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Evaluation of Ceftriaxone and Other Antibiotics against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* under In Vitro Conditions Simulating Those of Serious Infections

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In pursuit of an in vitro system capable of reliably predicting the activities of antibiotics in serious infections and in infections occurring in immunocompromised hosts, we evaluated the abilities of four drugs to achieve virtually complete killing of bacterial cells growing in human body fluids in amounts which are very high and close to those likely to be present in serious infections; drug concentrations varied with time as they vary in human bronchial secretions or blood or urine (dynamic concentrations). The rationale for such a test was (i) to set up in vitro conditions as close as possible to those the antibiotics encounter in serious infections and (ii) to hold the drugs capable of almost completely killing the bacteria used in the assay to be highly active in vitro and likely to be the most efficacious in the treatment of serious infections. Among the antibiotics used, ceftriaxone proved to be highly active under conditions simulating pulmonary infections and septicemias caused by *Streptococcus pneumoniae* (bacteria grown in bronchoalveolar fluid or blood; antibiotic concentrations varying with time as in human bronchial secretions or blood) and under conditions simulating blood and urinary infections caused by *Escherichia coli* (bacteria grown in human blood or urine; antibiotic concentrations varying as in the various fluids). Gentamicin (not tested against pneumococci) appeared to be highly active only under conditions simulating urinary infections caused by *E. coli*; aztreonam (not tested against pneumococci) and ampicillin (tested only against pneumococci) did not appear to be highly active under any of the test conditions. Only the combination of gentamicin plus either ceftriaxone or aztreonam appeared to be highly active under conditions simulating serious septicemias and urinary infections caused by *Pseudomonas aeruginosa*.

The antibiotic treatment of mild bacterial infections is generally successful, particularly if the drug used is chosen on the basis of in vitro susceptibility tests. By contrast, the treatment of serious infections is often ineffective, especially in immunocompromised hosts, even though the microorganisms responsible prove in vitro to be susceptible to the antibiotic used (11, 23). These failures may well depend on the fact that the conditions under which the activities of antibiotics are evaluated by standard in vitro tests enable drugs to express greater antimicrobial activities than do the conditions these drugs encounter in infected organisms (23). In common mild infections, host defenses, for which no allowance is made in in vitro tests, are very likely to offset the less favorable conditions under which antibiotics have to act. This is not the case when host defenses are inadequate, i.e., in infections occurring in immunocompromised hosts and in other serious infections.

To have an acceptable predictive value in serious infections, the activity of an antibiotic should probably be evaluated under in vitro conditions closer to those occurring in such cases. For instance, the sizes of bacterial populations in the test should be close to those of populations present in the various natural infections; bacteria should grow in the same media in which they grow in the infections, and antibiotic concentrations should vary with time as they vary in the infected body sites (dynamic concentrations). Unfortunately, tests of this type cannot be performed for each patient just before therapy is started, mainly because the tests are time-consuming and seriously infected patients need immediate antibiotic therapy.

An alternative possibility is the preliminary evaluation, under the aforementioned conditions, of the capabilities of the antibiotics to inhibit bacteria of the species most often responsible for serious infections, as a part of the antimicrobial activity characterization of drugs. Thus, in conditions closely simulating those of serious infections, we analyzed the antimicrobial activities of two new β-lactam antibiotics, ceftriaxone and aztreonam, both endowed with very low MICs for most susceptible microorganisms and both reaching concentrations in blood and other body sites manyfold higher than the MICs. We also compared the antimicrobial activities of these drugs with those of ampicillin and gentamicin.

(These data were presented in part at the 4th Mediterranean Congress of Chemotherapy, 19 to 25 October 1984, Rhodes, Greece [G. Satta, G. Cornaglia, G. Foddis, and R. Pompei, Proc. 4th Mediterr. Congr. Chemother., p. 238–240, 1984].)

MATERIALS AND METHODS

Test microorganisms. A total of five strains (two *Streptococcus pneumoniae*, two *Escherichia coli*, and one *Pseudomonas aeruginosa*) were used in this work. All were isolated from specimens of various human origins and were identified according to current criteria (18).

The MICs were determined by the macrodilution broth method (19) with Todd-Hewitt broth supplemented with 5%

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horse serum for *S. pneumoniae* and Mueller-Hinton broth supplemented with Mg^{2+} and Ca^{2+} for *E. coli* and *P. aeruginosa*. Inocula were prepared to contain about 5×10^6 CFU/ml; thus, they were a half $\log_{10}$ higher than recommended (19).

The strains used in our work are shown in Table 1, along with the MICs of the antibiotics used. For all strains, the susceptibilities to the drugs used in the experiments were within the range for the respective species, except for *E. coli* 2, which was chosen for certain experiments because of its significantly lower-than-average susceptibility to ceftriaxone.

Growth conditions. All the microorganisms were grown in the same biological fluids to be used for the experiments, and their numbers were determined on the basis of growth kinetics tests performed in advance. In all the experiments, appropriate amounts of bacteria were resuspended in 50 ml of fluid 1 h before the addition of the antibiotic to ensure a logarithmic growth. In the case of the highest inocula, we centrifuged appropriate amounts of exponentially growing bacteria and resuspended them in the 50 ml of culture fluid to be used for the experiment (see below).

S. pneumoniae strains were grown in some experiments in bronchoalveolar fluid and in some experiments in human blood supplemented with sterile bacteriostat-free heparin, in all cases at 37°C. *E. coli* and *P. aeruginosa* strains were grown either in heparinized human blood or in human urine at 37°C. Human blood was obtained from healthy volunteers who were on no medication. The blood was inactivated by heat at 56°C for 30 min to eliminate intrinsic bactericidal activity against the test strains, and then it was used immediately. Urine was also collected from healthy volunteers and was sterilized by filtration on Millipore membranes of 0.22-μm pore size; the pH was adjusted to 6.8 by the addition of either HCl (0.1 M) or NaOH (0.1 M).

