

# Efficacy of Ampicillin-Sulbactam Is Not Dependent upon Maintenance of a Critical Ratio between Components: Sulbactam Pharmacokinetics in Pharmacodynamic Interactions

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**An in vitro pharmacokinetic model (IVPM) and a mouse model of lethal bacteremia were used to compare the pharmacodynamics of ampicillin-sulbactam when the two components were dosed simultaneously and in sequence against TEM-1-producing *Escherichia coli*. The challenge isolates included three strains of *E. coli* producing various levels of  $\beta$ -lactamase. Human pharmacokinetics of ampicillin-sulbactam (1.5- and 3.0-g intravenous doses) were simulated in each model, and pharmacodynamic interactions were evaluated over one 6-h dosing interval. Against all three strains, the sequential dosing of sulbactam prior to ampicillin did not alter the pharmacodynamics of these combinations from comparison with results obtained with the simultaneous administration of the two components. Similar pharmacodynamics were observed for the two dosing regimens regardless of the ampicillin-sulbactam dose used or whether the bacteria were treated in an immunocompetent mouse or in the absence of immune defenses in the IVPM. When antibacterial activity was lost and regrowth of the inoculum was observed, viable bacterial counts increased in both the simultaneous and sequential regimens at a point when sulbactam levels fell below a critical concentration. These data suggest that the efficacy of ampicillin-sulbactam is not dependent upon the maintenance of a constant 2:1 ratio for the two components. Rather, the efficacy of ampicillin-sulbactam appears to be dependent upon the maintenance of one or both components above a critical concentration. Furthermore, the pharmacokinetics of sulbactam, specifically, how long sulbactam levels remain above a minimum critical concentration, appears to dictate how long antibacterial activity is maintained with the combination.**

*Escherichia coli* is the leading cause of gram-negative bacteremia in both community-acquired and nosocomial settings (9, 10, 19). Among ampicillin-resistant isolates of this pathogen, the most common mechanism of resistance is the production of plasmid-mediated  $\beta$ -lactamases, especially those of the TEM-1 and TEM-2 classes (17). Since 1984, these two enzymes have been reported in 61 to 98% of ampicillin-resistant *E. coli*. One approach used to circumvent this resistance problem is to combine ampicillin with sulbactam, an inhibitor of the  $\beta$ -lactamase.

Current therapeutic practice with ampicillin-sulbactam, as with other  $\beta$ -lactam- $\beta$ -lactamase-inhibitor combinations, is to dose the two components simultaneously. For ampicillin-sulbactam, the two components are dosed simultaneously at a ratio of 2:1 (ampicillin to sulbactam). Because of their similar pharmacokinetics in humans, these two components theoretically maintain a 2:1 ratio at the site of infection over the entire dose interval. To date, the optimal dosing and utilization of  $\beta$ -lactamase-inhibitor- $\beta$ -lactam combinations have not been extensively studied. Cavalieri et al. previously reported that the sequential dosing of sulbactam prior to cefoperazone and ticarcillin in mice enhanced the activities of these combinations against some strains of *Enterobacter cloacae* and *Serratia marcescens* producing inducible group 1  $\beta$ -lactamases (5). This increase in efficacy was seen despite the fact that group 1  $\beta$ -lactamases are the least susceptible of all  $\beta$ -lactamases to inhibition by sulbactam. It is possible that the increased efficacy observed with sequential dosing of sulbactam prior to cefoperazone or ticarcillin reflected the increased penetration of

each drug through the gram-negative outer membrane, which would occur in this setting but not with simultaneous administration of both components. In the latter setting, competition between the two components for entry into the cells would be maximized.

The purpose of this study was to determine if sequential dosing of sulbactam prior to ampicillin would affect the bactericidal activity of this combination against TEM-1-producing *E. coli*. Pharmacodynamic interactions between ampicillin-sulbactam and *E. coli* were evaluated using an in vitro pharmacokinetic model (IVPM) and a mouse model of lethal bacteremia, both of which simulated the human pharmacokinetics of ampicillin-sulbactam after 1.5- and 3.0-g intravenous doses. The data generated in such studies should begin to address the issue of the relative importance of each component in efficacy of the combination.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** Two clinical isolates, *E. coli* SC1359 and *E. coli* 149, were chosen for this study on the basis of their levels of TEM-1  $\beta$ -lactamase production, susceptibility to ampicillin-sulbactam, and virulence in mice. A third strain, *E. coli* AFE-MV1, was a laboratory strain which hyperproduces TEM-1 from a recombinant plasmid. All three isolates were stored at  $-70^{\circ}\text{C}$  in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 50% sterile horse serum. Strain purity was confirmed by subculturing onto Trypticase soy agar supplemented with 5% sheep blood (BAP; BBL). Strain AFE-MV1 was also subcultured onto Mueller-Hinton agar (MHA; BBL) supplemented with 50  $\mu\text{g}$  of ampicillin per ml for plasmid maintenance.

For in vitro pharmacodynamic studies, logarithmic-phase cultures were prepared by inoculating colonies from an overnight BAP culture into 70 ml of Mueller-Hinton broth (MHB; BBL) to equal an optical density at 540 nm of 0.1. The broth culture was then incubated at  $37^{\circ}\text{C}$  with shaking for 2 h and diluted in sterile MHB to approximately  $5 \times 10^5$  CFU/ml.

For animal studies, logarithmic-phase cultures were prepared by inoculating colonies from an overnight BAP culture into 70 ml of MHB to equal an optical

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density at 540 nm of 0.1. The broth culture was then incubated at 37°C with shaking for 2 h, and the bacteria were collected by centrifugation at  $8,000 \times g$  for 20 min, washed twice with sterile phosphate buffer ( $\text{KH}_2\text{PO}_4$  at 4.0 and  $\text{K}_2\text{HPO}_4$  at 13.6 g/liter), and resuspended to a concentration of  $\sim 10^9$  CFU/ml in 45 ml of phosphate buffer.

**Ampicillin-sulbactam preparation.** Ampicillin sodium and sulbactam sodium were supplied by Roerig-Pfizer, New York, N.Y. Antibiotic powder was reconstituted with distilled water and sterilized by passage through a 0.20- $\mu\text{m}$ -pore-size Acrodise filter membrane syringe (Gelman Sciences, Ann Arbor, Mich.) and diluted to desired concentrations with sterile water.

**Antimicrobial susceptibility testing and  $\beta$ -lactamase characterization.** All susceptibility testing with ampicillin, sulbactam, and ampicillin-sulbactam was performed by agar dilution according to the procedure recommended by the National Committee for Clinical Laboratory Standards (14). Susceptibility testing with ampicillin-sulbactam was performed using fixed 2:1 and 1:1 ratios of ampicillin to sulbactam, as well as with fixed concentrations of sulbactam (2, 4, 8, 16, 32, and 64  $\mu\text{g}/\text{ml}$ ) combined with various concentrations of ampicillin. Characterization of  $\beta$ -lactamases involved analysis of sonic extracts from cells by isoelectric focusing with TEM-1, TEM-2, and SHV-1 enzymes as standards (18) and measurement of nitrocefin hydrolysis by spectrophotometric assay (16).

