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Net Effect of Inoculum Size on Antimicrobial Action of Ampicillin-Sulbactam: Studies Using an In Vitro Dynamic Model

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To examine the predictable effect of inoculum size on the kinetics of the antimicrobial action of ampicillin-sulbactam, five TEM-1 beta-lactamase-producing *Escherichia coli* strains were studied in an in vitro dynamic model at two different initial inocula (N_0s). All bacteria were exposed to ampicillin-sulbactam in a simulated system reflecting the pharmacokinetic profiles in human tissue after the administration of a single intravenous dose of ampicillin (2 g) plus sulbactam (1 g). Each strain was studied at low (4.0 to 5.2 log CFU/ml) and high (5.0 to 7.1 log CFU/ml) N_0s. Despite pronounced differences in susceptibilities, the patterns of the killing curves observed with a given strain at different N_0s were similar. As expected, viable bacterial counts increased with inoculum size. Striking visual contrasts in the respective curves for each organism were reflected by the area under the bacterial count-time curve (AUBC) but not by the difference between the N_0 and the lowest bacterial counts (N_{min}) at the nadir of the killing curve: the N_0-associated changes in the AUBC on average were 75%, versus 2.5% for $\log N_0 - \log N_{min}$. To examine qualitative differences in antimicrobial effects at different N_0s (i.e., the net effect of the inoculum), the difference in the high and low N_0s was subtracted from each point on the killing curve obtained at the higher N_0 for each strain. These adjusted curves were virtually superimposable on the observed killing curves obtained at the lower N_0. Moreover, by using adjusted data, the AUBC values were similar at the two inocula, although slight (average, 11%) but systematic increases in the AUBC occurred at high N_0s. Thus, there was only a weak net effect of inoculum size on the antibacterial effect of ampicillin-sulbactam. Due to similar slopes of the AUBC-log N_0 plots, the antibacterial action at different N_0s may be easily predicted by an approximate equation; the predicted AUBCs were unbiased and well correlated with the observed AUBCs ($r = 0.997$). Compiled data obtained with normalized AUBCs for different strains at different N_0s yielded a positive correlation ($r = 0.963$) between the N_0-normalized AUBC and the MIC of ampicillin-sulbactam. The adjustment and normalization procedure described might be a useful tool for revealing the net effect of the inoculum and to predict the inoculum effect if there are no qualitative differences in antimicrobial action at different inocula.

Traditionally, the activities of beta-lactam antibiotics with beta-lactamase inhibitors are related to inoculum size in most in vitro test systems. The "inoculum effect" usually describes the increased requirement for beta-lactam antibiotics in the presence of large numbers of bacteria presumably associated with an increased amount of bacterial enzyme and subsequent drug hydrolysis and inactivation. These effects are usually noted by standard in vitro susceptibility tests which expose bacteria only to a static concentration of antibiotic.

Several methods have been described to study in vitro bacterial killing kinetics in the presence of changing concentrations of antibiotic in an attempt to mimic the various levels of antibiotics actually "seen" by the organisms during treatment of a clinical infection (4, 6, 10, 11, 13). However, the impact of inoculum size on antibacterial effect has been studied only infrequently in these dynamic models that stimulate pharmacokinetics in humans (1, 6, 14, 15). In three of these studies the establishment of the inoculum-induced changes in bacterial killing was based only on visual comparison of killing curves obtained at different inocula (1, 14, 15). The only attempt to relate the inoculum size to parameters of the bacterial killing-regrowth curve was undertaken in a study that simulated sisomicin pharmacokinetic profiles (6). The intensity of the antibacterial effect (I_E; the area between the curve representing bacterial killing and regrowth and that representing normal growth up to the point when bacterial counts in the regrowth curve reach the maximal values observed in the absence of antibiotic [5]) was evaluated in this study. Comparison of the I_E data obtained at different inocula showed a distinct inoculum effect for the action of sisomicin against *Escherichia coli*. Also, concentration-dependent relations between initial inocula and I_E were described (6). In spite of these studies, the problem as to how best to determine quantitative predictions of the effect of inoculum size on antibacterial action remains.