Bronchoalveolar fluid was obtained from healthy volunteers by lavage with a fiberoptic bronchoscope (29, 35), which is acceptable for use even without general anesthesia. Lidocaine (1% solution) was instilled in the naso-oropharynx as well as in the trachea and bronchi to obtain local anesthesia and reduce coughing. For each patient, 200 to 300 ml of sterile saline in aliquots of 100 ml was used, with recovery of about 50% of the fluid. Fluid was aspirated by hand suction into a syringe after the patient had been encouraged to take one or two normal breaths to obtain more mixing in the lung and, consequently, a better specimen. All the samples recovered from the patients were pooled and centrifuged at 8,000 × *g* for 5 min to eliminate the cellular fraction. The acellular supernatant fluid was sterilized by ultrafiltration through Millipore membranes of 0.22-μm pore size and, because it consisted mostly of saline, was concentrated 10-fold by centrifugation at 30,000 × *g* for 30 min. The concentrated fluid was stored frozen at −20°C for no longer than 1 week.

Antimicrobial agents. Ceftriaxone was a generous gift of Prodotti Roche S.p.A., kindly donated by G. Marini. The dry powder was dissolved in phosphate buffer (pH 6.0; 0.1 M) and diluted in sterile water.

Ampicillin, aztreonam, and gentamicin were obtained as dry powders from commercial sources. Ampicillin was dissolved in phosphate buffer (pH 8.0; 0.1 M) and diluted in phosphate buffer (pH 6.0; 0.1 M). Aztreonam was dissolved in saturated sodium bicarbonate (0.5 g/ml) and diluted in sterile water. Gentamicin was dissolved and diluted in sterile water.

Each antibiotic dilution was prepared just before being used and added at the proper concentration to the biological fluid to be used for the experiment.

The antibiotic concentrations selected for testing were chosen so that they approximated the peak concentrations achieved in the various body fluids after intravenous (i.v.) administration of single doses. For ceftriaxone, the simulated peak concentrations in blood (0.5-, 1-, 1.5-, and 3-g doses) were 90 (31), 150 (26), 280 (33), and 400 (33) μg/ml; the simulated peak concentrations in bronchial fluid (0.5- and 1-g doses) were 0.9 and 1.5 μg/ml (10); and the simulated peak concentrations in urine (1-, 1.5-, and 3-g doses) were 1,000 (26), 1,500, and 3,000 μg/ml. For ampicillin (2-g dose), the simulated peak concentrations were 24 μg/ml in blood (17) and 1 μg/ml in bronchial fluid. For gentamicin (1.5-mg/kg [body weight] dose), the simulated peak concentrations were 2.7 μg/ml in blood (F. D. Daschner, B. Metz, M. Schmuziger, and V. Schlosser, Program Abstr. 20th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 173, 1980) and 200 μg/ml in urine (3). For aztreonam (1-g dose), the simulated peak concentrations were 125 μg/ml in blood (34) and 3,000 μg/ml in urine (34).

All the simulated peak concentrations corresponded to those reported in the literature whenever data were available. Bronchial concentrations following administration of ampicillin at 2 g i.v. and ceftriaxone at 0.5 g i.v. and urine concentrations following administration of ceftriaxone at 1.5 and 3 g i.v. were not found in the literature and were calculated according to the pharmacokinetic data for similar dosages.

All the concentrations were allowed to vary according to the pharmacokinetics of each drug in the experimental system described below.

Evaluation of antimicrobial activity. Antimicrobial activities of the drugs were evaluated with antibiotic concentrations that varied with time. To obtain these dynamic concentrations, we used apparati similar to those previously described by others (14, 25, 30).

The two systems we used are shown in Fig. 1. Apparatus A was used to simulate antibiotic concentrations in blood, in which the peak is reached immediately after i.v. administration and then decreases with time. The apparatus consisted of two Erlenmeyer flasks connected via plastic tubing. Flask

TABLE 1. MICs of ceftriaxone, ampicillin, aztreonam, and gentamicin for the strains used in the various experiments[a]

Strain	MIC (μg/ml)			
	Ceftriaxone	Ampicillin	Aztreonam	Gentamicin
Streptococcus pneumoniae 37	0.06	0.015		
Streptococcus pneumoniae 45	0.03	0.015		
Escherichia coli 2	0.06		0.125	0.5
Escherichia coli 10	4			
Pseudomonas aeruginosa 69	8		4	1

[a] Blanks mean that the strain was not tested with the drug.

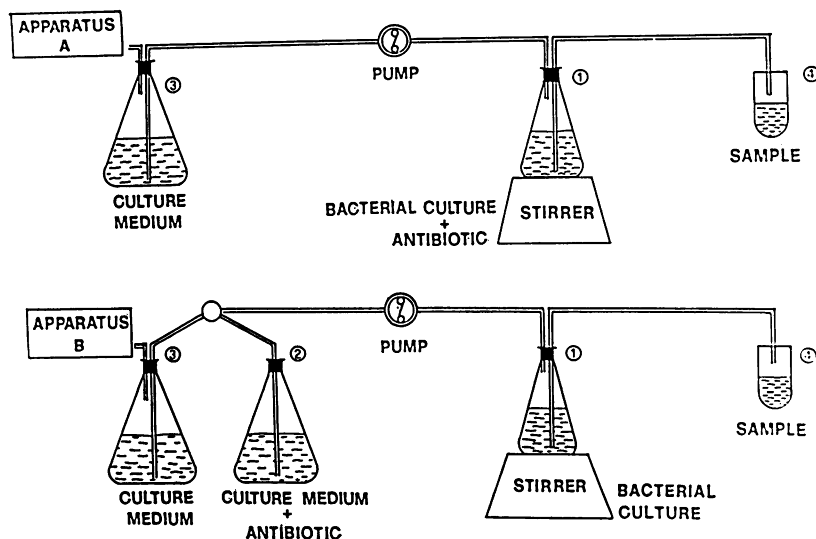

FIG. 1. Scheme of in vitro model used to obtain antibiotic concentrations varying with time. See the text for full description.