**IVPM.** The IVPM used in these studies was a modification of the original model described by Blaser et al. (1). A hollow-fiber cartridge (Amicon, Inc., Beverly, Mass.) was connected by a continuous loop of silicone tubing to a central reservoir. At the start of each experiment, peak ampicillin-sulbactam concentrations in MHB in the central reservoir were pumped through the hollow fibers of the cartridge and back into the central reservoir. As drug-containing MHB passed through the hollow fibers, pores in the fiber walls allowed diffusion of ampicillin-sulbactam from the lumen of the fibers into the peripheral compartment of the cartridge. Comparison of ampicillin and sulbactam levels in the peripheral compartment with those in the central reservoir at 15-min intervals after dosing demonstrated that equilibrium between the two compartments was achieved at approximately 1 h (data not shown). The exclusion size of the pores in the fiber walls prohibited bacteria introduced into the peripheral compartment from entering the hollow fibers. Thus, the drug concentration in the peripheral compartment could be altered without disrupting bacterial growth.

Human elimination pharmacokinetics of ampicillin-sulbactam were simulated in the peripheral compartment by a process of dilution and elimination of drug in the central reservoir. The ampicillin-sulbactam concentration in the central reservoir (and peripheral compartment as equilibrium is maintained) was decreased by the addition of drug-free MHB from a dilution reservoir. To maintain a constant volume in the central reservoir, drug-containing MHB was pumped from the central reservoir into an elimination reservoir. The rate at which drug was eliminated from the central reservoir and peripheral compartment by this method was determined by the flow rate of the peristaltic pumps. This rate was calculated from an equation for clearance by monoexponential decline based on the half-life of the ampicillin-sulbactam in humans and the volume of media in the central reservoir (1). An elimination half-life of 1 h for ampicillin-sulbactam was simulated by this method.

**Model of lethal *E. coli* bacteremia in mice.** Bacteremia was established by an intraperitoneal injection of 0.2 ml of logarithmic-phase *E. coli* diluted in 4.5% hog gastric mucin (Sigma Chemical Co., St. Louis, Mo.). To measure virulence of test strains, groups of 10 mice were infected with 10-fold serial dilutions of each strain and the minimum dose required to kill 100% of infected mice ( $\text{MLD}_{100}$ ) was determined 2 days postinfection. All mortality was shown to occur within 48 h of infection.

**Pharmacokinetics of ampicillin-sulbactam in the IVPM.** For studies with the IVPM, peak levels of ampicillin-sulbactam observed in human serum after intravenous administration of 1.5- and 3.0-g doses were introduced into the central compartment of the IVPM. The range of peak levels of ampicillin and sulbactam desired were 40 to 71 and 21 to 40  $\mu\text{g}/\text{ml}$ , respectively, when the 1.5-g dose was simulated (4, 7, 15). The corresponding ranges for the 3.0-g dose were 109 to 150  $\mu\text{g}/\text{ml}$  for ampicillin and 48 to 88  $\mu\text{g}/\text{ml}$  for sulbactam. For simultaneous dosing, both ampicillin and sulbactam were introduced into the central compartment at 0 h. For sequential dosing, sulbactam was administered at 0 h and ampicillin was 1 h. This sequential dosing strategy created a pharmacokinetic profile in which ampicillin levels in the peripheral compartment were rising to peak concentrations as sulbactam levels were declining from peak concentrations. To measure the levels of ampicillin and sulbactam in the IVPM, samples were removed from the peripheral compartment at 0, 1, 2, 3, 4, and 6 h. Ampicillin and sulbactam concentrations were measured by bioassay with *Bacillus subtilis* and *Acinetobacter calcoaceticus*, respectively. Samples assayed for sulbactam were first treated for 20 min with a Bush group 1 cephalosporinase (type III; Sigma) to inactivate the ampicillin. Preliminary tests indicated that this treatment did not significantly alter sulbactam concentrations.

**Pharmacokinetics of ampicillin-sulbactam in mice.** Human pharmacokinetics were simulated in male CF1 mice (25 to 28 g; Sasco Inc., Omaha, Neb.) by administering two subcutaneous doses, separated by 1 h, of reconstituted ampicillin-sulbactam at 40 mg/kg of body weight (1.5-g human equivalent) or 80 mg/kg (3.0-g human equivalent) as previously described (12). These doses reflect the ampicillin content of the dose. On the basis of the 2:1 ratio of ampicillin to sulbactam in parenteral preparations of this combination, the corresponding doses of sulbactam administered were 20 and 40 mg/kg, respectively. For se-

quential dosing regimens, sulbactam was administered subcutaneously 0.25 h prior to ampicillin so that ampicillin levels were rising to peak concentrations as sulbactam levels were declining from peak concentrations. At 0.25-h intervals after the first dose, mice were anesthetized with ethyl ether (Aldrich Chemical Company, Inc., Milwaukee, Wis.) and blood was obtained by direct cardiac puncture using a 1-ml tuberculin syringe and 26-gauge needle. Blood samples were allowed to stand undisturbed at room temperature for 30 min until clotted. Sera were then collected by centrifugation at  $1,300 \times g$  in a microcentrifuge and assayed by bioassay as described above.

**Pharmacodynamics of ampicillin-sulbactam against *E. coli* in an IVPM.** Logarithmic-phase cultures ( $5 \times 10^5$  to  $1 \times 10^6$  CFU/ml) of *E. coli* SC1359, *E. coli* 149, and *E. coli* AFE-MV1 were introduced into the peripheral compartment of the IVPM and were exposed to ampicillin-sulbactam as described above. As a control, cultures were also exposed to ampicillin alone, with peak levels and elimination kinetics mimicking those observed with both the 1.5- and 3.0-g doses of ampicillin-sulbactam. At 0, 1, 2, 4, and 6 h, 400  $\mu\text{l}$  of samples taken from the peripheral compartment of the IVPM was treated for 15 min at 37°C with 100  $\mu\text{l}$  of concentrated penicillinase from culture supernatants of *Bacillus cereus* (BBL) to inactivate ampicillin. Viable bacterial counts were measured by plating serial 10-fold dilutions of each sample into MHA (BBL). The least-diluted sample plated was 1 ml of undiluted sample from the peripheral compartment. Since 30 colonies is the lower limit of accurate quantitation by pour plate methodology, the lowest number of bacteria that could be accurately counted (limit of accurate counts) was 30 CFU/ml. The lowest level of detection, although actual counts were inaccurate, was 1 CFU/ml.

Samples taken at 1 h after dosing (observed pharmacokinetic peak) were filter sterilized to remove bacteria, and ampicillin and sulbactam concentrations were measured by bioassay to ensure that peak levels were within the desired range. Preliminary experiments indicated that the pharmacokinetics of ampicillin and sulbactam in the peripheral compartment were unaffected by the introduction of the challenge strains into the IVPM, except at time points when bacterial counts exceeded  $10^7$  CFU/ml. Therefore, drug levels were not measured at other time points during pharmacodynamic experiments.

