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MINIREVIEW

Role of Pharmacokinetics in the Outcome of Infections

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INTRODUCTION

When antimicrobial agents are administered for the treatment of infections, determination of the ultimate patient outcome is a function of multiple variables. Indeed, the multiplicity of variables has led some infectious disease clinicians to despair of finding rational methods for administration of these antimicrobial agents. However, much of the outcome can be explained on the basis of the intrinsic microbiologic activity of the agent administered for the infecting pathogen, the serum concentration-time profile in vivo, the protein binding of the antibiotic in question, and other factors (such as postantibiotic effect [PAE] and postantibiotic leukocyte enhancement) which may assist host defenses.

The beta-lactam and aminoglycoside antibiotics are two classes of agents which have been scrutinized with regard to the factors which influence outcomes of infections. These agents are widely used and, overall, have compiled an excellent record of success in the therapy of infection. In this paper, I attempt to review some of the information which has been compiled for each of these drugs in vitro, in vivo in animal models, and, finally, in the therapy of human infection. These data are examined for the drugs both alone and in combination.

BETA-LACTAM ANTIBIOTICS

Antibacterial properties. The last decade has witnessed the emergence of multiple new beta-lactam antibiotics. These drugs have been major advances over previous agents in our therapeutic armamentarium in that they have broadened spectra of activity and improved pharmacokinetic properties (11, 12, 39, 41). However, although they are bactericidal, it is a characteristic of beta-lactams that kill rates (with some exceptions, such as imipenem) are not as rapid as those seen with aminoglycosides and are characterized by the presence of a lag time to the onset of bacterial killing (7). Indeed, in a study by Bustamante et al. (7), ceftazidime at 65 μg/ml exhibited less than a 1-log kill for multiple strains of *Pseudomonas aeruginosa* after 1 h of incubation. This is in contrast to the aminoglycoside amikacin, which exhibited a greater-than-3-log kill on average for four *Pseudomonas* strains at 20 μg/ml for the same interval. On the other hand, while some regrowth was noted during exposure for the amikacin experiment, the ceftazidime-exposed organisms continued to die slowly over the first 6 to 7 h of incubation (7).

Further, virtually all the new beta-lactam antibiotics have been tested for the presence of a PAE against aerobic or facultative gram-negative bacilli. Again, with the single exception of penem or carbapenem antibiotics, all the beta-lactams have exhibited either no PAE or a very short PAE (6, 7). Consequently, because beta-lactam action on aerobic or facultative gram-negative bacilli is not, in the main, rapidly bactericidal or highly concentration dependent (i.e., higher peak concentrations in serum do not necessarily gain more rapid or complete killing) and because of the lack of PAE, the most rational course for beta-lactam administration seems to be administration either on a frequent schedule or as a constant infusion.

Pharmacokinetics of some new beta-lactam drugs. At the University of Maryland, we have examined the pharmacokinetics of multiple new beta-lactams when given intravenously to normal volunteers (11, 12, 39, 41). The pharmacokinetic parameters for these drugs show certain similarities (Table 1). Volumes of distribution tend to be similar and indicate that in the postdistributive phase beta-lactams distribute to the extracellular water. However, there is great variability among these new agents with regard to serum clearance, mechanisms of disposition (renal versus nonrenal clearance), and terminal elimination half-life. The acylureidopenicillins have a pharmacokinetic disposition profile very similar to that seen with older carboxypenicillins, and their main advantage comes from increased microbiologic activity. With the expanded-spectrum cephalosporins, along with an increased activity and broadened spectrum we also have somewhat better pharmacokinetic properties. However, clearances still vary over a wide range, from 14.04 ± 1.42 (standard deviation) liters/h per 1.73 m^2 for cefotaxime to 4.04 ± 0.58 liters/h per 1.73 m^2 for cefoperazone. Also, terminal elimination half-lives range from 1.2 h for cefotaxime to 2.5 h for moxalactam. Mechanisms of disposition are also quite variable, ranging from greater than 80% renally cleared for ceftazidime to 26% renally cleared for cefoperazone.

Protein binding. The importance of protein binding for clinical outcome in infected patients has always been a matter of great debate. However, considerable clinical data exist both for animals and for humans that increasingly protein-bound drugs penetrate to a lesser extent to the interstitial space. Wise and colleagues generated multiple studies examining the influence of protein binding on drug penetration (45–47). Hoffstedt and Walder examined the influence of serum protein binding on penetration of five cephalosporins into subcutaneous tissue fluid in humans (20). These and other animal and human studies have shown repeatedly that increasing protein binding adversely affects the ability of a drug to penetrate into the interstitial space. One can say that the area under the concentration-time curve of free drug should be approximately equal in the interstitial space and in serum (Fig. 1).

In addition, the microbiologic activity of beta-lactam agents is adversely affected by protein binding. In 1947, Ralph Tompsett and colleagues examined the efficiency of

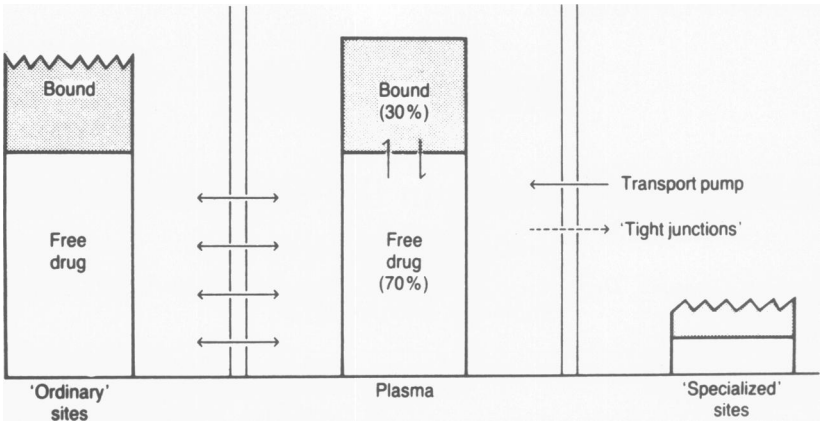

FIG. 1. Equilibrium distribution of an antibiotic which is 30% protein bound in serum, showing interstitial fluid concentrations at "ordinary" and "specialized" sites. Levels of free drug in plasma and ordinary sites are equal, despite differences in total drug concentration due to protein binding. Levels in specialized sites are much lower because of the tight capillary junctions (lipid barrier) and, in some instances, active transport out of the site (after Barza and Cuchural, reference 3).

kill for *Staphylococcus aureus* of different penicillins with different degrees of protein binding (40). Serum was added in a sequentially increasing fashion, and the effect of decreasing free drug was ascertained. Decreasing the amounts of free drug impaired the ability of all penicillins to kill the *S. aureus*. What is also clear is that the most heavily bound drugs were most severely impaired in their ability to kill (40).