1 contained a 50-ml suspension with the desired total number of microorganisms and the peak concentration of the antibiotic, and flask 3 contained the same fluid but neither bacteria nor antibiotic. The fluid from flask 3 was pumped into flask 1 by a peristaltic pump, thus causing continuous dilution of the antibiotic. Since flask 1 was tightly sealed, the diluent caused an increase in pressure, which in turn caused expulsion of an equal amount of fluid, so that the volume in flask 1 did not vary. This apparatus was regulated so as to have a starting concentration of antibiotic corresponding to the peak concentration following i.v. administration of a single dose of the drug. The dilution rate was calculated as described by others (14, 30) according to the following equation: $F = V_1 \times 0.693/t_{1/2}$, where F is the flow rate, V_1 is the volume of fluid contained in flask 1, and $t_{1/2}$ is the half-life postulated for the antibiotic.

Apparatus B was used to simulate dynamic drug concentrations in bronchial aspirate and urine. In these cases, the antibiotic concentration after i.v. injection rises with time to a peak and then decreases. The apparatus consisted of three Erlenmeyer flasks connected via plastic tubing. Flask 3 contained the diluent fluid only, and flask 2 contained an adequate concentration of antibiotic in the same diluent. The two flasks were connected to a switch so that the pump could push either the antibiotic solution or the diluent fluid into flask 1, which contained 50 ml of bacterial suspension. This system was regulated so that in the earlier phases of the experiment the antibiotic solution in flask 2 was pumped at a constant rate into flask 1 until the antibiotic concentration in the latter reached the peak value. At this point, we switched over from pumping the antibiotic solution to pumping the diluent fluid from flask 3 to flask 1 at a rate allowing an antibiotic dilution similar to the in vivo clearance of the drug being tested.

Combinations of antibiotics were tested by using the model proposed by Blaser for simultaneous simulation of the kinetics of two drugs (4).

All the experiments were performed in a warm room at 37°C, and a magnetic stirrer beneath flask 1 in each apparatus ensured both uniform distribution of the antibiotic and good aeration of the microbial culture.

With both apparati, 1-ml samples were taken at fixed intervals for 24 h and, in several cases, for 48 h. To minimize any carryover of antibiotic capable of preventing growth, samples were centrifuged and pellets were washed three to five times in saline solution, resuspended in 0.5 ml of the diluent, and plated. Plates were incubated for 48 h at 37°C, and the colonies formed were counted.

RESULTS

Activity of dynamic concentrations of ceftriaxone and ampicillin against *S. pneumoniae*. Figure 2 shows the effect of ceftriaxone on two *S. pneumoniae* strains at antibiotic concentrations varying with time as they vary in human bronchoalveolar secretions and in human blood after administration of i.v. doses of 1 and 0.5 g. The antibiotic clearly caused a very rapid, irreversible inhibition of a total of 10^{10} cells, grown either in blood or in bronchoalveolar fluid, even at the reduced dose of 0.5 g. Such inhibition was probably complete, since no regrowth was observed even as much as 48 h after addition of the antibiotic.

By contrast, ampicillin failed to kill a substantial portion of bacterial populations of the same size, grown either in bronchoalveolar fluid or in blood, at the dynamic concentrations it attains in the fluids after administration of a full daily dose (2 g every 4 h).

The strong killing activity of ceftriaxone probably did not depend on antibacterial effects of the biological fluids used, because such activity was even higher when similar experiments were performed in Todd-Hewitt broth (results not shown).

Activity of dynamic concentrations of ceftriaxone, aztreonam, and gentamicin against *E. coli*. Figure 3 shows that a dynamic concentration of ceftriaxone simulating the drug levels in blood and in urine after the i.v. injection of a single 1-g dose achieved, within 16 h (or less), virtually complete and irreversible killing of 1×10^{10} and 5×10^{10} cells of *E. coli* 2 grown in blood and urine, respectively. Ceftriaxone concentrations simulating those attained after administration of a 3-g dose were, as expected, even more efficacious, achieving virtually complete killing of amounts of bacteria unlikely to be exceeded in human infections (Fig. 3). In this respect, it should also be noted that killing of such large populations was achieved within the relatively short period of 16 h and that no regrowth was observed as much as 24 h

