg.

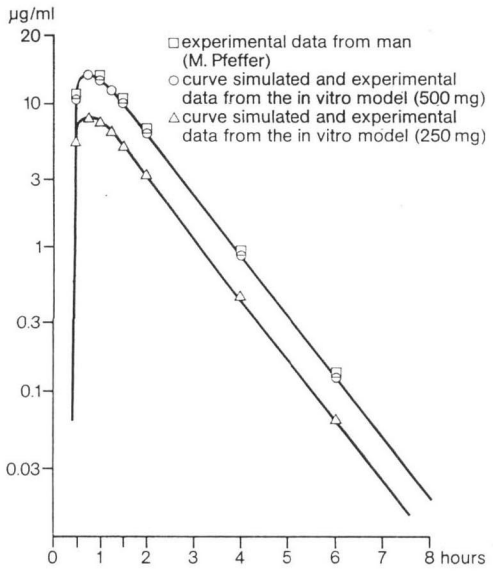


Fig. 6. Growth curves for *S. typhimurium* 277 in an undiluted static system (▲) and a dynamic system (○).

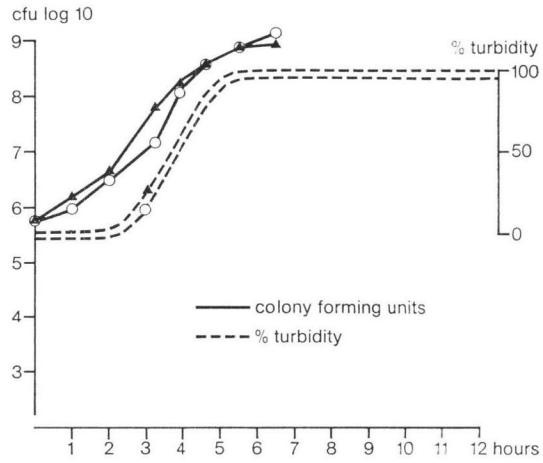


Fig. 7. Kinetics of the turbidity of a culture of *E. coli* 205 after exposure to antibiotic concentrations corresponding to the plasma concentration - time curve produced by a single oral dose of 250 mg in man.

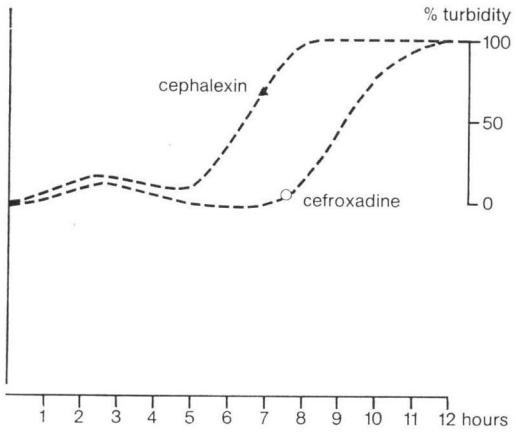
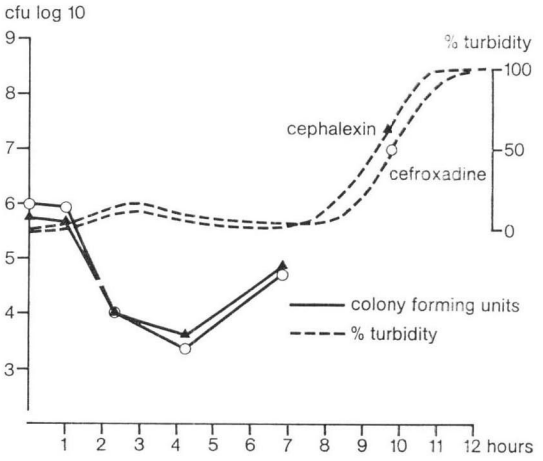


Fig. 8. Growth kinetics of *E. coli* 205 after exposure to concentrations corresponding to the plasma concentration - time curves produced by a single oral dose of 250 mg cefroxadine and 500 mg cephalexin in man.



Cephradine in concentrations simulating these produced by an oral dose of 500 mg inhibited *E. coli* 205 and *K. pneumoniae* 327 less effectively than did half the dose of cefroxadine (Figs. 13, 14). Under the same dynamic conditions (Fig. 15), cefroxadine displayed antibacterial activity against *S. aureus* 14 Smith equal to that of cephradine at a dose 1.5 times higher.

Fig. 9. Growth kinetics of *S. aureus* 14 Smith after exposure to concentrations corresponding to the plasma concentration - time curves produced by a single oral dose of 250 mg cefroxadine and 500 mg cephalexin in man.