The question always arises as to the in vivo import of this in vitro phenomenon. Merrikin and colleagues examined this issue in an intraperitoneal infection mouse model with *S. aureus* (27). By using a group of penicillins all belonging to the same chemical class, antibacterial activity against *S. aureus* was determined in vitro and in vivo. An excellent relationship was determined between the percentage of free antibiotic in mouse serum and the ultimate CD_{50} (protective dose for 50% of animals) as determined in the animal model (27). It appears, therefore, that protein binding affects the antimicrobial activity of beta-lactams both in vitro and in an in vivo animal model, at least for gram-positive cocci. For aerobic or facultative gram-negative bacilli, however, the results are much less clear-cut. Moody and colleagues examined the influence of protein binding on a gram-negative-bacillary infection in rabbits (J. A. Moody, L. R. Peterson, D. N. Gerding, and C. E. Fasching, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 557, 1985). They found that free drug concentrations of cefoperazone correlated better with gram-negative-rod-cell kill than did total drug levels in their rabbit model. Conversely, however, Lang and colleagues found an anomalous effect of serum on the antimicrobial activity of cefoperazone for multiple gram-negative organisms in vitro (26). They merely speculated that variables other than protein binding were responsible for the anomalous activity against the gram-negative organisms that they studied but did not identify the causative factors.

There is some evidence that protein binding may adversely affect antimicrobial activity clinically. Chambers and colleagues (9) demonstrated that cefonicid, a highly protein-bound cephalosporin, failed to clear the blood of organisms or cause a resolution of clinical findings by day 5 of therapy in three of four patients with staphylococcal endocarditis. This was despite measured (in two patients) peak concentrations of drug 20 to 40 times the MIC for the infecting strain; trough concentrations were 7 to 14 μg/ml in these patients. When protein was added to the medium, MICs for the isolates rose four- to eightfold and bactericidal titers were <1:8 at peak in three patients.

In a study evaluating another highly bound antibiotic (teicoplanin), Calain et al. (8) examined nine patients. Six were assessable for outcome. All six failed. Measured trough concentrations of total teicoplanin exceeded the MIC for the infecting pathogen by 5 to 35 times in four of these patients. As teicoplanin is very highly bound, however, free drug was less than the MIC (even at peak). Overall, it may be said that the evidence is relatively clear-cut that protein binding adversely affects the antimicrobial activity of beta-lactams against *S. aureus* and that there is probably an adverse effect on the antimicrobial activity for gram-negative organisms, although it is not as mathematically predictable as that seen for *S. aureus*. Nevertheless, integration of protein-binding

TABLE 1. Pharmacokinetic parameters for some new beta-lactam antibiotics[a]

Antibiotic	V_1 (liter/kg)	V_{ss} (liter/kg)	V_{area} (liter/kg)	$t_{1/2}$ (h)	CL (liters/h per 1.73 m^2)	CL_R (% of CL)	Reference
Moxalactam	0.16 ± 0.03	0.22 ± 0.04	0.27 ± 0.05	2.51 ± 0.21	4.72 ± 0.08	70.5	39
Cefotaxime	0.13 ± 0.03	0.23 ± 0.07	0.38 ± 0.13	1.18 ± 0.34	14.04 ± 1.42	48.7	39
Cefoperazone	0.09 ± 0.02	0.14 ± 0.02	0.17 ± 0.03	1.87 ± 0.28	4.04 ± 0.58	25.7	39
Ticarcillin	0.10 ± 0.04	0.15 ± 0.01	0.16 ± 0.02	1.32 ± 0.18	5.78 ± 0.95		41
Mezlocillin	0.12 ± 0.04	0.18 ± 0.04	0.28 ± 0.10	1.24 ± 0.49	10.47 ± 2.32		41
Piperacillin	0.10 ± 0.02	0.15 ± 0.02	0.19 ± 0.04	1.12 ± 0.21	7.89 ± 0.99		41
Ceftazidime	0.13 ± 0.07	0.21 ± 0.04	0.24 ± 0.05	1.75 ± 0.21	6.4 ± 0.7	80.0	12
Imipenem	0.16 ± 0.05	0.24 ± 0.04	0.23 ± 0.03	0.93 ± 0.09	12.1 ± 0.6	54.0	11

[a] V_1, Volume of distribution in the central compartment; V_{ss}, volume of distribution at steady state; V_{area}, volume of distribution of drug in the body; $t_{1/2}$, terminal half-life; CL, serum clearance; CL_R, renal clearance. Values are means ± standard deviations.

properties of a drug is clearly the most conservative way to evaluate new agents.

Animal models evaluating beta-lactam dosing schemes. A number of investigators have examined the impact of dosing schedule on the outcome of infections in animals. However, certain attempts have most clearly delineated the effect between different schedules and outcomes for aerobic or facultative gram-negative bacilli. Rollinson examined the impact of schedule on cell kill in a *Pseudomonas* mouse thigh infection (33). Carbenicillin was used as the probe agent. When counts at the infection site were done, it was clear that as drug concentration dropped below the MIC, the organisms returned to log growth at the infection site after a single dose of antibiotic. In a multiple-dosing study, when the schedule was such that drug concentration was allowed to drop below the MIC for approximately half the dosing interval, there was cycling of the number of organisms at the primary site of infection. The beta-lactam was unable to eradicate the organism at the site of infection because of regrowth. When the dosing interval was shortened, such that concentrations in serum remained above the MIC, the number of CFU at the site of infection continued to decline with continued therapy. Unfortunately, this experiment was fatally flawed in its design in that the same drug dose was given more frequently, and, consequently, a larger total milligram-per-kilogram (body weight)-per-day dose of drug was being used in the group of animals with the better outcome.

Gerber and colleagues, however, repeated this experiment with design changes (15). While Rollinson used normal mice, Gerber et al. used granulocytopenic mice to remove the influence of some host defenses on infection outcome. These investigators also used the same total daily dose of beta-lactam drug (ticarcillin). They altered the schedule, dividing the same total dose on an every-3-h schedule or dividing the dose on an hourly schedule (a smaller dose more frequently). The results are shown in Fig. 2. While neither schedule caused eradication of the organisms at the site of infection with the dose used, clearly the more frequent schedule, resulting in drug concentrations remaining above the MIC for a longer period, resulted in statistically significantly better control of the experimental infection.

Similarly, Roosendaal et al. examined a *Klebsiella pneumoniae* pneumonia model in normal and neutropenic rats (34). Ceftazidime was the beta-lactam agent used as the probe. Constant infusion was compared with intermittent administration. The effect of neutropenia on outcome can be seen in Table 2. Clearly, organism load in the lung increases much more rapidly in the neutropenic host, bacteremia occurs more frequently and sooner, and organisms are found in the pleural space only in the granulocytopenic animals. Table 3 shows outcome of therapy. In normal animals, constant infusion was slightly more effective than intermittent administration. However, the neutropenic animals show the most dramatic outcome differences. Constant infusion required 1/15 as much drug to completely protect a cohort of animals compared with intermittent administration. This is powerful evidence for administering beta-lactam drugs in a manner which will continue to provide serum concentrations of drug (perhaps free drug) in excess of the MIC for the infecting pathogen for the whole dosing interval.

Data from human studies. While investigations into the relationship between schedule and outcome for beta-lactam drugs in serious human infections is scarce, some information does exist and the cumulative weight of evidence supports the conclusion drawn from the animal model, i.e., that concentrations in serum should remain always in excess of the MIC for infecting pathogens. Andersen et al. examined simultaneous antibiotic levels in breakthrough gram-negative-rod bacteremia (1). They divided the breakthrough bacteremias into early (≤3 days after admission) or late (thereafter) breakthroughs. Among the early breakthroughs, 12 of 19 patients had antibiotic concentrations in serum lower than the MIC for the infecting pathogen at the time of the positive blood culture. Many of these patients were receiving beta-lactam antibiotics as single agents. While consistent with the lessons learned in the animal model, this does not constitute proof of the hypothesis because of the lack of denominator information (number of patients with drug concentrations lower than the MIC without breakthrough bacteremia).