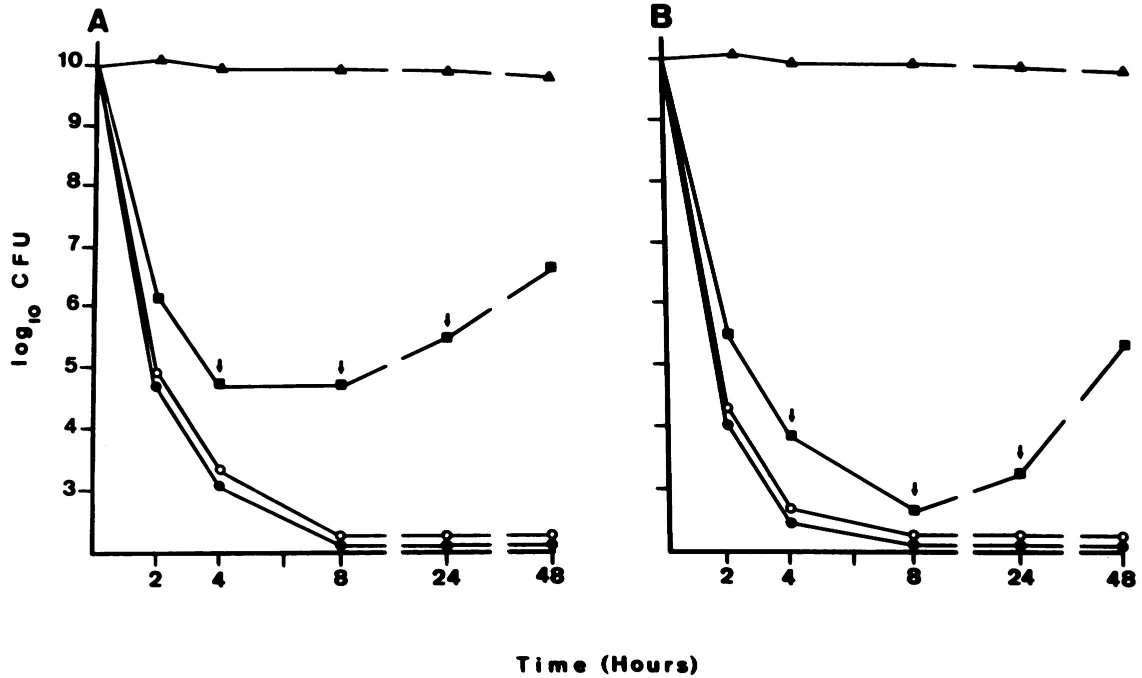

FIG. 2. Kill kinetics in human blood (A) and human bronchoalveolar fluid (B) of *S. pneumoniae* 37 in a dynamic system simulating in vivo pharmacokinetics of ceftriaxone and ampicillin in the same fluids. Symbols: ▲, growth without antibiotic in human blood (A) and human bronchoalveolar fluid (B); ■, ampicillin (simulated dosage, 2 g every 4 h i.v.); ○, ceftriaxone (simulated dosage, 0.5 g/day i.v.); ●, ceftriaxone (simulated dosage, 1 g/day i.v.). Arrows indicate subsequent administrations of ampicillin. Kill kinetics of the other *S. pneumoniae* strain undergoing the same assay (strain 45) were very similar.

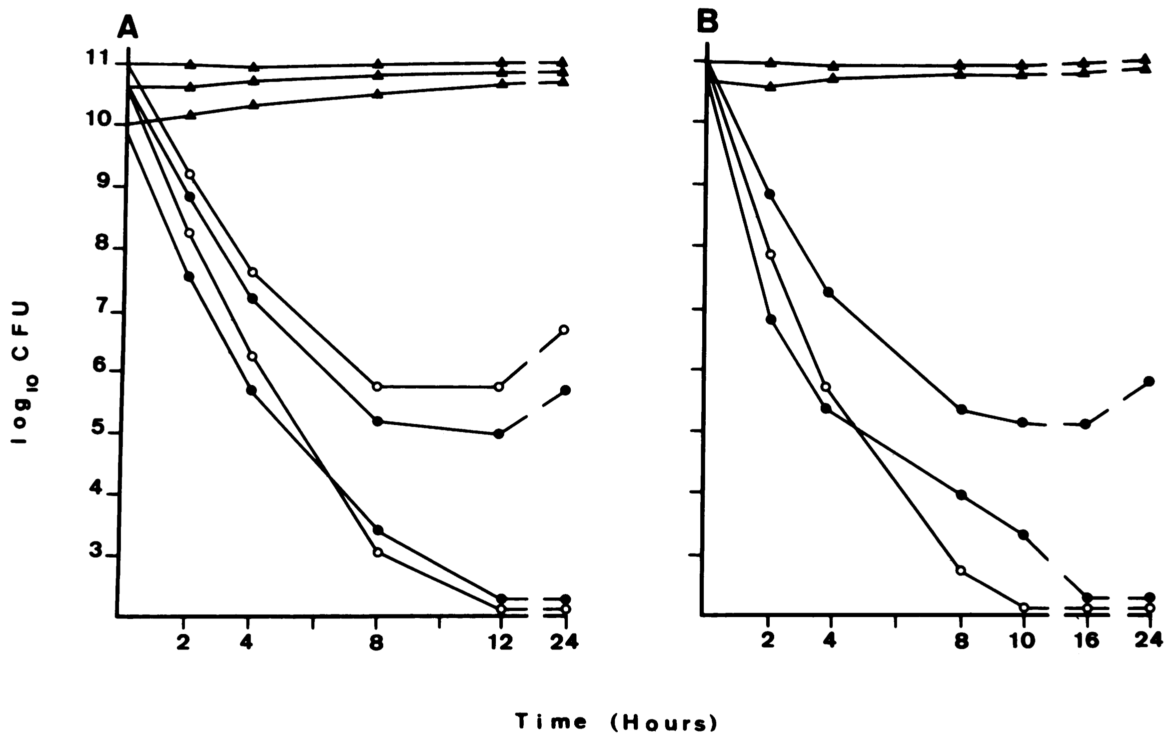

FIG. 3. Kill kinetics in human blood (A) and human urine (B) of an *E. coli* strain of average susceptibility (strain 2; MIC, 0.06 μg/ml) in a dynamic system simulating in vivo pharmacokinetics of ceftriaxone in the same fluids. Symbols: ▲, growth without antibiotic in human blood (A) and human urine (B); ●, ceftriaxone (simulated dosage, 1 g/day i.v.); ○, ceftriaxone (simulated dosage, 3 g/day i.v.).

later (results not shown). Moreover, ceftriaxone demonstrated an even greater antibacterial activity when the cells were grown in Mueller-Hinton broth (results not shown).