The present study was designed to discriminate between expected (quantitative) and unexpected (qualitative) changes in the antimicrobial action of ampicillin-sulbactam at different inocula and to examine whether or not these changes were predictable. The antibacterial effect of the antibiotic against *E. coli* strains producing TEM-1 beta-lactamases was investigated in an in vitro model simulating pharmacokinetic profiles in humans. The antibacterial effect was expressed by the area under the bacterial count-time curve (AUBC), an integral parameter of the bacterial killing-regrowth curve (6, 16, 17).

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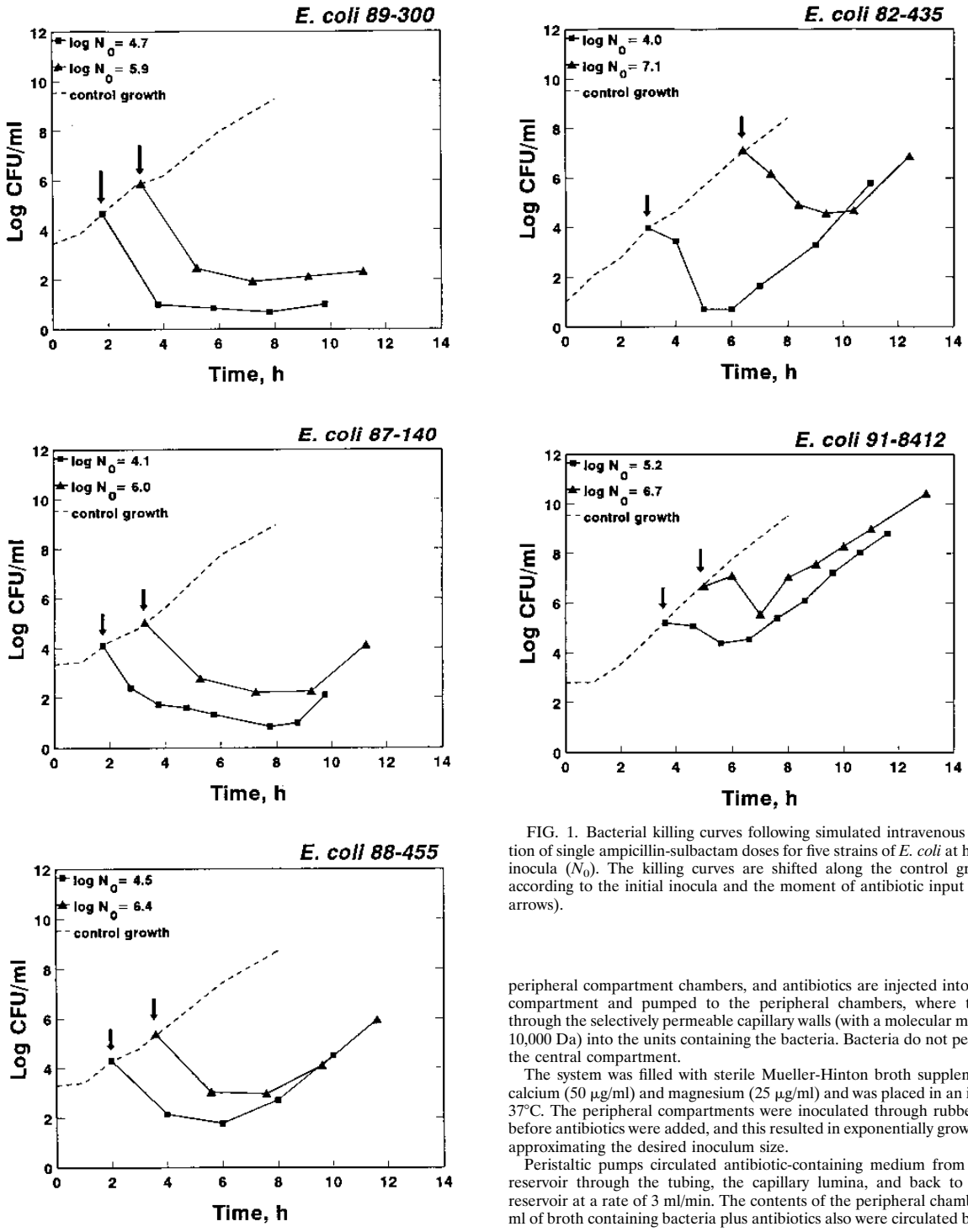

FIG. 1. Bacterial killing curves following simulated intravenous administration of single ampicillin-sulbactam doses for five strains of *E. coli* at high and low inocula (N_0). The killing curves are shifted along the control growth curve according to the initial inocula and the moment of antibiotic input (marked by arrows).