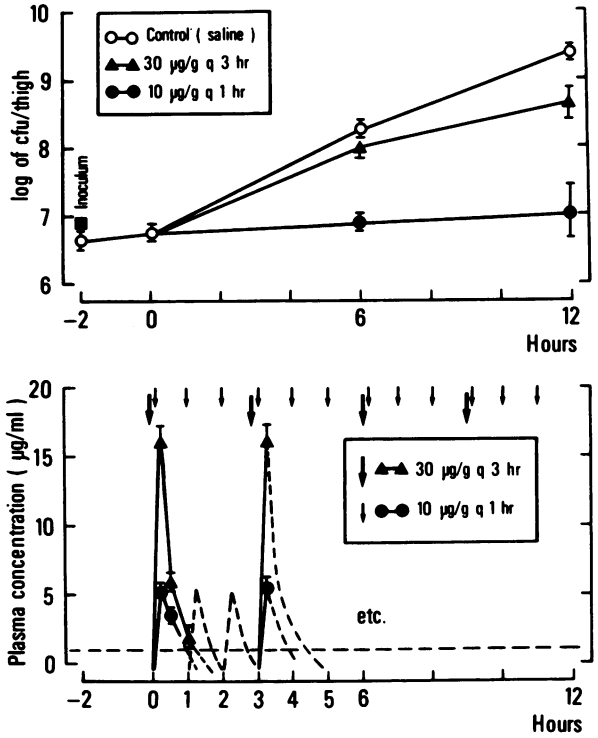

FIG. 2. Kinetics of sub-MICs of ticarcillin and the corresponding effect on *P. aeruginosa* ATCC 27853 in the same granulocytopenic mice. Top, Growth kinetics of *P. aeruginosa* in vivo. Each point represents the geometric mean ± standard deviation number of CFU per thigh in three mice. The differences among the three growth curves are significant ($P < 0.01$). Bottom, Plasma kinetics after repeated 3- and 1-h subcutaneous injections of ticarcillin (30 and 10 µg/g, respectively). Each point stands for the mean ± standard deviation plasma level in three mice. Limit of detectability (– – –), 1 µg/ml (after Gerber et al., reference 15).

Other information, however, is available. Warren and colleagues at the University of Maryland performed a study of cefoperazone versus cefamandole-tobramycin for the empiric therapy of suspected gram-negative-rod bacteremia (44). Patients were receiving cefoperazone on a schedule of 1.5 g intravenously every 6 h. As our group at Maryland had previously performed a cefoperazone pharmacokinetic evaluation (39), and as others have shown that altered renal function has little influence on cefoperazone pharmacokinetics, I conducted a simulation study looking at the expected trough concentration of free cefoperazone for their patients. I predicted in a blinded manner the outcome of patients with gram-negative-rod bacteremia receiving cefoperazone on this dosing schedule. Considering that predicted trough concentrations were 2 µg of free drug per ml, patients for whose organisms MICs were lower than or equal to this

TABLE 2. Course of *K. pneumoniae* in normal and leukopenic rats[a]

h after inoculation	Normal rats (n = 5)				Leukopenic rats (n = 5)			
	Log CFU in left lung (range)	Blood		No. of rats with bacteria in pleural fluid	Log CFU in left lung (range)	Blood		No. of rats with bacteria in pleural fluid
		No. of rats positive	Log CFU/ml[b]			No. of rats positive	Log CFU/ml[b] (range)	
5	5.7 (5.3–6.0)	0		0	5.7 (5.7–5.9)	0		0
10	5.8 (4.8–6.3)	0		0	6.5 (6.2–6.7)	0		0
18	5.6 (3.6–5.8)	0		0	7.5 (7.0–7.6)	1	1.5	0
24	6.5 (5.7–7.4)	0		0	8.5 (7.7–8.8)	5	2.3 (1.6–2.2)	0
29	6.3 (5.7–7.3)	1	1.9	0	8.7 (8.2–8.9)	5	3.0 (1.4–3.5)	2
34	8.3 (7.4–9.2)	1	1.9	0	8.9 (8.7–9.4)	5	3.3 (3.1–4.7)	3

[a] Groups of five rats each were studied after inoculation of the left lung with 8×10^4 CFU. The values given are median values. The mean times to death ± standard deviations were 5.1 ± 1.3 and 2.3 ± 0.3 days for normal and leukopenic rats, respectively.
[b] Calculated for positive cultures only (after Roosendaal et al., reference 34).

value were predicted to succeed; those with MICs greater than this were predicted to fail. Of 10 patients, 9 were correctly predicted (prediction of 6 successes and 4 failures; in reality, there were 5 successes and 5 failures). The single misprediction was an 85-year-old patient with septic shock, renal failure, and acidosis who died less than 24 h after admission. If one were to use a cefoperazone MIC of 16 μg/ml for the bacteremic organism to predict success or failure, one would expect nine successes and one failure. Again, however, this does not constitute proof of the hypothesis because of the small number of patients and the simulation nature of the study. However, again, it is consistent with lessons learned in the animal model. Perhaps the most dramatic data come from the study performed by Bodey et al. (5). They compared carbenicillin plus cefamandole with cefamandole given either as 3 g intravenously every 6 h (intermittent dosing) or as 12 g/day as a constant infusion to carbenicillin-tobramycin as the control regimen. Among patients who had the poorest prognosis, that is, patients whose initial neutrophil count was less than 100/μl and did not increase during the course of therapy, constant-infusion cefamandole was significantly more effective than intermittent-administration cefamandole (65% success rate versus 21%; $P = 0.03$). Considering that this study showed significantly better outcome results with constant-infusion therapy in this very impaired patient population, this is compelling evidence that the lessons learned in the animal model truly do translate to the clinical arena. Finally, Schentag et al. (35) examined the influence of maintaining beta-lactam concentrations above the MIC on length of time to eradication in patients with nosocomial pneumonias and demonstrated significant linear correlation between the time drug concentrations exceeded the MIC and time to eradication.

Our group has contended that different drugs could be compared for expected efficacy and that dosing schedules could be optimized by comparing times free drug concentrations remained above the MIC for 90% of clinically important pathogens (10). As many of the new drugs examined above were meant to be used in the empiric therapy situation, one would like to have an expectation that greater than 90% of the infections seen in the nosocomial setting would respond to the antibiotic used. In Table 4 is shown time above the MIC for 90% of nosocomial gram-negative pathogens most clinically important at my institution for the new beta-lactams which we have studied over the course of the last 5 years (10, 12, 38). Clearly, predictions about efficacy of these drugs against different pathogens have been borne out. The vast majority of the new cephalosporins used at recommended dose and schedule provide excellent clinical outcomes for members of the family *Enterobacteriaceae*. However, based on the data it is clear that the early broad-

TABLE 3. Efficacies of ceftazidime treatment schedules[a] on normal and leukopenic[b] rats