The activity of ceftriaxone was also evaluated against *E. coli* 10, a strain for which the MIC is 4 μg/ml, which is 40-fold higher than the reported MIC for 90% of isolates tested of this species (1). It was found that, even with this poorly susceptible strain, dynamic concentrations of ceftriaxone simulating those attained in blood and urine after i.v. administration of a single 1-g dose were capable of killing 1 $\times 10^9$ cells in blood and 5×10^{10} cells in urine, while concentrations simulating those attained in the aforementioned fluids after administration of a single 3-g dose killed a maximum of 1×10^{10} and 6×10^{10} cells in blood and urine, respectively (Fig. 4).

At this point, we evaluated the activities of dynamic concentrations of two other antibiotics, aztreonam and gentamicin, against *E. coli* 2, which demonstrated an average susceptibility to these drugs, too (Table 1). Aztreonam, a monocyclic β-lactam which (like ceftriaxone) has low MICs for *E. coli* (1) and attains high levels both in blood and in urine (34), was much less active than ceftriaxone and poorly active in absolute terms (at least as regards bactericidal activity) at concentrations simulating those attained in either blood or urine after a 1-g dose every 8 h. This antibiotic killed maximums of 1×10^6 and 5×10^7 *E. coli* cells in blood and urine, respectively (Fig. 5). Gentamicin, too, although known to be a highly bactericidal drug (5), showed inadequate activity, appearing more efficacious than aztreonam but definitely less so than ceftriaxone. At the concentration attained after administration of a 1.5-mg/kg dose every 8 h, it achieved virtually complete killing of maximums of 5×10^8 and 5×10^9 *E. coli* cells in blood and urine, respectively (Fig. 5).

Activities of dynamic concentrations of ceftriaxone, aztreonam, and gentamicin against *P. aeruginosa*. *P. aeruginosa* strains may be susceptible in vitro to ceftriaxone (15), and ordinary infections caused by these microorganisms can be treated with this drug (9). Furthermore, *P. aeruginosa* strains are generally susceptible to gentamicin (1), which has long been widely used in the treatment of *P. aeruginosa* infections (28), and to aztreonam (1), which looks very promising for such treatment (16, 24).

Figure 6 shows that ceftriaxone was definitely less active against *P. aeruginosa* than against *S. pneumoniae* and *E. coli*. This drug achieved virtually complete killing of a maximum of 10^7 cells in blood and 10^8 cells in urine at the dynamic concentrations attained after administration of a single 1-g dose and killed a maximum of 5×10^7 cells in blood and 5×10^8 cells in urine even at the concentrations attained after administration of 1.5 g every 12 h.

The activities of the other two drugs tested, although they had lower MICs for the *P. aeruginosa* strain used, did not prove to be higher than that of ceftriaxone. Aztreonam killed maximums of 10^5 and 10^6 *P. aeruginosa* cells in blood and urine, respectively (simulated dosage, 1 g every 8 h i.v.), and gentamicin showed a very similar activity, killing maximums of 1×10^6 and 5×10^8 *P. aeruginosa* cells in blood and urine, respectively, at the concentrations attained in the fluids after i.v. injection of a 1.5-mg/kg dose every 8 h (Fig. 7).

Activities of dynamic concentrations of antibiotic combinations against *P. aeruginosa* cells. Since none of the antibiotics evaluated proved capable of completely killing large populations of a susceptible *P. aeruginosa* strain when tested at dynamic concentrations, we evaluated the activities of combinations of drugs acting synergistically even against this species (12). Figure 8 shows that a combination of ceftriaxone plus gentamicin was capable of completely killing up to