MATERIALS AND METHODS

In vitro model, operating procedure, and simulated pharmacokinetic profiles. The in vitro pharmacokinetic-pharmacodynamic model described previously was used to simulate antibiotic profiles achieved in human tissue after the administration of single intravenous doses of ampicillin-sulbactam (2). Briefly, the model consists of a sterile central compartment representing the systemic circulation (which includes a central reservoir, tubing, and the lumina of artificial capillary bundles) and a peripheral compartment that represents extravascular sites of infection (which include six artificial hollow-fiber capillary units [Unisyn Fibertec Corporation, San Diego, Calif.]) placed in series (2, 3). Bacteria are placed in the peripheral compartment chambers, and antibiotics are injected into the central compartment and pumped to the peripheral chambers, where they diffuse through the selectively permeable capillary walls (with a molecular mass cutoff of 10,000 Da) into the units containing the bacteria. Bacteria do not penetrate into the central compartment.

The system was filled with sterile Mueller-Hinton broth supplemented with calcium (50 μg/ml) and magnesium (25 μg/ml) and was placed in an incubator at 37°C. The peripheral compartments were inoculated through rubber septa 2 h before antibiotics were added, and this resulted in exponentially growing cultures approximating the desired inoculum size.

Peristaltic pumps circulated antibiotic-containing medium from the central reservoir through the tubing, the capillary lumina, and back to the central reservoir at a rate of 3 ml/min. The contents of the peripheral chambers with 10 ml of broth containing bacteria plus antibiotics also were circulated by peristaltic pumps.

Broth in the central compartment was continuously eliminated and replaced with fresh medium from a diluent reservoir to mimic the drug concentration-time profiles in peripheral tissue observed in humans following the administration of a single intravenous dose of ampicillin-sulbactam (2 and 1 g, respectively). In all experiments the respective initial concentration of ampicillin was 100 μg/ml, and that of sulbactam was 50 μg/ml. Monoexponential decays of ampicillin and sulbactam concentrations were simulated with an elimination half-life of 1 h, because their half-lives in vivo are similar (12). Control growth in the absence of antibiotics was studied in the same model.

Ampicillin concentrations in the central and peripheral compartments were determined by bioassay (7). Antibiotic medium 1 (Difco) seeded with *Bacillus subtilis* ATCC 6633 (*B. subtilis* spore suspension; Difco) was used to prepare the bioassay plates. Wells were made in the agar and were filled with 25 μl of sample.

Plates were incubated overnight at 37°C. After overnight incubation the zone sizes were measured with calipers. Ampicillin concentrations were calculated against a four-point standard curve by linear regression. The calibration plots were linear within the range of 0.5 to 4.0 μg/ml, and samples were diluted when necessary. The R^2 for standard curves ranged from 0.84 to 0.99 (median = 0.91). The median error in the standard curves was 6.9% (range, 4.0 to 14.1%). The median coefficient of variation for standards and unknown drug concentrations was 5.81% (range, 0.0 to 22.1%) when three to four replicates per sample were studied. The observed concentrations were close to the designed values, with no systematic deviation from expected values. At concentrations between 4.7 and 75 μg/ml, sulbactam did not interfere with ampicillin concentration determinations.

Antibiotics. Analytical-grade powders of ampicillin and sulbactam (kindly supplied by Pfizer Laboratories) were used in the experiments.

Bacterial strains. Five strains of TEM-1 beta-lactamase-producing *E. coli* were selected from a collection of clinical isolates. The MICs determined at a constant (2:1) ratio of ampicillin to sulbactam by the broth microdilution technique with inocula of approximately 5×10^5 CFU/ml (9) ranged from 11 to 125 μg/ml. Geometric mean values of the MICs determined in duplicate or triplicate were calculated. Overnight cultures of the bacteria to be tested were diluted in fresh medium on the day that MIC determinations were made and were allowed to incubate at 37°C for 2 to 3 h to achieve the desired inoculum (see above). Each strain was studied in the in vitro dynamic model at low (4.0 to 5.2 log CFU/ml) and high (5.0 to 7.1 log CFU/ml) initial inocula (N_0). With four strains, including *E. coli* 89-300, 97-140, 88-455, and 91-8412, the difference between the low and high inocula was approximately 1 log CFU/ml, which is typical for time-kill studies. With one strain, *E. coli* 82-435, the range was extended up to 3 log CFU/ml.