**Efficacy of ampicillin-sulbactam in treatment of *E. coli* bacteremia in mice.** Prior to each experiment, all mice were weighed and divided into groups (untreated controls, simultaneous 40-mg/kg regimen, simultaneous 80-mg/kg regimen, sequential 40-mg/kg regimen, and sequential 80-mg/kg regimen) so that the mice in the treatment groups were equal in weight within  $\pm 1$  g. All mice were infected intraperitoneally with the observed  $\text{MLD}_{100}$  for each strain, and at 1 h postinfection, the simultaneous treatment groups received their first doses of ampicillin-sulbactam. This marked time 0 h of the experiment. The simultaneous treatment groups then received their second dose of ampicillin-sulbactam at 1 h. Mice in the sequential treatment group received their first dose of sulbactam at 0.75 h postinfection ( $-0.25$  h on the experimental time line) and their first dose of ampicillin at 1 h post infection (0 h). Second doses of sulbactam and ampicillin were given at 0.75 and 1 h, respectively. At times 0, 0.5, 1, 1.5, 2, 3, 4, and 6 h, two mice from each group were anesthetized with ethyl ether and blood was obtained by direct cardiac puncture. To prevent clotting and to eliminate ampicillin carryover, 100  $\mu\text{l}$  of blood was immediately diluted 10-fold into 800  $\mu\text{l}$  of sterile phosphate buffer plus 100  $\mu\text{l}$  of penicillinase and 100-fold into 9.8 ml of sterile phosphate buffer plus 100  $\mu\text{l}$  penicillinase. Viable bacterial counts were then measured by performing 10-fold serial dilutions from the 100-fold dilute sample and plating 1 ml in duplicate into MHA. Duplicate 1-ml aliquots of the original 10-fold dilute sample were also plated into MHA. The least-diluted sample plated was 1 ml of a 10-fold dilution of blood obtained by cardiac puncture. Since 30 colonies are the lower limit of accurate quantitation by pour plate methodology, the lowest number of bacteria that could be accurately counted (limit of accurate counts) was as follows:  $30 \text{ CFU} \times 10$  (dilution factor) = 300 CFU/ml. The lowest level of detection of bacteremia, although actual counts were inaccurate, was 10 CFU/ml ( $1 \text{ CFU} \times 10$ ). Each experiment was repeated twice to ensure accuracy and to provide results from four mice per datum point. As a control, five mice from each group were observed for 48 h to ensure lethality of the inoculum in untreated controls and to evaluate protection in the treatment groups.

Sera obtained at 0.25 h after dosing (observed pharmacokinetic peak) were filter sterilized to remove bacteria, and ampicillin and sulbactam concentrations were measured by bioassay to ensure that peak levels were within the desired range. Drug levels were not measured at other time points during pharmacodynamic experiments with mice.

## RESULTS

**Characterization of experimental strains.** Strains *E. coli* SC1359 and *E. coli* 149 were clinical isolates which expressed similar degrees of virulence in mice, with an  $\text{MLD}_{100}$  of  $10^6$  CFU per mouse. Strain *E. coli* AFE-MV1 was 100-fold more virulent with an  $\text{MLD}_{100}$  of  $10^4$  CFU per mouse. Table 1 summarizes the  $\beta$ -lactamase activities and susceptibilities of the challenge panel to ampicillin, sulbactam, and ampicillin-sulbactam. Ampicillin resistance ( $\text{MIC} > 256 \mu\text{g}/\text{ml}$ ) in all

TABLE 1.  $\beta$ -Lactamase activities and ampicillin-sulbactam susceptibilities of challenge panel

<i>E. coli</i> strain	$\beta$ -Lactamase activity <sup>a</sup>	MIC ( $\mu$ g/ml) <sup>b</sup>									
		Sulbactam	Ampicillin	Ampicillin + sulbactam (2:1 ratio)	Ampicillin + sulbactam (1:1 ratio)	Ampicillin + sulbactam (2)	Ampicillin + sulbactam (4)	Ampicillin + sulbactam (8)	Ampicillin + sulbactam (16)	Ampicillin + sulbactam (32)	Ampicillin + sulbactam (64)
SC1359	19	32	>256	8/4	4/4	16/2	4/4	0.25/8	<0.06/16	<0.06/32	<0.06/64
149	75	128	>256	16/8	8/8	>256/2	16/4	1/8	0.12/16	<0.06/32	<0.06/64
AFE-MV1	972	128	>256	128/64	64/64	>256/2	>256/4	>256/8	>256/16	>256/32	4/64

<sup>a</sup> TEM-1 was the only plasmid-mediated  $\beta$ -lactamase detected in these strains.  $\beta$ -lactamase activity was measured in nanomoles of nitrocefin hydrolyzed per milligram of protein.

<sup>b</sup> For ampicillin-sulbactam, MIC procedures were performed with fixed ratios of 2:1 and 1:1 (ampicillin to sulbactam), or a fixed concentration of 2, 4, 8, 16, 32, or 64  $\mu$ g of sulbactam per ml was combined with standard twofold serial dilutions of ampicillin.

three strains was mediated through the production of a TEM-1-like  $\beta$ -lactamase, and  $\beta$ -lactamase activity correlated with ampicillin-sulbactam MICs (Table 1). No other  $\beta$ -lactamases were detected in these strains. When ampicillin-sulbactam MICs obtained with a fixed 2:1 ratio were compared with MICs obtained with a fixed 1:1 ratio, the ampicillin component was twofold higher in the former, whereas the sulbactam component remained constant for both methods (Table 1). This suggested that the sulbactam component was more important in determining the MIC than the ampicillin component and that the minimal critical concentration (MCC) of sulbactam was identified by both tests. From the data in Table 1, it appears that the sulbactam MCC was 4  $\mu$ g/ml for *E. coli* SC1359, 8  $\mu$ g/ml for *E. coli* 149, and 64  $\mu$ g/ml for *E. coli* AFE-MV1. In tests using a fixed concentration of sulbactam with various twofold dilutions of ampicillin, the ampicillin component increased significantly when the concentration of sulbactam was just twofold below the MCC and decreased significantly when the concentration of sulbactam was just twofold above the MCC.

#### Pharmacokinetics of ampicillin-sulbactam in the IVPM.

The pharmacokinetic profiles of ampicillin-sulbactam (3.0 and 1.5 g) dosed simultaneously and sequentially into the IVPM are shown in Fig. 1. Peak levels of ampicillin and sulbactam (mean  $\pm$  standard error [SE]) achieved in the peripheral compartment of the IVPM when the 3.0-g dose of ampicillin-sulbactam was simulated were 121  $\pm$  2 and 54  $\pm$  4  $\mu$ g/ml, respectively. Corresponding peak levels for the 1.5-g dose were 64  $\pm$  4  $\mu$ g/ml for ampicillin and 31  $\pm$  3  $\mu$ g/ml for sulbactam. Calculated half-lives (mean  $\pm$  SE) were 58  $\pm$  1 min for ampicillin and 64  $\pm$  3 min for sulbactam.

**Pharmacokinetics of ampicillin-sulbactam in mice.** The pharmacokinetic profiles of ampicillin-sulbactam (3.0 and 1.5 g) dosed simultaneously and sequentially into mice are shown in Fig. 2. Human ampicillin-sulbactam pharmacokinetics were simulated in mice by administering two subcutaneous injections of 40 mg/kg (1.5-g dose) and 80 mg/kg, separated by 1 h as previously described. This protocol produced mean  $\pm$  SE peak ampicillin-sulbactam levels of 79  $\pm$  5–34  $\pm$  4  $\mu$ g/ml with the 40-mg/kg dose and 144  $\pm$  12–72  $\pm$  4  $\mu$ g/ml with the 80-mg/kg dose. The areas under the curve from 0 h to infinity for ampicillin and sulbactam with the 40-mg/kg dose were 81  $\mu$ g  $\cdot$  h/ml and 49  $\mu$ g  $\cdot$  h/ml, respectively. With the 80-mg/kg dose, these values were 160  $\mu$ g  $\cdot$  h/ml for ampicillin and 90  $\mu$ g  $\cdot$  h/ml for sulbactam.