Dosage (mg/kg per day)	Normal rats (n = 10)				Leukopenic rats (n = 10)			
	Intermittent administration		Continuous administration		Intermittent administration		Continuous administration	
	No. of survivors	Time to death (days)[c]	No. of survivors	Time to death (days)[c]	No. of survivors	Time to death (days)[c]	No. of survivors	Time to death (days)[c]
0.06	0	8.9 ± 2.0	0	5.5 ± 0.7				
0.12	1	9.5 ± 3.1	2	5.8 ± 0.7				
0.23	4	8.2 ± 3.3	1	6.8 ± 1.6				
0.47	6	8.8 ± 1.5	6	9.0 ± 1.4			0	2.8 ± 0.4
0.94	9	8	10				1	5.2 ± 1.9
1.88	9	8					7	9.3 ± 3.5
3.75	10						10	
7.50					0	3.4 ± 1.2		
15.00					1	3.5 ± 0.7		
30.00					7	5.3 ± 2.1		
60.00					10			

[a] To groups of 10 rats each, ceftazidime was administered either as intermittent bolus injections at 6-h intervals or as continuous infusions (infusion rate, 0.113 ml/h) over a period of 4 days. Treatment was started 5 h after inoculation of the left lung with 8×10^4 CFU of *K. pneumoniae*. The 50% protective doses (milligrams per kilogram per day) for intermittent administration and continuous administration for normal rats were 0.35 and 0.36, respectively (99.9% confidence limits, 0.19 to 0.67 and 0.21 to 0.61, respectively); the corresponding values for leukopenic rats were 24.37 and 1.52 (16.07 to 36.97 and 1.00 to 2.31).
[b] Cyclophosphamide was administered intraperitoneally in two doses of 90 and 60 mg/kg at 5 days and 1 day before bacterial inoculation, respectively.
[c] Mean ± standard deviation; based on the time of bacterial inoculation (day 0) (after Roosendaal et al., reference 34).

TABLE 4. Duration free drug concentrations of new beta-lactams exceed MIC for 90% of important gram-negative pathogens in volunteers

Antibiotic	Dose (g)	h of free drug concn for:					Reference
		Escherichia coli	*Klebsiella pneumoniae*	*Enterobacter cloacae*	*Serratia marcescens*	*Pseudomonas aeruginosa*	
Moxalactam	2	12	12	12	12	0.4	10
Cefotaxime	2	8.9	8.9	6.5	6.5	0.2	10
Cefoperazone	2	5.3	5.3	3.6	0.6	0.6	10
Ticarcillin	5	0	0	0	0	0.1	41[a]
Mezlocillin	5	2.0	3.8	2.0	0	1.1	41[a]
Piperacillin	5	1.4	4.4	0.6	0	3.3	41[a]
Ceftazidime	2	12	12	12	12	5.8	12
Imipenem	1	6	6	6	5.6	4.5	34

[a] As derived from parameters in reference 41.

spectrum compounds would not be likely to provide adequate therapy against *P. aeruginosa*. Conversely, ceftazidime and imipenem would be predicted to have the best response rates for *P. aeruginosa* when used in schedules of 2 g every 8 h (ceftazidime) or 1 g every 6 h (imipenem). The borderline activity of moxalactam and ceftazidime for *S. aureus*, as well as the inadequacy of the acylureidopenicillins for single-agent empiric therapy of serious nosocomial infections, is also pointed up by these studies.

In conclusion then, outcomes for patients with serious infections being treated with beta-lactam antibiotics as single agents can probably be optimized by producing serum concentration-time profiles such that free drug concentrations always remain above the MIC for the infecting pathogen. Whether this should be by intermittent administration (for logistical ease) or by constant infusion requires further study. Cure at specialized sites (e.g., central nervous system) or secretory fluids (e.g., prostate) may require higher concentrations.

Feedback (adaptive) control algorithms have been proposed for alteration of antimicrobial therapy. For the beta-lactam antibiotics, however, one simple adaptive control approach would be to examine the trough serum bactericidal titer produced by a specific dosing schedule. Patients having serum bactericidal titers ≥1:2 at trough would, in general, not require major adjustment of therapy except if their infections were in specialized sites (3). If adjustment is required, our group has shown that a simple mathematical relationship is demonstrated between concentration achieved in serum and serum bactericidal titer (13). Indeed, the relationship demonstrated between outcome and peak serum bactericidal titer (24, 37) may simply be one of time above the MIC (or MBC for cidal titers), with one twofold dilution representing one terminal half-life worth of coverage for the pathogen in question.

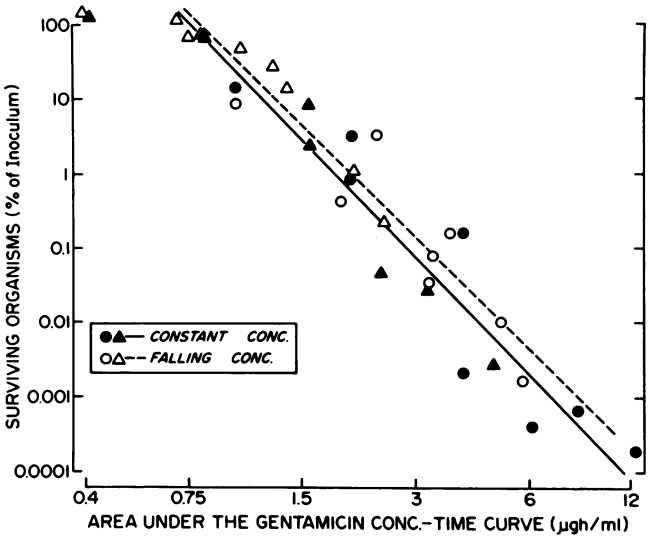

FIG. 3. Effect of gentamicin on large inocula of *P. aeruginosa* ATCC 9721 in a kinetic model with unsupplemented Mueller-Hinton broth. Regression lines are calculated from data obtained with an area under the curve of gentamicin concentration-time equal to and greater than the minimal effective value (0.75 μg/ml). ○, 4× the minimal active concentration (MAC) with a declining profile; ●, 4× the MAC with a constant profile; △, 8× the MAC with a declining profile; ▲, 8× the MAC with a constant profile (after Gerber et al., reference 18).

AMINOGLYCOSIDE ANTIBIOTICS

Antibacterial properties. Aminoglycoside antibiotics differ markedly from beta-lactams in that their kill of aerobic or facultative gram-negative bacilli is relatively rapid (7). Again, in contrast to the beta-lactams, aminoglycosides tend to induce a prolonged PAE against aerobic or facultative gram-negative rods. Indeed, Vogelman and Craig examined this in detail (42) and showed convincingly that they also exert this PAE in vivo in the neutropenic mouse thigh infection model.

Gerber et al. examined the kill of *P. aeruginosa* in an in vitro system as a function of the mode of exposure (constant infusion versus declining concentration) (18). They found that the log kill of *P. aeruginosa* for aminoglycosides was independent of the mode of exposure (Fig. 3) and correlated very closely with the area under the curve of drug exposure. This independence from mode of exposure may be tied to the presence of a PAE. Obviously, such a finding immediately presents a hypothesis which should be tested in an animal model.