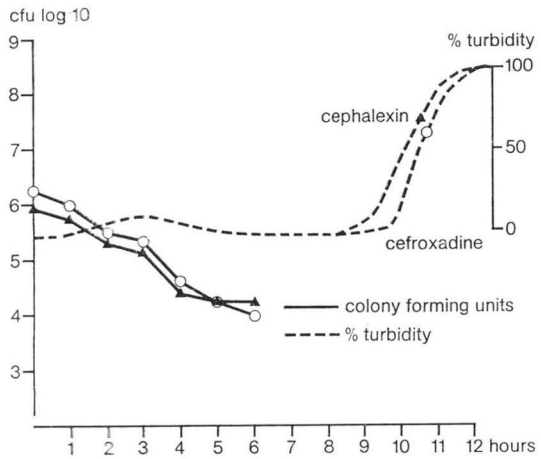


Fig. 10. Growth kinetics of *P. mirabilis* 564 after exposure to concentrations corresponding to the plasma concentration - time curves produced by a single oral dose of 250 mg cefroxadine and 500 mg cephalexin in man.

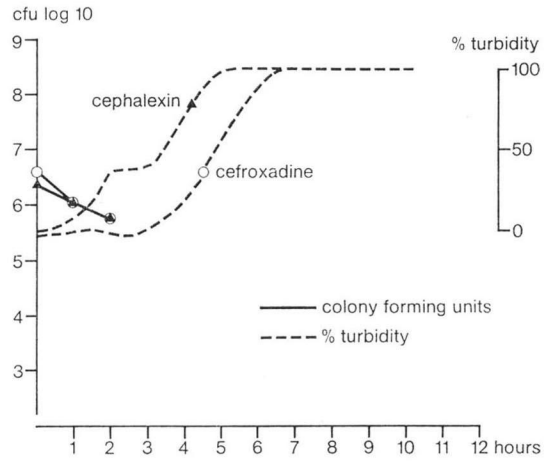


Fig. 11. Growth kinetics of *P. mirabilis* E30 after exposure to concentrations corresponding to the plasma concentration - time curves produced by a single oral dose of 250 mg cefroxadine and 500 mg cephalexin in man.

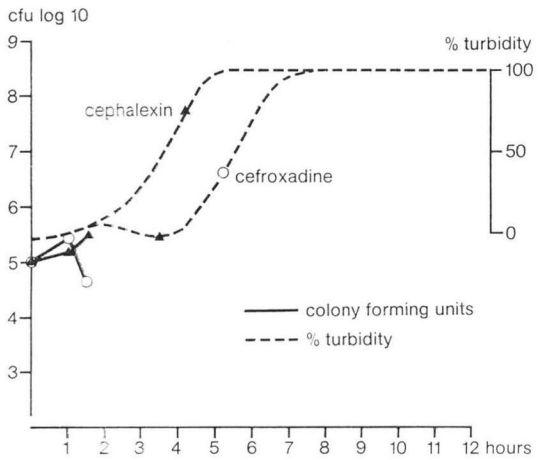
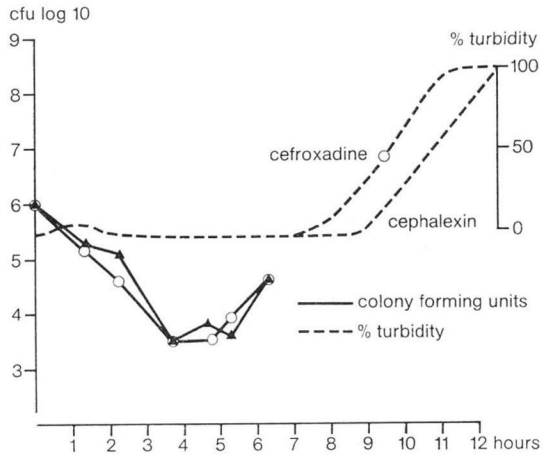


Fig. 12. Growth kinetics of *S. typhimurium* 277 after exposure to concentrations corresponding to the plasma concentration - time curves produced by a single oral dose of 250 mg cefroxadine and 500 mg cephalexin in man.



Discussion

Comparison of the serum profiles produced by equi-effective doses of the three drugs shows that cephalexin and cephradine produce higher peaks and greater AUC's than cefroxadine and remain present for longer periods at levels above the MIC. Since the MIC of the three drugs are equal for many strains, it can be assumed that they exert similar inhibitory effects at constant concentrations over an incubation period of 18 hours. Under dynamic conditions, however, when the concentrations of the drugs decreased below the MIC after a few hours, the effects of cefroxadine were clearly longer-lasting, so that

Fig. 13. Growth kinetics of *E. coli* 205 after exposure to concentrations corresponding to the plasma concentration - time curves produced by single oral doses of cefroxadine and cephradine in man.