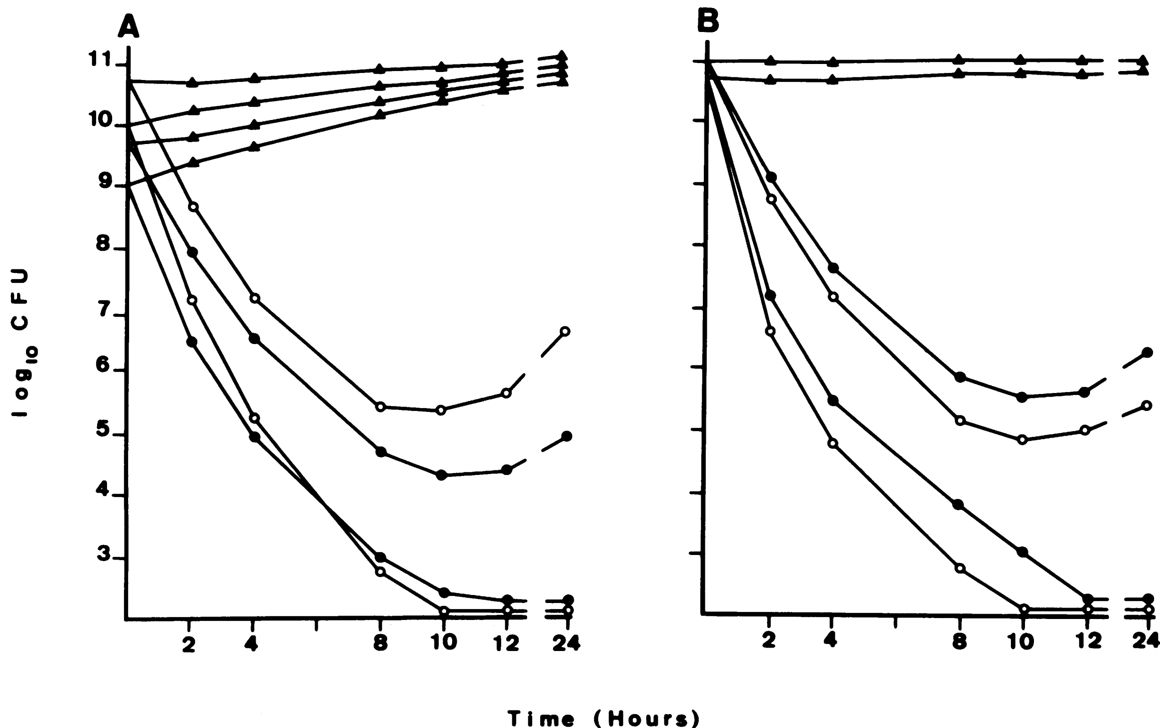

FIG. 4. Kill kinetics in human blood (A) and human urine (B) of an *E. coli* strain of low susceptibility (strain 10; MIC, 4 μg/ml) in a dynamic system simulating in vivo pharmacokinetics of ceftriaxone in the same fluids. Symbols: ▲, growth without antibiotic in human blood (A) and human urine (B); ●, ceftriaxone (simulated dosage, 1 g/day i.v.); ○, ceftriaxone (simulated dosage, 3 g/day i.v.).

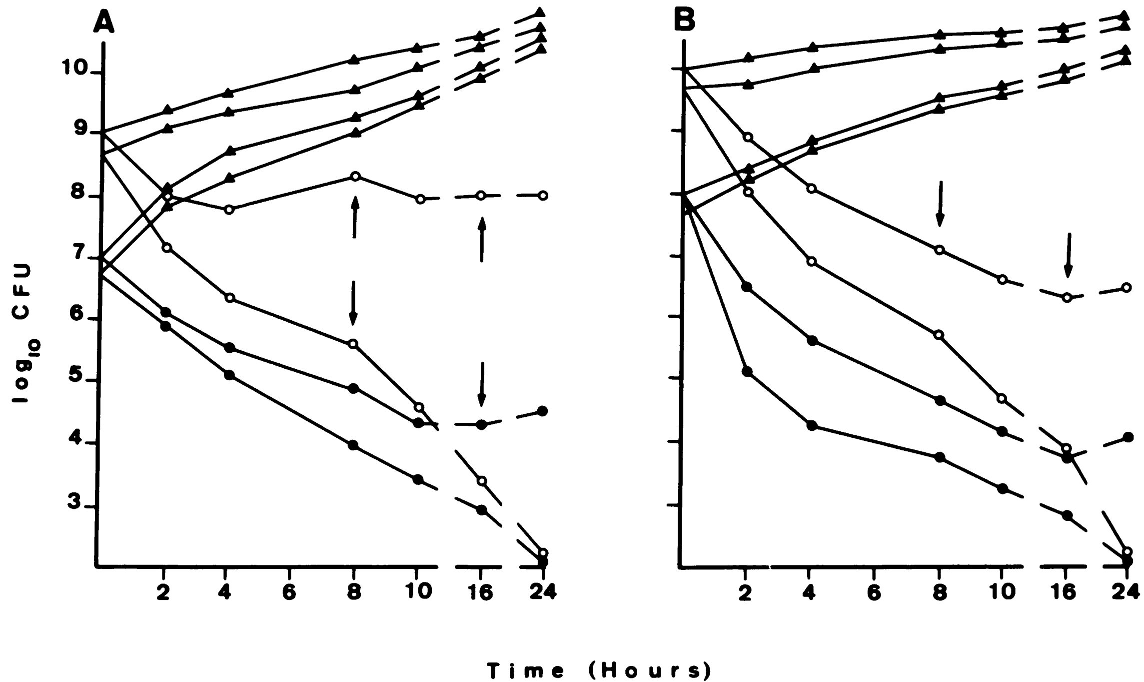

FIG. 5. Kill kinetics in human blood (A) and human urine (B) of an *E. coli* strain of average susceptibility (strain 2; aztreonam MIC, 0.125 μg/ml; gentamicin MIC, 0.5 μg/ml) in a dynamic system simulating in vivo pharmacokinetics of aztreonam and gentamicin in the same fluids. Symbols: ▲, growth without antibiotic in human blood (A) and human urine (B); ●, aztreonam (simulated dosage, 1 g every 8 h i.v.); ○, gentamicin (simulated dosage, 1.5 mg/kg every 8 h). Arrows indicate subsequent administrations of the drugs.

1×10^{10} and 5×10^{10} *P. aeruginosa* cells in blood and urine, respectively. A similar combination of aztreonam plus gentamicin also proved to be highly active, with complete inhibition of up to 5×10^9 and 1×10^{10} cells in blood and urine, respectively.

DISCUSSION

In the present work, we evaluated the antibacterial activities of several antibiotics by using a novel system. We used the antibiotics at concentrations varying with time as they