Animal models. Gerber and Feller-Segessenmann used the granulocytopenic mouse thigh infection model subsequently and in this examination found that outcome for the animal could be correlated directly with peak concentrations of aminoglycoside in serum (16). However, because the pharmacokinetics of aminoglycosides in small animals reveal an extremely short half-life, there would be a significant covariance between the peak concentration achieved in serum and the total area under the curve. Consequently, it is not surprising that peak concentrations in serum can be shown to correlate with cell kill at the site of infection. Vogelman and colleagues showed that, as in the in vitro setting, cell kill at the infection site correlates well with the area under the curve of drug exposure (B. Vogelman, S. Gudmundsson, J.

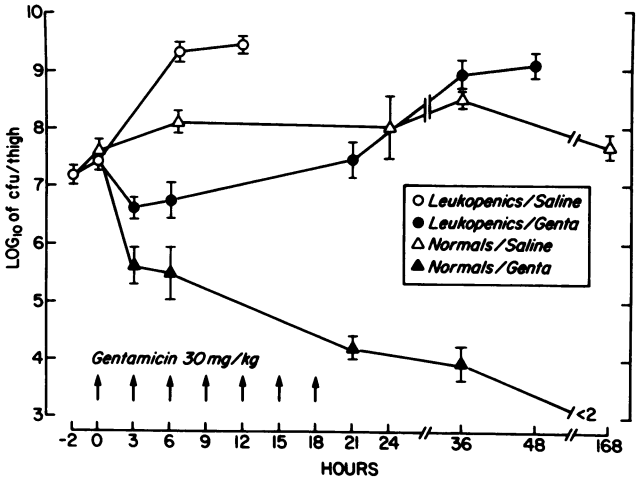

FIG. 4. Effect of multiple injections of gentamicin (3-hourly subcutaneous injections of 30 mg/kg) on *P. aeruginosa* ATCC 27853 in normal and leukopenic mice. Data are geometric mean ± standard deviation values obtained from three animals. The greatest standard deviation was found in normal mice (0.6 log in untreated normal mice at 24 h, 0.45 log in treated normal mice at 6 h). Regrowth of the organism in leukopenic mice between 3 and 21 h of treatment was significant ($P = 0.0104$). Gentamicin-treated leukopenic mice (Genta) that were dissected at 36 h had died between 33 and 36 h; gentamicin-treated mice dissected at 48 h had died between 42 and 48 h. No deaths occurred in normal mice whether treated or untreated (after Gerber et al., reference 17).

Legett, K. Totsuka, and W. A. Craig, Clin. Res. **34**:408A, 1986).

Gerber et al. also examined the influence of multiple-dose administration of aminoglycosides on infection outcome in the neutropenic mouse thigh infection model (17). Clearly, a large body of clinical experience has been garnered with the aminoglycoside antibiotics in the therapy of serious gram-negative-bacillary infections in humans. In general, so long as host defenses remain intact, aminoglycosides have done quite well in providing a good therapeutic outcome. However, data from neutropenic cancer patients indicate that response rates of 20 to 40% could be expected when therapy was initiated, with aminoglycosides being the only gram-negative-bacillus-active agent for patients with a gram-negative-rod bacteremia (25, 36). Clearly, some mechanism must underlie this marked discrepancy in clinical outcome. In examining Fig. 4, it becomes apparent that, in contrast to normal mice with *Pseudomonas* thigh infections, granulocytopenic mice have a much more rapid increase in the number of CFU at the infection site in the absence of therapy. Indeed, the curve stops at 12 h, because the animals have all died from sepsis secondary to *Pseudomonas* infection. This is quite consistent with the clinical situation, since untreated patients with gram-negative-rod bacteremia and profound persistent neutropenia usually have a very poor outcome in a short period of time. Normal animals administered aminoglycosides have an excellent therapeutic response, with good kill of organisms at the site of infection and continuing kill with repeated administration of drug. Of perhaps the greatest interest is the group of animals which are neutropenic and which received multiple doses of aminoglycoside. Here, slow regrowth of the organism is seen through repeated aminoglycoside administration. Clearly, this is very consistent with the early experience in neutropenic patients treated with aminoglycosides. Gerber et al. made further inroads into this problem by examining the population of organisms at the site of infection and the influence of continued aminoglycoside therapy (17). It was clear that the dense population of organisms at the site of infection had a number of mutants which were resistant to twice and even four times the MIC. These small-colony-variant resistant organisms slowly grew up and took over the population at the primary site of infection through continued aminoglycoside administration. When the investigators rechecked the site of infection 24 h after initiating therapy, the vast majority of organisms at the infection site were resistant (Fig. 5).

Clinical experience with combinations of beta-lactams plus aminoglycosides in granulocytopenic cancer patients has shown excellent efficacy (25, 36, 43). When this was examined in the mouse thigh infection model, it was shown that addition of the beta-lactam (ticarcillin) resulted in a markedly increased rate of kill of organisms at the infection site (17). More importantly, however, it was shown that the addition of the beta-lactam also resulted in suppression of

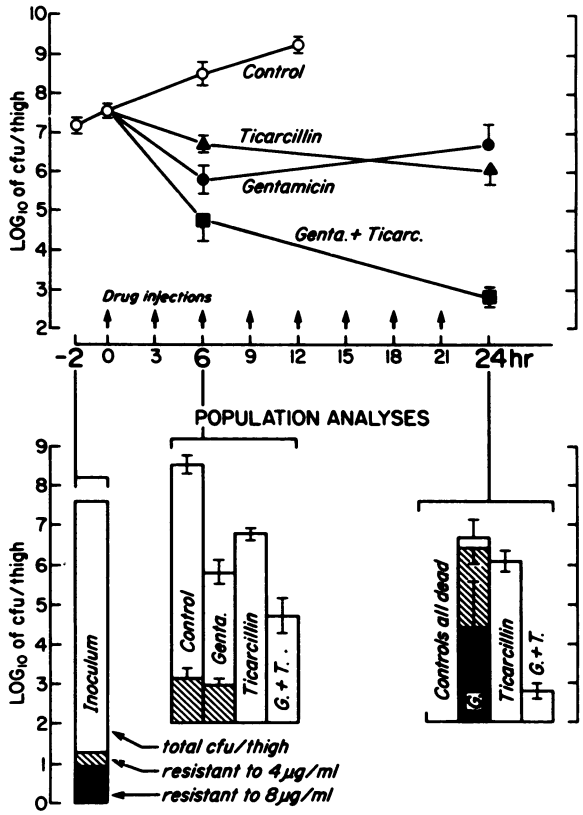

FIG. 5. Selection of gentamicin-resistant variants and gentamicin-ticarcillin synergy in vivo. Top, Effect of three treatment regimens on colony counts of *P. aeruginosa* ATCC 27853 from the infected thighs of leukopenic mice. Treatment consisted of gentamicin (3-hourly injections of 30 mg/kg), ticarcillin (3-hourly injections of 1,000 mg/kg), or gentamicin (3-hourly injections of 30 mg/kg) plus ticarcillin (3-hourly injections of 1,000 mg/kg). Each sampling point was based on colony counts obtained from three mice (geometric mean ± standard deviation). Regrowth was significant in leukopenic, gentamicin-treated mice between 6 and 24 h ($P = 0.071$). Bottom, Bacterial population analysis with regard to gentamicin susceptibility of inoculum and isolates (thigh tissue homogenates) from treated and untreated mice. Bars represent the geometric mean ± standard deviation of colony counts from three mice. The lower limit for detection of organisms was 10^2 bacteria per thigh. The bacterial population shift in gentamicin-treated mice to variants resistant to ≥4 μg of gentamicin per ml was highly significant ($P < 0.001$) (after Gerber et al., reference 17).

emergence of resistance of the small-colony-variant organisms (Fig. 5). Consequently, the animal data are in excellent concordance with clinical data showing that combinations of beta-lactams and aminoglycosides provide superior therapeutic outcomes in this patient population.