Quantitation of bacterial growth and killing. In each experiment 0.3-ml samples were withdrawn from the peripheral compartments seven to eight times over the 8-h period after antibiotic input into the model. This period reflects the usual dosing interval for patients treated with ampicillin-sulbactam. These samples were subjected to serial 10-fold dilutions with chilled, sterile 0.9% NaCl and were plated in triplicate on Mueller-Hinton agar. In addition, 100 μl of each sample was filtered through a 0.45-μm-pore-size membrane filter (47 mm in diameter; HA; Millipore Corp., Bedford, Mass.) and was also plated on agar. After incubation at 37°C the resulting bacterial colonies were counted, and the numbers of CFU per milliliter were calculated. Incubation times varied from 16 to 48 h depending on the strain and plating method (direct or filter). The experiments with each strain were performed in duplicate (two chambers for each organism). The limit of detection was 10 CFU/ml (1 log), and the standard deviation of the bacterial counts for replicate samples averaged 0.32 log CFU.

Quantitative evaluation of antibacterial effect. To compare bacterial killing at different N_0s, an integral parameter was used to reflect the AUBC (6, 16, 17). On the basis of the bacterial count-time data obtained in the presence of ampicillin-sulbactam, trapezoidal areas under the log CFU per milliliter-time curves for the 8-h observation period were calculated. The difference between log N_0 and the lowest bacterial counts (log N_{min}) at the nadir of the killing curve was also estimated for each strain.

Correlation and regression analyses of the relations between the observed and predicted AUBCs as well as between AUBC and MIC were performed by using STATGRAF software (STSC, Inc., Clinton, Wa.).

RESULTS

Figure 1 shows the control growth of *E. coli* and the killing curves in the presence of ampicillin-sulbactam at the low and high inocula. Despite pronounced differences associated with the different susceptibilities of the strains (weak or almost negligible regrowth of *E. coli* 89-300 and 87-140 [geometric mean MICs, 11 and 15.6 μg/ml, respectively] and obvious regrowth of *E. coli* 88-455, 82-435, and 91-8412 [geometric mean MICs, 44.2, 62.5, and 125 μg/ml, respectively), the patterns of the curves observed with a given strain at different N_0s were generally similar. However, inoculum-induced differences in the positions of the curves were shifted along the *y* axes, with the higher inoculum in the upper position.

Despite striking visual contrasts in the respective curves for each organism, this was not reflected by differences between N_0 and N_{min} at the nadir of the killing curve (Fig. 2). For each strain log N_0 − log N_{min} measured at different N_0s were minor (average, 2.5%) and not systematic. Thus, the true effect of inoculum size may not be demonstrated by log N_0 − log N_{min}.

The effect of inoculum size was also expressed by the integral parameter AUBC, another measurement of antibiotic-induced antibacterial action. As seen in Fig. 3, for each strain the different inocula had a systematic and pronounced effect

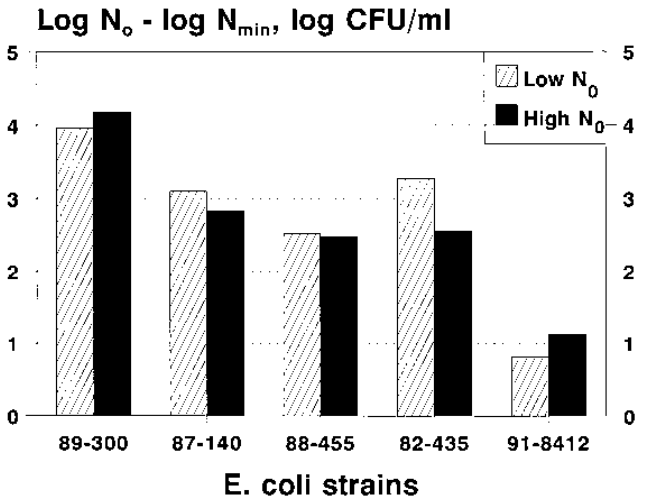

FIG. 2. Antibacterial effect of ampicillin-sulbactam determined by log N_0 − log N_{min} for five strains of *E. coli* at high and low inocula.

on the AUBC (average, 75%). Larger inocula were associated with larger AUBC determinations, reflecting less bacterial killing and/or more bacterial regrowth in the face of antibiotic administration.