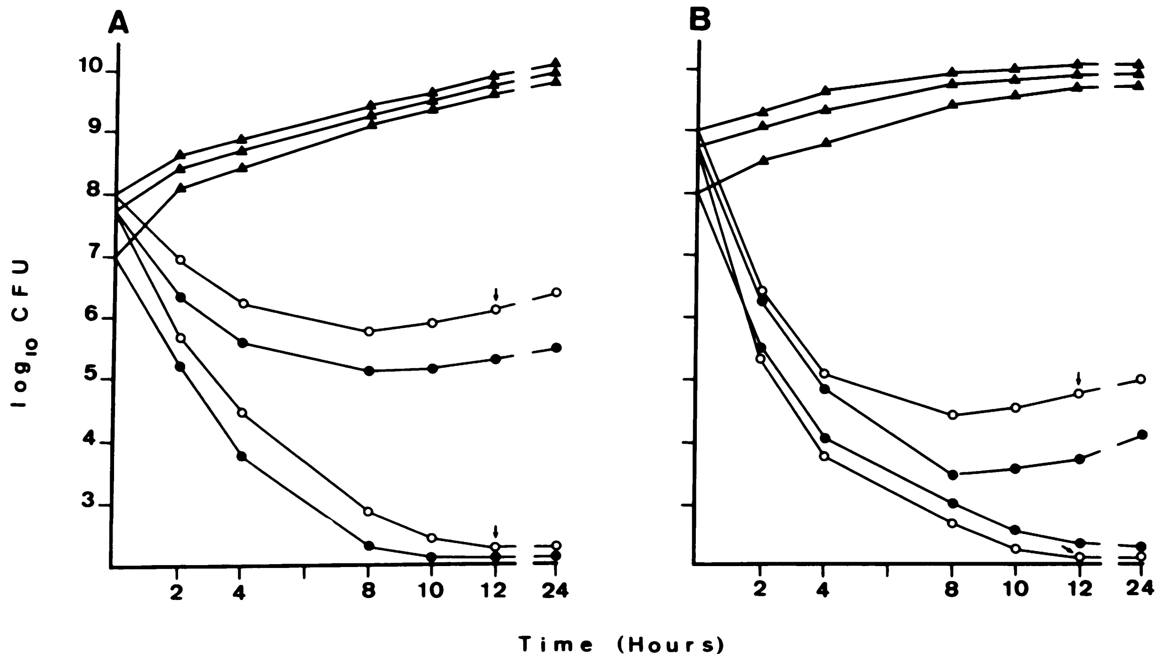

FIG. 6. Kill kinetics in human blood (A) and human urine (B) of *P. aeruginosa* 69 (MIC, 8 μg/ml) in a dynamic system simulating in vivo pharmacokinetics of ceftriaxone in the same fluids. Symbols: ▲, growth without antibiotic in human blood (A) and human urine (B); ●, ceftriaxone (simulated dosage, 1 g/day i.v.); ○, ceftriaxone (simulated dosage, 1.5 g every 12 h i.v.). Arrows indicate subsequent administrations of the drug (higher dosage).

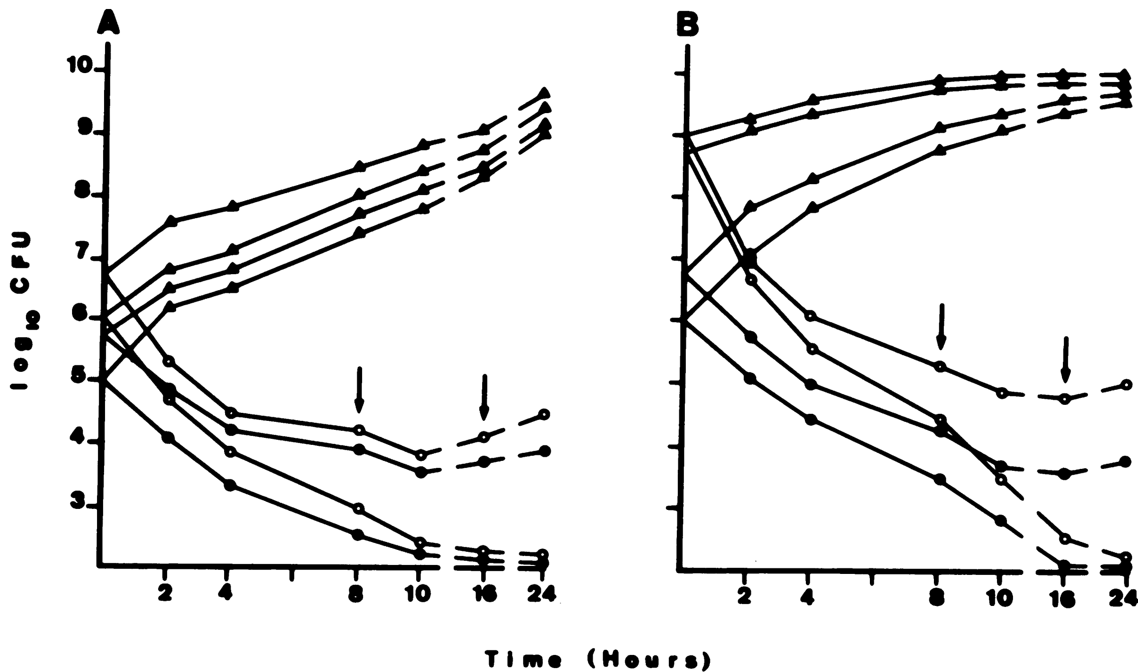

FIG. 7. Kill kinetics in human blood (A) and human urine (B) of *P. aeruginosa* 69 (aztreonam MIC, 4 μg/ml; gentamicin MIC, 1 μg/ml) in a dynamic system simulating in vivo pharmacokinetics of aztreonam and gentamicin in the same fluids. Symbols: ▲, growth without antibiotic in human blood (A) and human urine (B); ●, aztreonam (simulated dosage, 1 g every 8 h i.v.); ○, gentamicin (simulated dosage, 1.5 mg/kg every 8 h). Arrows indicate subsequent administrations of the drugs.

vary in some body sites and evaluated their abilities to achieve virtually complete killing of bacterial populations grown in fluids of human origin and close in size to populations occurring in serious infections. Other researchers previously evaluated antibiotic properties, such as bactericidal effect (compared with growth inhibition), factors influencing the apparent antimicrobial activity (such as number of bacterial cells and presence of various body fluids), and antibacterial activities of dynamic concentrations (4, 7, 8, 13, 20, 21, 32). However, in our work all these factors were combined to create conditions simulating as closely as possible those which antibiotics encounter in serious infections. As far as we know, no similar analysis has previously been performed.