Data developed from clinical trials. Since aminoglycosides have been available for serious gram-negative-bacillary infections in the clinical arena much longer than extended-spectrum beta-lactams, there has been more opportunity to examine the relationship between achieved concentrations in serum and therapeutic outcome. Noone and colleagues retrospectively examined patients with severe gram-negative-bacillary infections and reported that outcome was significantly correlated with achieving peak serum concentrations of aminoglycosides greater than 5 μg/ml sometime in the first 3 days of therapy (31). They further reported that the exception to this rule was gram-negative-bacillary pneumonia, in which early peaks of 8 μg/ml or greater were necessary for an optimal clinical outcome. Subsequently, Keating and colleagues examined the influence of aminoglycoside serum concentrations on outcome in cancer patients with serious gram-negative-bacillary infections (23). In a randomized trial of constant-infusion therapy, they were able to show that there was a gradation of response which was proportional to the achieved constant concentration in serum divided by the MIC for the organism. As this was a constant-infusion study, there would then be clearcut association of the AUC/MIC ratio with clinical outcome. In addition, for the reasons outlined for the animal model discussed above, it would not be surprising to find an association in a bolus-administration study between peak serum concentration or peak serum concentration/MIC ratio and outcome.

In three studies published between 1984 and 1987, Moore et al. examined the relationship between outcome and concentrations achieved in serum and the MIC for the organism in patients with bacteremia and with gram-negative-bacillary pneumonia (28–30). In all three studies, a significant relationship was found between the peak concentration achieved in serum and clinical outcome by either logistic regression analysis or a stepwise discriminate function analysis. In their latest examination of the data, these investigators also showed that the peak serum concentration-to-MIC ratio was perhaps the best predictor of outcome.

For the aminoglycosides then, data are available from in vitro studies which have been examined in animal models and which have been to a great degree validated in clinical trials, indicating that outcome is influenced by the area under the curve of drug in the serum or, perhaps, peak concentration achieved in serum relative to the MIC for the infecting pathogen. The practical implications of this are that aminoglycosides may not need to be given on a frequent-dosing schedule to keep drug concentrations always above the MIC, as is the case with beta-lactams. Obviously, the PAE of aminoglycosides is key in determining the optimal dosing interval for these drugs. Further investigation needs to be performed relating time above the MIC plus PAE and optimal outcome for this class of drugs.

COMBINATION THERAPY

Little has been done to elucidate the optimal dose and schedules for patients receiving combinations of antimicrobial agents, particularly beta-lactams plus aminoglycosides. Indeed, the only accepted and practical method for integrating the activity of these drugs in combination is the use of serum bactericidal titers. A number of institutions have validated the usefulness of serum bactericidal titers, particularly in the neutropenic cancer patient. Klastersky and colleagues have led the way in this regard and have shown that for the vast majority of neutropenic patients, peak serum bactericidal titers of 1:8 or greater were associated with statistically significantly better outcome (24). For patients who are more profoundly and persistently neutropenic, serum cidal titers greater than 1:16 seem to be most appropriate (37). The dynamic nature of the interaction when two drugs are used in these patients has been recognized by Barriere and colleagues, and they have proposed integrating the activity and pharmacokinetics of multidrug therapy through the use of multiple serum bactericidal titers with determination of the area under the curve for reciprocal cidal titer over time (2).

Other inroads are being made into this area. In the in vitro setting, Blazer (4) and, more recently, Dudley and colleagues (14) have described an elegant in vitro capillary model which allows elucidation of the effects of two different drugs with different serum half-lives to be examined on a population of organisms. The animal model referred to above (17) provides a powerful tool for probing different doses and schedules to optimize outcome for combination therapy. Gerding and colleagues used a rabbit model to examine combination therapy as well with very interesting results (19). At my institution, Johnson and colleagues (21, 22) have used the neutropenic rat model of sepsis to probe outcome differences between single- and combination-agent therapy. Finally, at this institution we have begun to use logistic regression analysis to examine in a quantitative sense the interaction between two drugs as it exists in a checkerboard synergy plate by the method of Reller and Stratton (32). This analysis fits the growth-no growth situation (growth scored as 1 or no growth scored as 0) on the 96-well synergy plate to the following equation:

$$L = \alpha + \beta_1 \mathrm{Ln}(\text{piperacillin}) + \beta_2 \mathrm{Ln}(\text{amikacin}) + \beta_3 \mathrm{Ln}(\text{piperacillin})\mathrm{Ln}(\text{amikacin}) \quad (1)$$

where L is the natural log of the probability of growth divided by the probability of no growth. The coefficients α, β_1, β_2, and β_3 were fit by a nonlinear least-squares regression technique utilizing the SAS package of programs. One can then express equation 1 in probalistic form and write an explicit expression for the probability of no growth as a function of the concentrations of the antimicrobial agents used. This expression is seen in equation 2:

$$P\,(\text{ng}) = \frac{1}{1 + e^{L}} \quad (2)$$

Because of the logarithmic transformation in equation 1, wells containing zero concentration of either drug were not analyzed in this calculation. Consequently, we have been able to ascertain the serum concentration-time profile of patients on combination antimicrobial chemotherapy (Fig. 6) and integrate this with the microbiologic activity of each drug and the interaction of the drugs. In so doing we have been able to present plots (Fig. 7) of the probability of a no-growth situation being present in the blood of a patient over time during a dosing interval. In this actual clinical example, even though serum concentrations of both drugs declined below MICs at 4 h, drug interaction continued to produce a high probability of a no-growth situation out to 6 h (end of interval). We have begun to examine the relationship between these predictions of no growth (i.e., good clinical outcome) and actual outcome in septic neutropenic cancer patients. Early results have been quite promising.

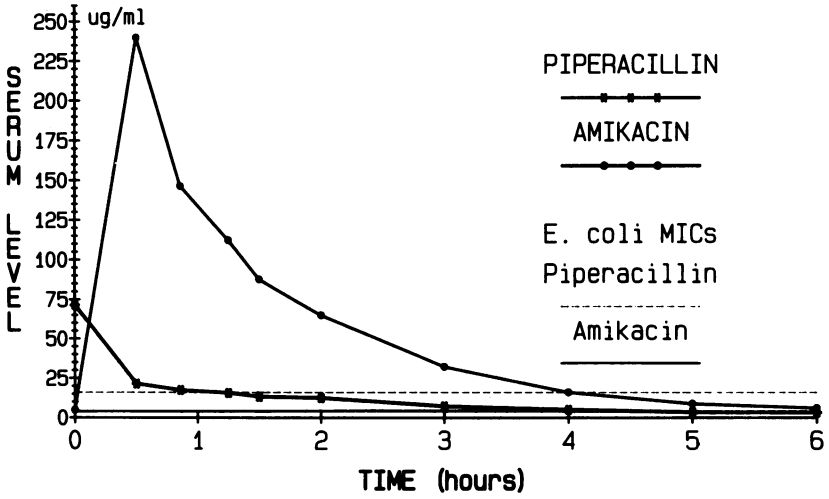

FIG. 6. Steady-state concentration-time profile for piperacillin-amikacin in a granulocytopenic cancer patient bacteremic with *Escherichia coli*. Serum concentrations of both drugs declined below their respective MICs for the *E. coli* (piperacillin MIC, 16 μg/ml; amikacin MIC, 4 μg/ml) at 4 h.