To examine for qualitative differences in antimicrobial effects at different inocula, i.e., the net effect of the inoculum, the respective killing curves were superimposed for each strain. The difference in N_0 was subtracted from each point on the killing curve obtained at the higher inoculum. As seen in Fig. 4, the adjusted curves are virtually superimposable on the observed killing curves obtained at the lower inoculum. Moreover, when the adjusted data were used, AUBC values were similar at the two inocula, although a slight (average, 11%) but systematic increase in the AUBC at high N_0s occurred (Fig. 5). Thus, there was only a weak net influence of inoculum on the antibacterial effect of ampicillin-sulbactam.

This also may be illustrated by the AUBC-log N_0 dependencies for five strains of *E. coli* (Fig. 6). Similar slopes (average, 9.4 h) were inherent in the plots (Fig. 6). Hence, when con-

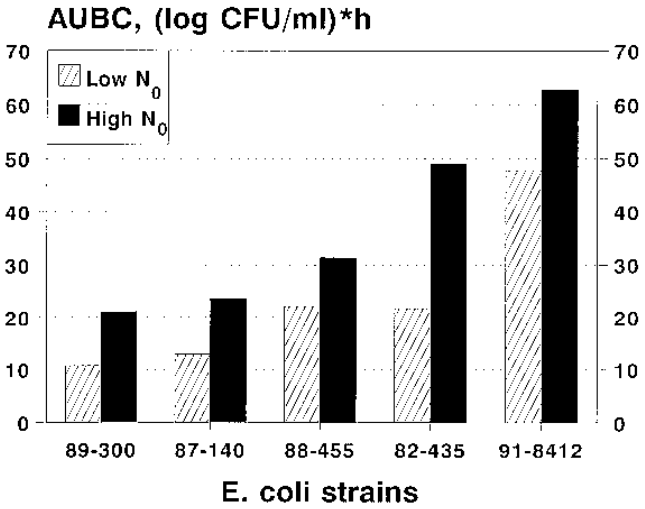

FIG. 3. Antibacterial effect of ampicillin-sulbactam determined by the AUBC for five strains of *E. coli* at high and low inocula.