**Pharmacodynamics of ampicillin alone in the IVPM.** The growth of all three challenge strains was unaffected by ampicillin alone when dosed into the IVPM at either concentration used in the ampicillin-sulbactam combinations (data not shown).

**Pharmacodynamics of ampicillin-sulbactam against *E. coli* SC1359.** In studies with *E. coli* SC1359 in the IVPM, the pharmacodynamics observed after sequential dosing of ampicillin-sulbactam were similar to the pharmacodynamics observed when the drugs were dosed simultaneously (Fig. 3A and B).

Although there was a 1-h delay in killing when ampicillin-sulbactam was administered sequentially, viable bacterial counts decreased 4 to 5 logs by 2 h with all four dosing regimens and remained below the limit of accuracy throughout the remainder of the 6-h dosing interval.

In the treatment of lethal *E. coli* SC1359 bacteremia in mice, the pharmacodynamics of ampicillin-sulbactam dosed sequentially were similar to those observed when the combination was dosed simultaneously (Fig. 3C and D). All four dosing regimens rapidly decreased bacterial counts 3 to 4 logs over the first hour of the dosing interval and maintained bacterial counts below the limit of accuracy for the remaining 5 h. All four regimens were 100% protective against lethal infection with *E. coli* SC1359.

**Pharmacodynamics of ampicillin-sulbactam against *E. coli* 149.** In studies with *E. coli* 149 in the IVPM, the pharmacodynamics observed after sequential dosing of ampicillin-sulbactam were similar to the pharmacodynamics observed when the drugs were dosed simultaneously (Fig. 4A and B). With the 1.5-g dose (Fig. 4A), a 1-h delay in killing was followed by a 3- to 4-log decrease in viable bacterial counts by 4 h. Between 4 and 6 h, a 1- to 2-log regrowth of the inoculum was observed with both dosing regimens. In contrast to studies with SC1359, the delay in killing of *E. coli* 149 was observed with both the simultaneous and sequential dosing regimens. With the 3.0-g dose (Fig. 4B), the bactericidal activity observed with both dosing regimens over the first hour of the dosing interval was delayed or diminished compared to the killing of *E. coli* SC1359. Viable bacterial counts then decreased 3 to 4 logs over the next 3 h. Between 4 and 6 h, viable bacterial counts continued to fall in cultures treated with the sequential dosing regimen, whereas a 1-log regrowth of the inoculum was observed in cultures treated with the simultaneous dosing regimen. Viable bacterial counts at 6 h did not differ significantly between the two dosing regimens.

In the treatment of lethal *E. coli* 149 bacteremia in mice, the pharmacodynamics observed with the simultaneous and sequential dosing regimens were similar (Fig. 4C and D). With the 1.5-g dose (Fig. 4C), both simultaneous and sequential dosing of ampicillin-sulbactam decreased bacterial counts 1.5 to 2 logs by 1 h, after an initial 0.5-h delay in killing. Bacteriostasis was then observed over the remainder of the 6-h dosing interval and both dosing regimens were 100% protective against lethal *E. coli* 149 infection. With the 3.0-g dose (Fig. 4D), a similar 0.5-h delay in killing was observed with both dosing regimens, followed by a rapid 2.5 to 3 log decrease in viable bacterial counts by 2 h. Both dosing regimens maintained viable bacterial counts below the limit of accurate

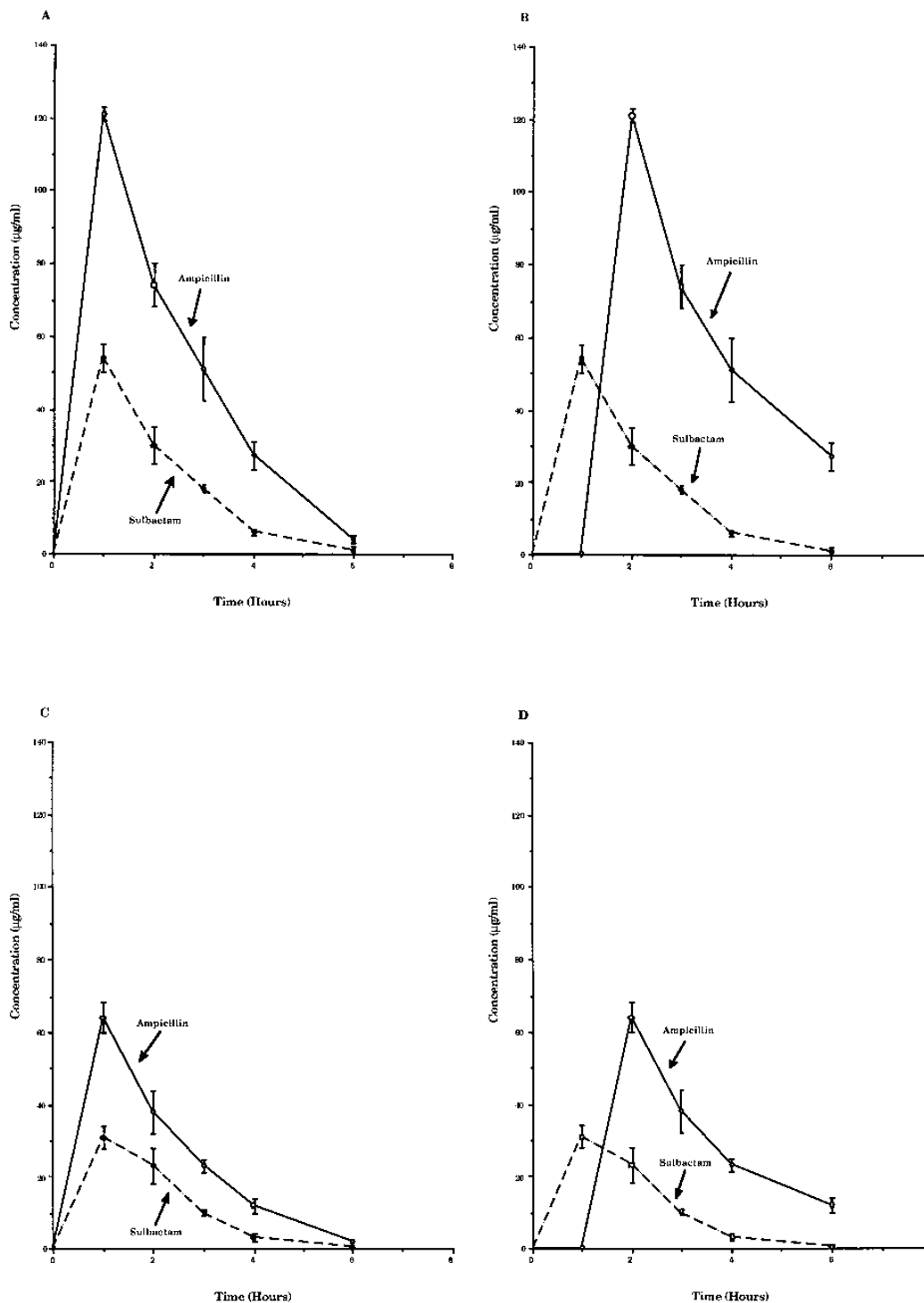


FIG. 1. Pharmacokinetics of ampicillin and sulbactam in the peripheral compartment of the IVPM after dosing peak concentrations of ampicillin-sulbactam into the central reservoir. Simulated 3.0-g dose of ampicillin-sulbactam in the IVPM when dosed simultaneously (A) and in sequence (B); simulated 1.5-g dose of ampicillin-sulbactam in the IVPM when dosed simultaneously (C) and in sequence (D). Drug levels were measured by bioassay. Each datum point represents the mean drug level in the peripheral compartment for four experimental runs. Error bars show SE.

counts over the remainder of the 6-h dosing interval and were 100% protective against the lethal *E. coli* 149 infection.