In summary, much work has been done with beta-lactams and aminoglycosides. Beta-lactams should probably be given in doses and schedules which maintain the drug concentration (and perhaps the free drug concentration) in excess of the MIC for the specific pathogen for the entire dosing interval. Possible exceptions to this rule may be penem and carbapenem antibiotics which do possess a PAE against gram-positive and gram-negative organisms. For aminoglycosides, it is less important to maintain serum concentrations above the MIC, and area under the curve in the serum relative to the MIC probably correlates best with outcome. However, some would argue that the more easily measurable peak serum concentration relative to the MIC should be the standard for adjustment of therapy.

With regard to combination therapy, much needs to be investigated. Several tools are available to allow the optimal dose and schedule for combinations of drugs to be elucidated. Whether this will be constant infusion of beta-lactams with intermittent bolus of aminoglycosides or staggered schedules of bolus administration of each drug remains to be seen. Clearly, the in vitro model of Blazer (4) and Dudley et al. (14), the animal models of infection, and the technique of logistic regression will offer insights into optimal modes of therapy for combinations of agents.

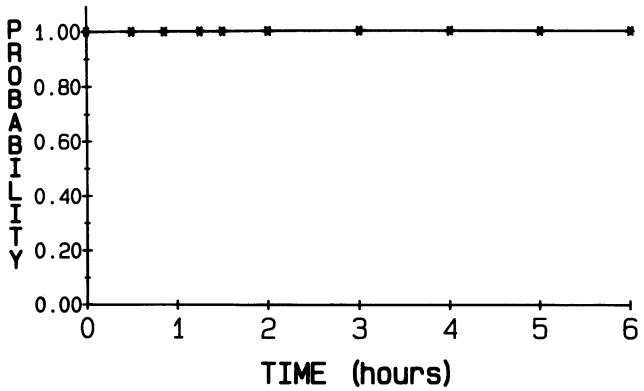

FIG. 7. Plot of the probability of obtaining a no-growth situation in the blood of the patient described in Fig. 6. The probability was calculated by inserting assayed serum values into equation 1. Coefficients were determined by analysis of a checkerboard plate with the patient's *E. coli*.

LITERATURE CITED

1. **Andersen, E. T., L. S. Young, and W. L. Hewitt.** 1976. Simultaneous antibiotic levels in "breakthrough" Gram-negative rod bacteremia. Am. J. Med. **61**:493–497.
2. **Barriere, S. L., E. Ely, J. E. Kapusnik, and J. G. Gambertoglio.** 1985. Analysis of a new method for assessing activity of combinations of antimicrobials: area under the bactericidal activity curve. J. Antimicrob. Chemother. **16**:49–59.
3. **Barza, M., and G. Cuchural.** 1985. General principles of antibiotic tissue penetration. J. Antimicrob. Chemother. **15**:59–75.
4. **Blaser, J.** 1985. In vitro model for simultaneous simulation of the serum kinetics of two drugs with different half-lives. J. Antimicrob. Chemother. **15**:125–130.
5. **Bodey, G. P., S. J. Ketchel, and V. Rodriguez.** 1979. A randomized study of carbenicillin plus cefamandole or tobramycin in the treatment of febrile episodes in cancer patients. Am. J. Med. **67**:608–616.
6. **Bundtzen, R. W., A. U. Gerber, D. L. Cohn, and W. A. Craig.** 1981. Postantibiotic suppression of bacterial growth. Rev. Infect. Dis. **3**:28–37.
7. **Bustamante, C. I., G. L. Drusano, B. A. Tatem, and H. C. Standiford.** 1984. Postantibiotic effect of imipenem on *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **26**:678–682.
8. **Calain, P., K. H. Krause, P. Vaudaux, R. Auckenthaler, D. Lew, F. Waldvogel, and B. Hirschel.** 1987. Early termination of a prospective, randomized trial comparing teicoplanin and flucloxacillin for treating severe staphylococcal infections. J. Infect. Dis. **155**:187–191.
9. **Chambers, H. F., J. Mills, T. A. Drake, and M. A. Sande.** 1984. Failure of a once-daily regimen of cefonicid for treatment of endocarditis due to *Staphylococcus aureus*. Rev. Infect. Dis. **6**(Suppl. 4):S870–S874.
10. **Drusano, G. L., P. A. Ryan, H. C. Standiford, M. R. Moody, and S. C. Schimpff.** 1984. Integration of selected pharmacologic and microbiologic properties of three new β-lactam antibiotics: a hypothesis for rational comparison. Rev. Infect. Dis. **3**:357–363.
11. **Drusano, G. L., H. C. Standiford, C. Bustamante, A. Forrest, G. Rivera, J. Leslie, B. Tatem, D. Delaportas, R. R. MacGregor, and S. C. Schimpff.** 1984. Multiple-dose pharmacokinetics of imipenem-cilastatin. Antimicrob. Agents Chemother. **26**:715–721.
12. **Drusano, G. L., H. C. Standiford, B. Fitzpatrick, J. Leslie, P.**

Tangtatsawasdi, P. Ryan, B. Tatem, M. R. Moody, and S. C. Schimpff. 1984. Comparison of the pharmacokinetics of ceftazidime and moxalactam and their microbiological correlates in volunteers. Antimicrob. Agents Chemother. **26**:388–393.