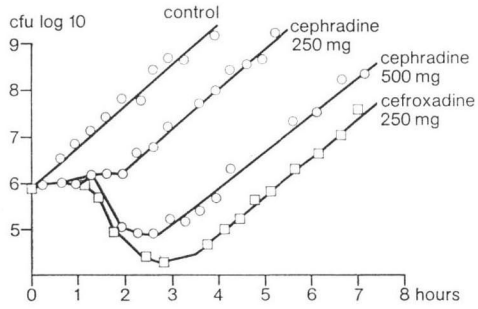


Fig. 14. Growth kinetics of *K. pneumoniae* 327 after exposure to concentrations corresponding to the plasma concentration - time curves produced by single oral doses of cefroxadine and cephradine in man.

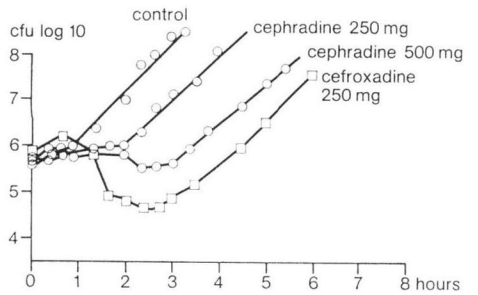


Fig. 15. Growth kinetics of *S. aureus* 14 Smith after exposure to concentrations corresponding to plasma concentration - time curves produced by single oral doses of cefroxadine and cephradine in man.

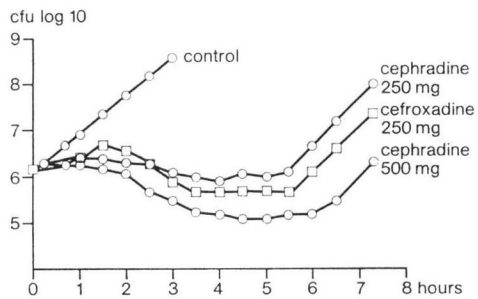


Fig. 16. Sub-MIC effect of cefroxadine on *E. coli* 205 after exposure to concentrations corresponding to the plasma concentration - time curve produced by a single oral dose of 250 mg in man and addition of β-lactamase at the time when the concentration was approximately 1 MIC (4 μg/ml).

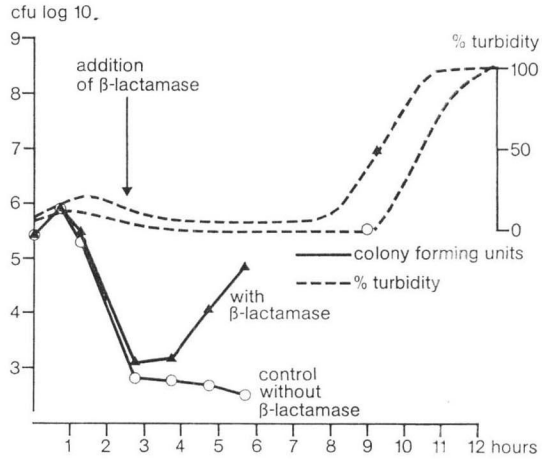
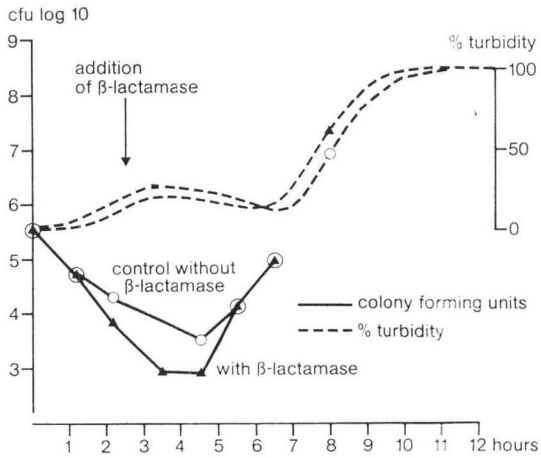


Fig. 17. Sub-MIC effect of cephalexin on *E. coli* 205 after exposure to concentrations corresponding to the plasma concentration - time curve produced by a single oral dose of 500 mg in man and addition of β-lactamase at the time when the concentration was approximately 1 MIC (8 μg/ml).



cefroxadine seem to be more effective than cephalexin and cephradine at sub-MIC levels. This assumption was borne out by the results of two additional experiments in which a β-lactamase from *E. cloacae* P99 was added to the medium containing the antibiotics at the time when the concentrations were not higher than about 1 MIC (Figs. 16, 17). A clear-cut sub-MIC effect of cefroxadine was evident from the

fact that the viable-cell count increased distinctly after the addition of the β-lactamase, but not at all without β-lactamase. No significant sub-MIC effect of cephalexin was found.

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