**Pharmacodynamics of ampicillin-sulbactam against *E. coli* AFE-MV1.** In studies with *E. coli* AFE-MV1 in the IVPM, the pharmacodynamics of ampicillin-sulbactam were similar wheth-

er the combination was dosed simultaneously or in sequence (Fig. 5A and B). With the 1.5-g dose (Fig. 4A), neither dosing regimen was active, and viable bacterial counts increased at a rate similar to those for the growth control. With the 3.0-g dose (Fig. 5B), a 1-h delay in killing was followed by a slight 1-log

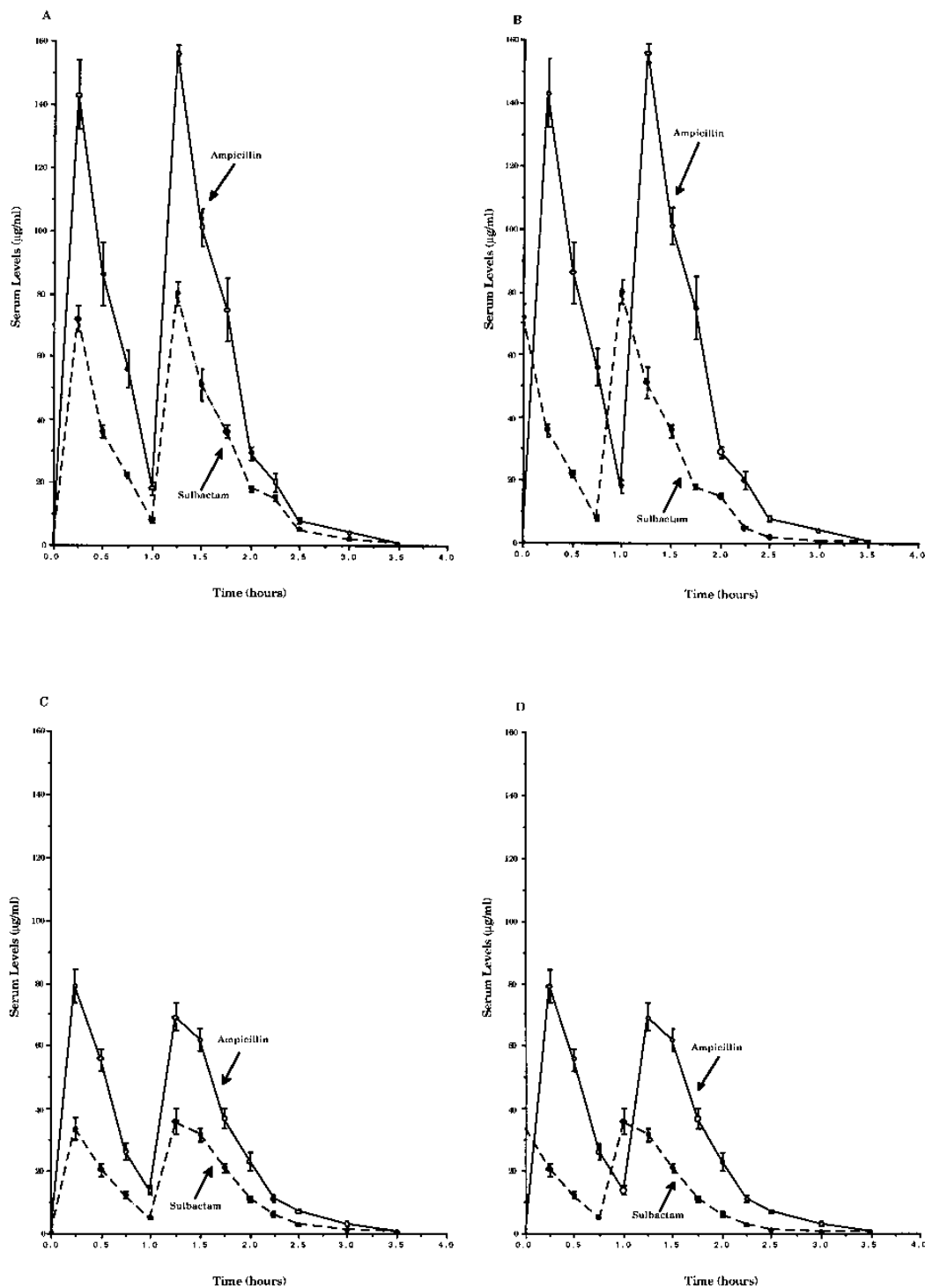


FIG. 2. Pharmacokinetics of ampicillin and sulbactam in mice. Simulated 3.0-g dose of ampicillin-sulbactam in mice when dosed simultaneously (A) and in sequence (B); simulated 1.5-g dose of ampicillin-sulbactam in mice when dosed simultaneously (C) and in sequence (D). Drug levels were measured by bioassay. Each datum point represents the mean serum drug level for four mice. Error bars show SE.

reduction in viable counts by 2 h. Between 2 and 6 h, a 2- to 2.5-log increase in bacterial counts was observed with both dosing regimens.

In the treatment of lethal *E. coli* AFE-MV1 bacteremia in mice, the pharmacodynamics of ampicillin-sulbactam dosed sequentially were similar to those observed when the combi-

nation was dosed simultaneously (Fig. 5C and D). With the 1.5-g dose (Fig. 5C), a 0.5-h delay in bacterial killing was followed by a 2-log decrease in viable bacteria counts through 3 h. Bacteriostasis was maintained between 2 and 4 h, at which time a 1- to 1.5-log increase in bacterial counts was observed through 6 h. Both dosing regimens were 100% protective