13. **Drusano, G. L., H. C. Standiford, P. Ryan, W. McNamee, B. Tatem, and S. Schimpff.** 1986. Correlation of predicted serum bactericidal activities and values measured in volunteers. Eur. J. Clin. Microbiol. **5**:88–92.
14. **Dudley, M. N., H. D. Mandler, D. Gilbert, J. Ericson, K. H. Mayer, and S. H. Zinner.** 1987. Pharmacokinetics and pharmacodynamics of intravenous ciprofloxacin. Am. J. Med. **82**:363–368.
15. **Gerber, A. U., W. A. Craig, H. P. Brugger, C. Feller, A. P. Vastola, and J. Brandel.** 1983. Impact of dosing intervals on activity of gentamicin and ticarcillin against Pseudomonas aeruginosa in granulocytopenic mice. J. Infect. Dis. **147**:910–917.
16. **Gerber, A. U., and C. Feller-Segessenmann.** 1985. In vivo assessment of in vitro killing patterns of Pseudomonas aeruginosa. J. Antimicrob. Chemother. **15**:201–206.
17. **Gerber, A. U., A. P. Vastola, J. Brandel, and W. A. Craig.** 1982. Selection of aminoglycoside-resistant variants of Pseudomonas aeruginosa in an in vivo model. J. Infect. Dis. **146**:691–697.
18. **Gerber, A. U., P. Wiprachtiger, U. Stettler-Spichiger, and G. Lebek.** 1982. Constant infusion vs. intermittent doses of gentamicin against Pseudomonas aeruginosa in vitro. J. Infect. Dis. **145**:554–560.
19. **Gerding, D. N., L. R. Peterson, J. A. Moody, and C. E. Fasching.** 1985. Mezlocillin, ceftizoxime, and amikacin alone and in combination against six Enterobacteriaceae in a neutropenic site in rabbits. J. Antimicrob. Chemother. **15**:207–219.
20. **Hoffstedt, B., and M. Walder.** 1981. Influence of serum protein binding and mode of administration on penetration of five cephalosporins into subcutaneous tissue fluid in humans. Antimicrob. Agents Chemother. **20**:783–786.
21. **Johnson, D. E., and B. Thompson.** 1986. Efficacy of single-agent therapy with azlocillin, ticarcillin, and amikacin and beta-lactam/amikacin combinations for treatment of *Pseudomonas aeruginosa* bacteremia in granulocytopenic rats. Am. J. Med. **80**:53–58.
22. **Johnson, D. E., B. Thompson, and F. M. Calia.** 1985. Comparative activities of piperacillin, ceftazidime, and amikacin, alone and in all possible combinations, against experimental *Pseudomonas aeruginosa* infections in neutropenic rats. Antimicrob. Agents Chemother. **28**:735–739.
23. **Keating, M. J., G. P. Bodey, M. Valdivieso, and V. Rodriguez.** 1979. A randomized comparative trial of three aminoglycosides—comparison of continuous infusions of gentamicin, amikacin and sisomicin combined with carbenicillin in the treatment of infections in neutropenic patients with malignancies. Medicine (Baltimore) **58**:159–170.
24. **Klastersky, J., D. Daneau, G. Swings, and D. Weerts.** 1974. Antibacterial activity in serum and urine as a therapeutic guide in bacterial infections. J. Infect. Dis. **129**:187–193.
25. **Klastersky, J., M. P. Glauser, S. C. Schimpff, S. H. Zinner, H. Gaya, and the European Organization for Research on Treatment of Cancer Antimicrobial Therapy Project Group.** 1986. Prospective randomized comparison of three antibiotic regimens for empirical therapy of suspected bacteremic infection in febrile granulocytopenic patients. Antimicrob. Agents Chemother. **29**:263–270.
26. **Lang, S. D. R., G. L. Cameron, and P. R. Mullins.** 1981. Anomalous effect of serum on the antimicrobial activity of cefoperazone. Drugs **22**:52–59.
27. **Merrikin, D. J., J. Briant, and G. N. Rolinson.** 1983. Effect of protein binding on antibiotic activity in vivo. J. Antimicrob. Chemother. **11**:233–238.
28. **Moore, R. D., P. S. Lietman, and C. R. Smith.** 1987. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. J. Infect. Dis. **155**:93–99.
29. **Moore, R. D., C. R. Smith, and P. S. Lietman.** 1984. The association of aminoglycoside plasma levels with mortality in patients with Gram-negative bacteremia. J. Infect. Dis. **149**: 443–448.
30. **Moore, R. D., C. R. Smith, and P. S. Lietman.** 1984. Association of aminoglycoside plasma levels with therapeutic outcome in Gram-negative pneumonia. Am. J. Med. **77**:657–662.
31. **Noone, P., T. M. C. Parsons, J. R. Pattison, R. C. B. Slack, and D. Garfield-Davies.** 1974. Experience in monitoring gentamicin therapy during treatment of serious Gram-negative sepsis. Br. Med. J. **1**:477–481.
32. **Reller, L. B., and C. W. Stratton.** 1977. Serum dilution test for bactericidal activity. II. Standardization and correlation with antimicrobial assays and susceptibility tests. J. Infect. Dis. **136**: 196–204.
33. **Rolinson, G. N.** 1980. Basis and results of therapy with β-lactam antibiotics in experimental infections. Infection **8**:30–34.
34. **Roosendaal, R., I. A. J. M. Bakker-Woudenberg, M. van den Berghe-van Raffe, and M. F. Michel.** 1986. Continuous versus intermittent administration of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia in normal and leukopenic rats. Antimicrob. Agents Chemother. **30**:403–408.
35. **Schentag, J. J., I. L. Smith, D. J. Swanson, C. DeAngelis, J. E. Fracasso, A. Vari, and J. W. Vance.** 1984. Role for dual individualization with cefmenoxime. Am. J. Med. **77**(Suppl. 6a):43–50.
36. **Schimpff, S., W. Satterlee, V. M. Young, and A. Serpick.** 1970. Empiric therapy with carbenicillin and gentamicin for febrile patients with cancer and granulocytopenia. N. Engl. J. Med. **284**:1061–1065.
37. **Sculier, J. P., and J. Klastersky.** 1984. Significance of serum bactericidal activity in Gram-negative bacillary bacteremia in patients with and without granulocytopenia. Am. J. Med. **76**: 429–435.
38. **Standiford, H. C., G. L. Drusano, C. I. Bustamante, G. Rivera, A. Forrest, B. Tatem, J. Leslie, and M. Moody.** 1986. Imipenem coadministered with cilastatin compared with moxalactam: integration of serum pharmacokinetics and microbiologic activity following single-dose administration to normal volunteers. Antimicrob. Agents Chemother. **29**:412–417.
39. **Standiford, H. C., G. L. Drusano, W. B. McNamee, B. Tatem, P. A. Ryan, and S. C. Schimpff.** 1982. Comparative pharmacokinetics of moxalactam, cefoperazone, and cefotaxime in normal volunteers. Rev. Infect. Dis. **4**:585–594.
40. **Tompsett, R., S. Shultz, and W. McDermott.** 1947. The relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, dihydro F, and K. J. Bacteriol. **53**:581–595.
41. **Viollier, A. F., H. C. Standiford, G. L. Drusano, B. A. Tatem, M. R. Moody, and S. C. Schimpff.** 1985. Comparative pharmacokinetics and serum bactericidal activity of mezlocillin, ticarcillin and piperacillin, with and without gentamicin. J. Antimicrob. Chemother. **15**:597–606.
42. **Vogelman, B. S., and W. A. Craig.** 1985. Postantibiotic effects. J. Antimicrob. Chemother. **15**:37–46.
43. **Wade, J. C., S. C. Schimpff, K. A. Newman, C. L. Fortner, H. C. Standiford, and P. H. Wiernik.** 1981. Piperacillin or ticarcillin plus amikacin—a double blind prospective comparison of empiric antibiotic therapy for febrile granulocytopenic cancer patients. Am. J. Med. **71**:984–990.
44. **Warren, J. W., E. H. Miller, Jr., B. Fitzpatrick, D. E. DiFranco, E. S. Caplan, J. H. Tenney, and W. C. Anthony.** 1983. A randomized, controlled trial of cefoperazone vs. cefamandole-tobramycin in the treatment of putative, severe infections with Gram-negative bacilli. Rev. Infect. Dis. **5**:173.
45. **Wise, R.** 1985. The relevance of pharmacokinetics to in vitro models: protein binding—does it matter? J. Antimicrob. Chemother. **15**:77–83.
46. **Wise, R., I. A. Donovan, N. S. Ambrose, and J. E. Allcock.** 1981. The penetration of cefoxitin into peritoneal fluid. J. Antimicrob. Chemother. **8**:435–437.
47. **Wise, R., A. P. Gillett, B. Cadge, S. R. Durham, and S. Baker.** 1980. The influence of protein binding upon tissue fluid levels of six β-lactam compounds. J. Infect. Dis. **142**:77–82.