The philosophy underlying this type of assay is that very large bacterial populations may grow in infections in which host defenses are not (or are not very) efficacious, and such infections can be overcome only if the antibiotics are capable of killing all or almost all the infecting microorganisms. Consequently, in serious infections, the antibiotics that are most likely to prove efficacious should be those which, under in vitro conditions simulating as closely as possible the situations encountered in the human body, prove capable of completely (or almost completely) killing bacterial populations at least as large as those which may be present in the most serious infections. From this point of view, the findings of our work indicate that ceftriaxone is likely to be efficacious in serious respiratory and blood infections caused by *S. pneumoniae* and in serious blood and urinary infections caused by *E. coli*; ampicillin is unlikely to be active either in *S. pneumoniae* septicemias or in serious respiratory infections caused by this microorganism; gentamicin is likely to be able to cure serious urinary infections caused by *E. coli* but probably not the most serious *E. coli* septicemias; and

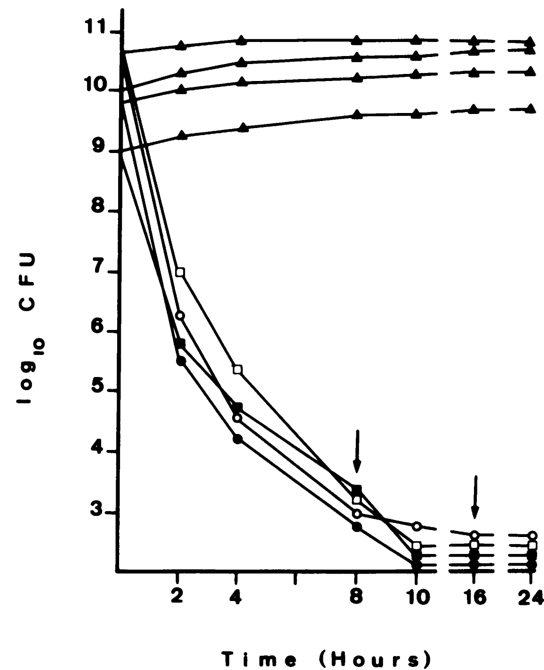

FIG. 8. Kill kinetics in human blood and human urine of *P. aeruginosa* 69 in a dynamic system simulating in vivo pharmacokinetics of antibiotic combinations in the same fluids. Symbols: ▲, growth without antibiotic in human blood and human urine; □ and ○, combination of ceftriaxone (simulated dosage, 1 g/day i.v.) plus gentamicin (simulated dosage, 1.5 mg/kg every 8 h); ■ and ●, combination of aztreonam (simulated dosage, 1 g every 8 h i.v.) plus gentamicin (simulated dosage, 1.5 mg/kg every 8 h); □ and ■, kinetics in blood; ○ and ●, kinetics in urine. Arrows indicate subsequent administrations of gentamicin and aztreonam.

aztreonam is unlikely to be adequate for treatment of serious septicemias and urinary tract infections caused by *E. coli*. Finally, none of the antibiotics assayed proved likely to be efficacious in the treatment of serious infections caused by *P. aeruginosa* when used in monotherapy. Only drug combinations (gentamicin plus either ceftriaxone or aztreonam) might be efficacious in the treatment of such infections. Although the predictive value of our in vitro test needs to be confirmed by clinical studies, it is important to note that it is generally held that serious infections caused by *P. aeruginosa* require combination therapy (12).

Of course, the low or relatively low antimicrobial activities demonstrated by some antibiotics in our experimental system do not imply that the same antibiotics are not sufficiently efficacious in the treatment of ordinary infections. In such infections, the number of bacteria present is generally distinctly lower and, what is more, killing (or even simple inhibition) of only some of them by the antibiotic may suffice for the rest to be eliminated by host defenses. On the other hand, the ability of an antibiotic to inhibit large populations of a strain of a given species under conditions mimicking a particular type of infection cannot be extrapolated either to strains of other species (whose response to the antibiotic might differ greatly) or to infections occurring in other body sites (where antibiotic pharmacokinetics may be very different). With this respect, it is in fact important to note that our data demonstrate that the abilities of antibiotics to kill large numbers of bacteria under conditions simulating serious infections cannot be predicted either solely on the basis of strong intrinsic bactericidal activities in the standard in vitro tests or solely on the basis of high ratios of concentrations in blood (and other fluids and tissues) to MICs. Thus, although gentamicin, with its strong intrinsic bactericidal activity, and aztreonam, with its high in vivo concentration/MIC ratio, are generally regarded as useful in the treatment of serious infections, both these drugs showed unexpectedly low activities in our experimental system. It seems very probable that the ability of an antibiotic to kill large numbers of bacteria under conditions simulating infections involves a combination of the factors mentioned above and other factors of particular importance, such as the half-life of the drug and influences of the environment in which the bacteria grow. Aztreonam, for instance, has a much shorter half-life and a much lower bactericidal activity for *E. coli* than ceftriaxone. On the other hand, the relative lack of activity shown in certain cases by gentamicin could depend on the fact that the activity of this drug, like those of all aminoglycosides, is greatly reduced when bacteria grow under conditions of microaerophily which may easily occur in serious infections among dense bacterial cultures; along with many other factors, such as changes in pH, concentration of divalent cations, etc.

As already discussed, to simulate in vivo conditions in our assays, we used large bacterial populations for all the infections. While it is probably clear that serious pulmonary and urinary infections may easily contain a total of bacteria close to the numbers we used in our assays, less clear is the maximum number of bacteria that may be present in serious septicemias. Colony counts exceeding 100/ml of blood are not frequent in the modern literature (6, 22), but the magnitude of bacteremia was much higher in the pre-antibiotic era (2). Moreover, very different colony counts have been observed from different arterial and venous sites, depending both on the mixing of the venous blood and on the sites of clearance of bacteria with respect to the sites of the infections (2, 27).

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