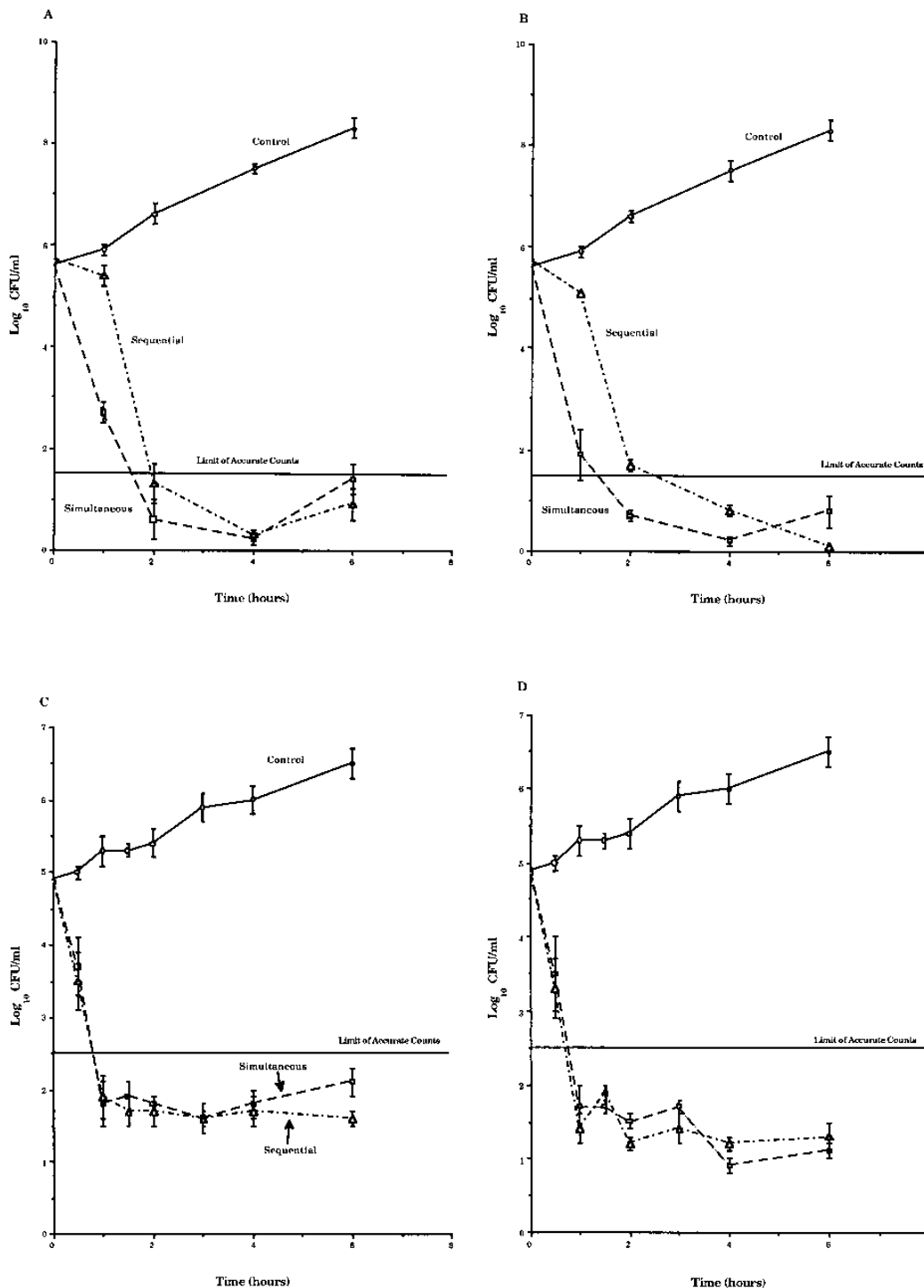


FIG. 3. Time-kill pharmacodynamics of simultaneous versus sequential dosing of ampicillin-sulbactam against *E. coli* SC1359. Pharmacodynamics of ampicillin-sulbactam at 1.5 g (A) and 3.0 g (B) in the IVPM; pharmacodynamics of ampicillin-sulbactam at 1.5 g (C) and 3.0 g (D) in mice. Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment of duplicate IVPM runs or blood from four mice. Error bars show SE.

against lethal *E. coli* AFE-MV1 infection. With the 3.0-g dose (Fig. 5D), a 0.5-h delay in killing was followed by a rapid 2-log decrease in viable bacterial counts. Bacteriostasis was maintained through the remainder of the 6-h dosing interval with both dosing regimens. Both dosing regimens were 100% protective against lethal *E. coli* AFE-MV1 infection.

## DISCUSSION

An IVPM and a mouse model of lethal bacteremia were used to compare the pharmacodynamics of simultaneous and sequential dosing of ampicillin-sulbactam against TEM-1-producing *E. coli*. In experiments with all three challenge strains in

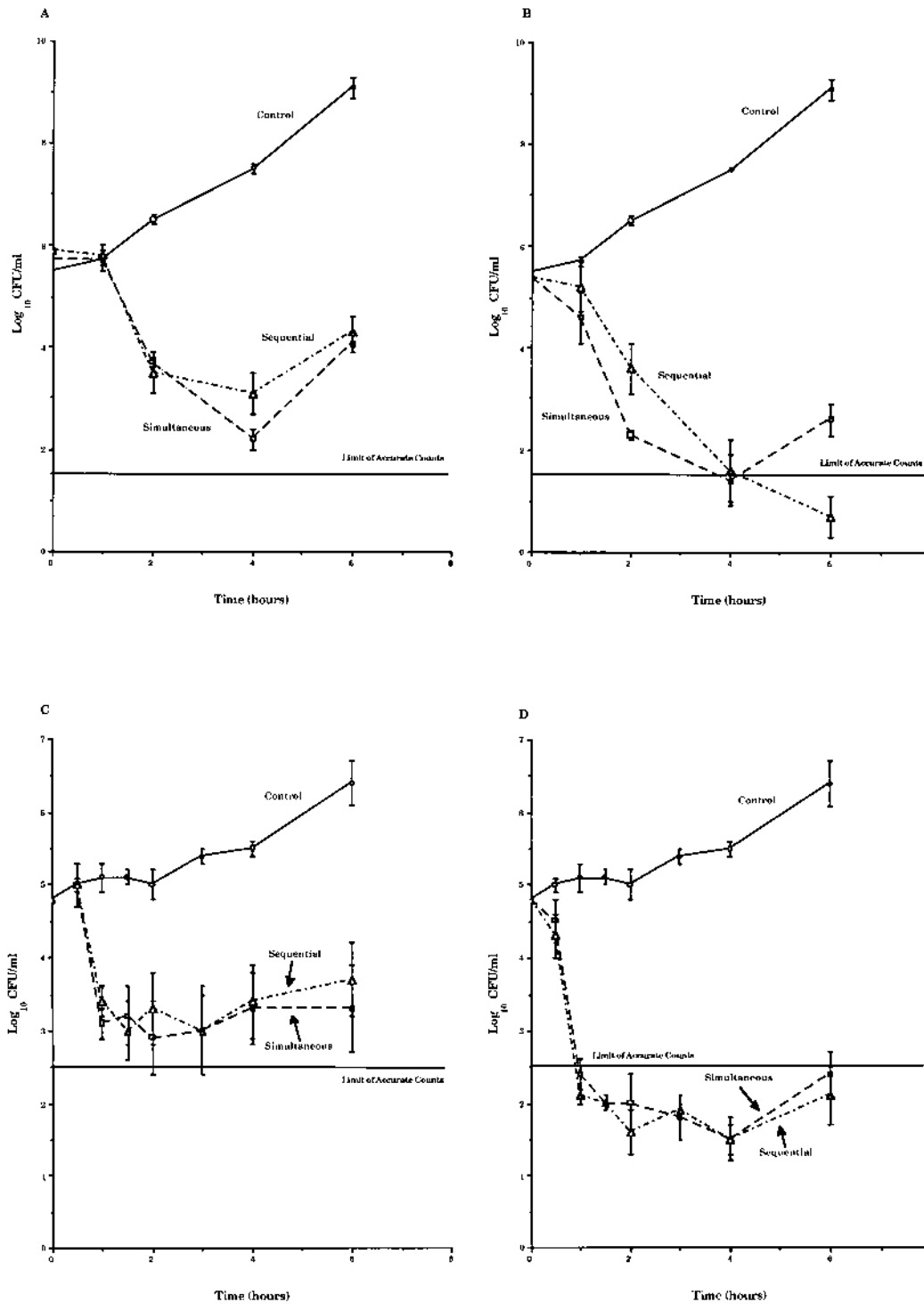


FIG. 4. Time-kill pharmacodynamics of simultaneous versus sequential dosing of ampicillin-sulbactam against *E. coli* 149. Pharmacodynamics of ampicillin-sulbactam at 1.5 g (A) and 3.0 g (B) in the IVPM; pharmacodynamics of ampicillin-sulbactam at 1.5 g (C) and 3.0 g (D) in mice. Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment of duplicate IVPM runs or blood from four mice. Error bars show SE.

the IVPM, the pharmacodynamics observed when sulbactam was dosed prior to ampicillin were similar to those observed when the combination was dosed simultaneously. The only significant difference between the two dosing regimens was the 1-h delay in bacterial killing observed with the sequential reg-

imen against *E. coli* SC1359. This delay in bacterial killing was not unexpected, however, since ampicillin was not dosed into the system until 1 h, after which viable bacterial counts fell rapidly to levels comparable with those in cultures treated with the simultaneous regimen. Delayed killing of *E. coli* SC1359

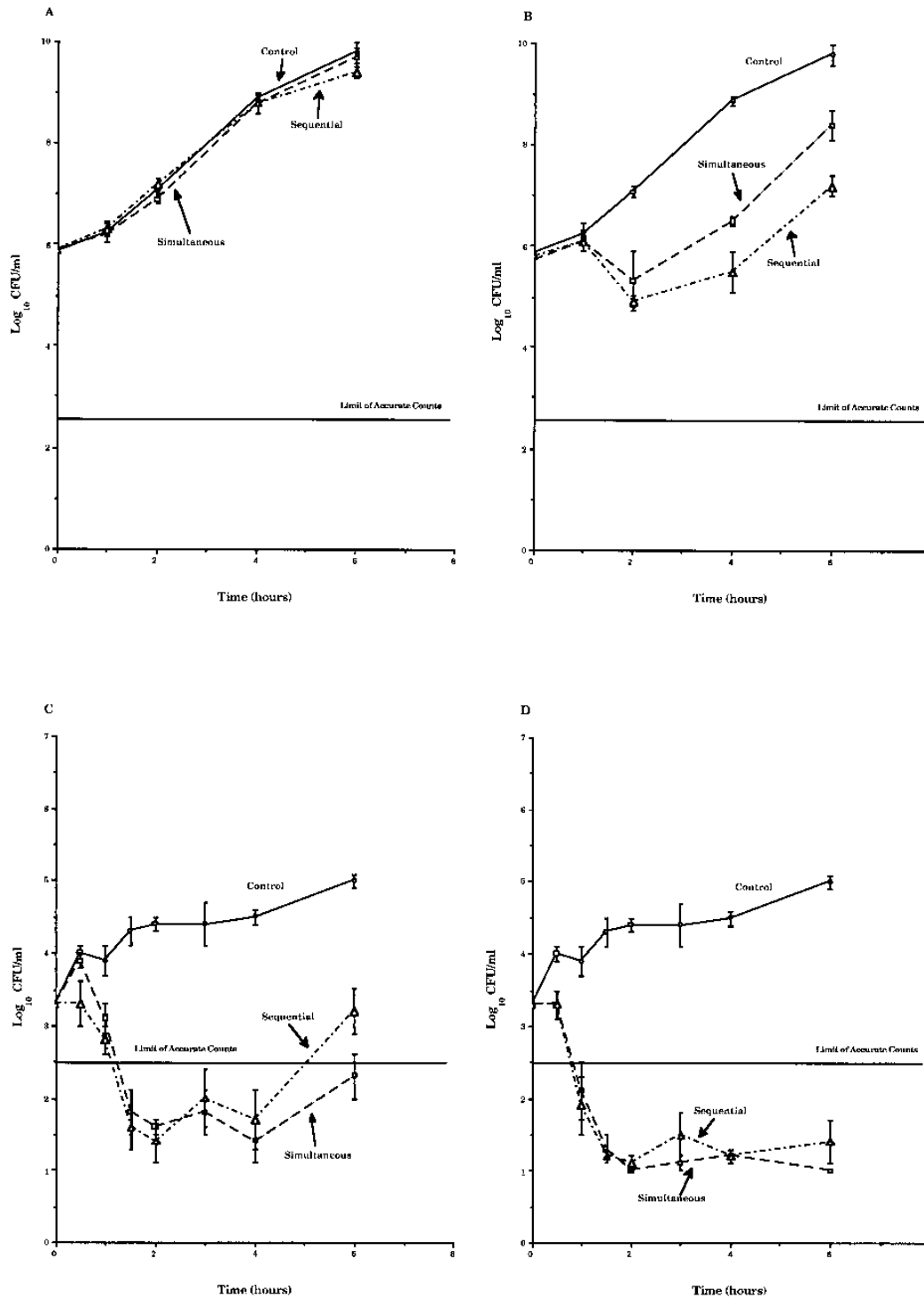


FIG. 5. Time-kill pharmacodynamics of simultaneous versus sequential dosing of ampicillin-sulbactam against *E. coli* AFE-MV1. Pharmacodynamics of ampicillin-sulbactam at 1.5 g (A) and 3.0 g (B) in the IVPM; pharmacodynamics of ampicillin-sulbactam at 1.5 g (C) and 3.0 g (D) in mice. Each datum point represents the mean CFU per milliliter of MHB from the peripheral compartment of duplicate IVPM runs or blood from four mice. Error bars show SE.

was not observed in the mouse model. However, this difference was most likely a reflection of the difference between the protocols used for sequential dosing of ampicillin-sulbactam in the two models. For sequential dosing regimens in the IVPM, ampicillin was not dosed into the IVPM until 1 h. For sequential dosing in mice, ampicillin was dosed at 0 h. Therefore, in

contrast to studies in the IVPM, *E. coli* SC1359 had already been exposed to peak levels of both ampicillin and sulbactam by 0.25 h in mice, and thus a delay in bactericidal activity would not have been detected.

In experiments with *E. coli* 149 and *E. coli* AFE-MV1, a similar delay in bacterial killing was observed over the first

hour in the IVP. In contrast to results with *E. coli* SC1359, however, the delay in bacterial killing was also observed with the simultaneous regimen in the IVP and over the first 0.5 h in mice with both regimens. The reason for the delayed killing of *E. coli* 149 and *E. coli* AFE-MV1 with the simultaneous regimens in the IVP and all regimens in the mouse model is unknown. However, it is possible that this delay in killing reflects the increased levels of TEM-1 production in these strains compared to *E. coli* SC1359. Enzyme kinetic studies with sulbactam and a closely related enzyme, TEM-2, have demonstrated that approximately 7,000 molecules of sulbactam must be hydrolyzed before a molecule of enzyme is inactivated (3). As the level of enzyme produced increases, the number of sulbactam molecules required in the periplasmic space also increases substantially. Without a change in outer membrane permeability, it is not surprising that a longer period would be required to achieve enzyme-inactivating levels of sulbactam in *E. coli* 149 and *E. coli* AFE-MV1, and thus a delay in the bactericidal activity of ampicillin would be expected.

Although sequential dosing of ampicillin 1 h after sulbactam did not enhance bacterial killing in these studies, sequential dosing did not diminish bactericidal activity either. This is an important observation, since bacteria in the sequential dosing regimen studies were exposed to a constant fluctuation in the ratio of ampicillin to sulbactam, in contrast to a fixed 2:1 ratio of ampicillin to sulbactam after simultaneous dosing. This was especially true during the initial 2 h, when maximum bactericidal activity was observed. These data suggest that the activity of ampicillin-sulbactam is not dependent upon the maintenance of a critical ratio between the components but rather is dependent upon the maintenance of both components above critical concentrations. This conclusion is supported by comparative MIC data from this study, in which MICs of ampicillin-sulbactam against the challenge panel did not differ more than twofold when fixed 2:1 and 1:1 ratios were tested. In a more expanded MIC study, Bradford and Sanders demonstrated that MICs of ampicillin-sulbactam against a panel of  $\beta$ -lactamase-producing gram-negative bacteria differed, on average, less than 2-fold whether the MICs were performed with ratios of 2:1 or 1:1 or with fixed sulbactam concentrations of 4 or 8  $\mu\text{g/ml}$  (2).

Which component of ampicillin-sulbactam is most critical for bactericidal activity is unknown. However, it is apparent from these studies that both must be above a critical concentration for the combination to be effective. The importance of ampicillin was illustrated by the lack of bactericidal activity during the first hour of experiments with *E. coli* SC1359 in the IVP. Bactericidal activity in these experiments was not detected until ampicillin was introduced into the model at 1 h. The importance of sulbactam was illustrated by pharmacodynamic data from the 1.5-g dose against *E. coli* 149 and the 3.0-g dose against *E. coli* AFE-MV1 in the IVP. In these experiments, bacterial regrowth was observed after both simultaneous and sequential dosing of ampicillin-sulbactam, with increases in viable bacterial numbers initiating at the same time point whether ampicillin was dosed simultaneously with sulbactam at 0 or 1 h after sulbactam. Since the pharmacokinetics of sulbactam are the same for the simultaneous and sequential regimens, bacterial regrowth in these experiments initiated when sulbactam levels fell below a critical concentration, suggesting that the maintenance of sulbactam levels above enzyme inhibitory concentrations was a critical pharmacodynamic parameter affecting the activity of ampicillin-sulbactam. The greater importance of the sulbactam concentration in determining efficacy of ampicillin-sulbactam was supported by the MIC

data obtained in a series of tests with ampicillin-sulbactam. These data defined the MCC for sulbactam and showed that the ampicillin concentration required increased significantly when the sulbactam concentration was below the MCC, and it was decreased significantly when the sulbactam concentration was above the MCC.

The relative importance of sulbactam over ampicillin in the pharmacodynamics of ampicillin-sulbactam is also supported by data from Dudley et al. with piperacillin-tazobactam (6). Using a similar IVP, Dudley et al. demonstrated that lower doses of piperacillin combined with usual doses of tazobactam provided bactericidal activity against piperacillin-resistant *E. coli* that was comparable to bactericidal activity observed against an isogenic piperacillin-susceptible strain. However, if the pharmacokinetics of tazobactam were altered by dosing at 12-h intervals rather than 8-h intervals, activity was diminished, even if the dose of piperacillin was doubled. In an earlier study by the same group (20), it was concluded that the area under the concentration curve for  $\beta$ -lactamase inhibitors was the important pharmacodynamic parameter determining activity of inhibitor-drug combinations against  $\beta$ -lactamase-producing bacteria. The relative importance of the area under the concentration curve for sulbactam in this study could not be assessed, since the pharmacokinetics of sulbactam were similar in all dosing regimens.

To substantiate the pharmacodynamics observed in vitro, similar studies were performed with a mouse model of lethal *E. coli* bacteremia. In the mouse model of bacteremia, the pharmacodynamics observed when sulbactam was dosed prior to ampicillin were similar to those observed when the combination was dosed simultaneously. These data also suggest that the bactericidal activity of ampicillin-sulbactam is independent of a critical ratio between the components whether the combination is dosed against TEM-1-producing *E. coli* in the presence or absence of host defense mechanisms.

The primary difference observed between the two models was the greater activity in vivo of both doses of ampicillin-sulbactam against *E. coli* AFE-MV1. In studies with *E. coli* AFE-MV1, a 2- to 3-log decrease in viable bacterial counts was observed in mice treated with the both the 1.5- and 3.0-g dose of ampicillin-sulbactam. In contrast, little if any bactericidal activity was observed with either dose in the IVP. It is possible that the pharmacodynamic differences observed between the two models were due to differences in the starting inoculum. However, when the original inoculum of *E. coli* AFE-MV1 in the IVP was decreased to  $10^3$  CFU/ml, the pharmacodynamic interactions observed were similar to those observed when the starting inoculum was  $10^6$  CFU/ml (data not shown). This suggests that in studies with *E. coli* AFE-MV1, the pharmacodynamic differences observed between the in vitro and in vivo models were not the result of an inoculum effect. Rather, these differences most likely reflect the cooperation between subinhibitory  $\beta$ -lactam antibiotics and phagocytic killing of *E. coli* by leukocytes (8, 13, 21) or other host defense mechanisms.

Previous studies in our laboratory with *E. coli* SC1359 and *E. coli* 149 have shown that the pharmacodynamics of the 1.5- and 3.0-g doses of ampicillin-sulbactam against TEM-1-producing *E. coli* in mice are similar (12). These studies confirmed the observations of Leggett et al., who also demonstrated with mice that there is no dose-dependent relationship between level of  $\beta$ -lactam concentrations above the MIC and bactericidal activity (11). In the IVP in the current study, similar conclusions were reached. The pharmacodynamics of the 1.5- and 3.0-g doses of ampicillin-sulbactam in the IVP were similar when the drugs were dosed against *E. coli* SC1359 and

*E. coli* 149. Furthermore, the pharmacodynamics of each dose were similar whether experiments were performed with mice or in the absence of host defenses in the IVP. These data further substantiate the IVP and suggest that the in vivo pharmacodynamic interactions of ampicillin-sulbactam against TEM-1-producing *E. coli* may be predicted by studies with an IVP, as long as the challenge organism does not express high-level resistance to the combination. As mentioned previously, the pharmacodynamics of ampicillin-sulbactam against *E. coli* AFE-MV1 differed substantially between the two models, which is not surprising considering the level of resistance displayed by this strain. However, despite the differences between the models, the conclusions reached concerning dose-response relationships with the 1.5- and 3.0-g doses were the same with both models.

In summary, the results of this investigation have shown that the pharmacodynamics of ampicillin-sulbactam against TEM-1-producing *E. coli* were similar whether the combinations were dosed simultaneously or in sequence. These data suggest that the antibacterial activity of ampicillin-sulbactam is not dependent upon the maintenance of a critical ratio between the two components but rather is dependent upon the maintenance of both components above critical concentrations. Furthermore, the pharmacokinetics of sulbactam appear to play a critical role in the pharmacodynamics of ampicillin-sulbactam. With the current strategy of administering ampicillin-sulbactam at a 2:1 ratio, antibacterial activity is lost when sulbactam levels fall below an MCC for the infecting organism. The MCC of sulbactam required for optimal antibacterial activity of ampicillin-sulbactam against TEM-1-producing *E. coli* or other species of bacteria is highly variable and probably depends upon the amount and type of  $\beta$ -lactamase produced and the intrinsic susceptibility of the host strain to ampicillin. However, data from this study suggest that ampicillin-sulbactam MIC data obtained with the recommended fixed 2:1 ratio can define the MCC of sulbactam for an individual strain. Further investigation of this critical pharmacodynamic parameter is essential to the understanding of ampicillin-sulbactam dynamics and to the ability to optimize dosing of this and other  $\beta$ -lactamase-inhibitor combinations for maximum antibacterial activity.

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