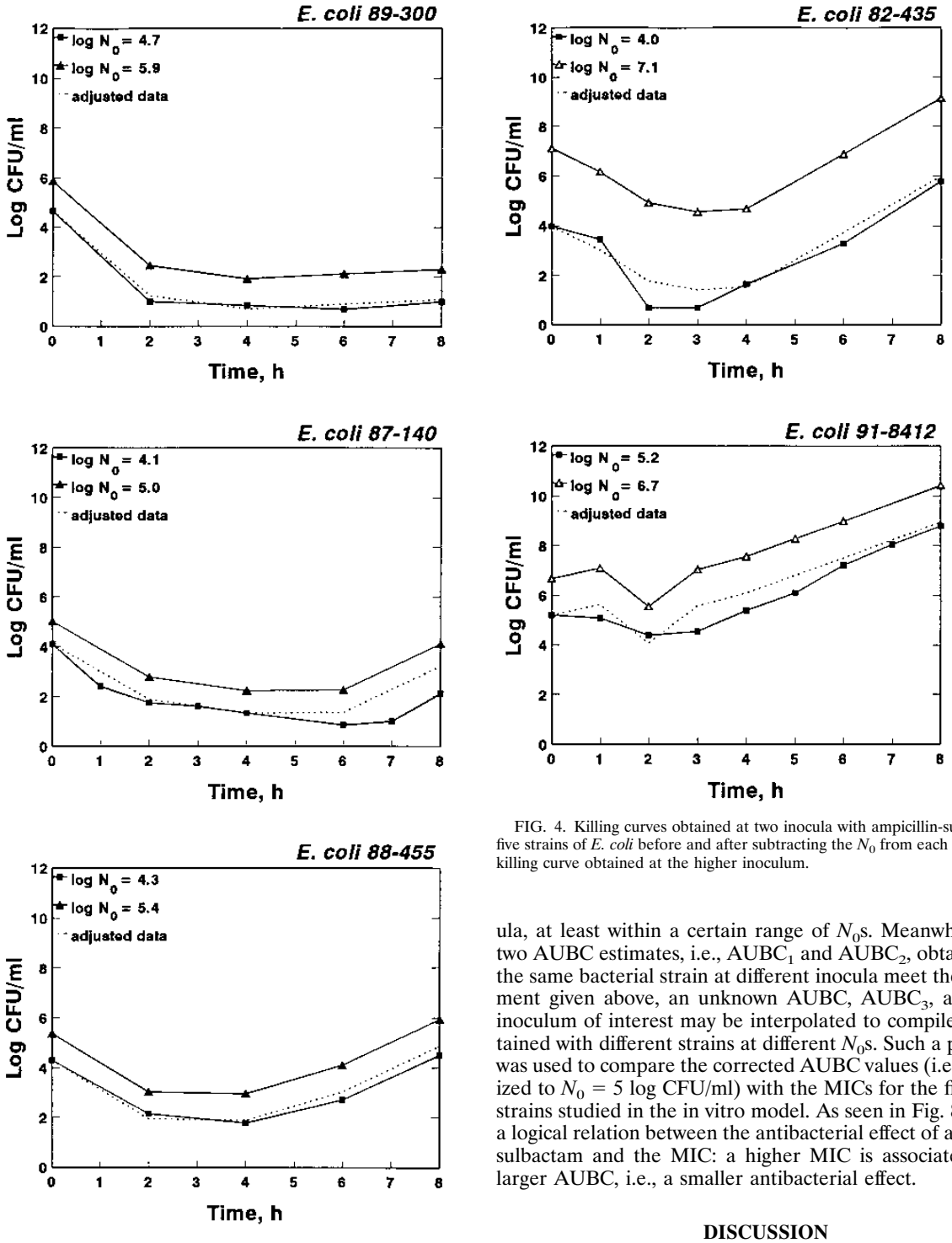

FIG. 4. Killing curves obtained at two inocula with ampicillin-sulbactam for five strains of *E. coli* before and after subtracting the N_0 from each point on the killing curve obtained at the higher inoculum.

sidering the AUBC observed at a given inoculum (log $N_{0,1}$), the antibacterial action at another inoculum (log $N_{0,2}$), $AUBC_2$, might be approximately predicted by the equation $AUBC_2 = AUBC_1 + 9.4 \times (\log N_{0,2} - \log N_{0,1})$. Figure 7 demonstrates a strong correlation ($r = 0.997$) between the observed and predicted values of AUBC.

The equation given above may be used if there are no qualitative differences in the antibacterial effects at different inoculum, at least within a certain range of N_0s. Meanwhile, when two AUBC estimates, i.e., $AUBC_1$ and $AUBC_2$, obtained with the same bacterial strain at different inocula meet the requirement given above, an unknown AUBC, $AUBC_3$, at another inoculum of interest may be interpolated to compile data obtained with different strains at different N_0s. Such a procedure was used to compare the corrected AUBC values (i.e., normalized to $N_0 = 5$ log CFU/ml) with the MICs for the five *E. coli* strains studied in the in vitro model. As seen in Fig. 8, there is a logical relation between the antibacterial effect of ampicillin-sulbactam and the MIC: a higher MIC is associated with a larger AUBC, i.e., a smaller antibacterial effect.

DISCUSSION

The role of inoculum size in in vitro antibiotic susceptibility studies has been emphasized for many years (8). However, most studies to date have used methods which expose bacteria to a static concentration of antibiotic, whereas in vivo and during the treatment of an infection, bacteria are exposed to concentrations of antibiotics that change according to the pharmacokinetic properties of the drug. Only a few such investigations have been performed with in vitro-simulated antibiotic pharmacokinetics (1, 6, 14, 15). A pharmacokinetic-pharmacodynamic model was used in the present study to

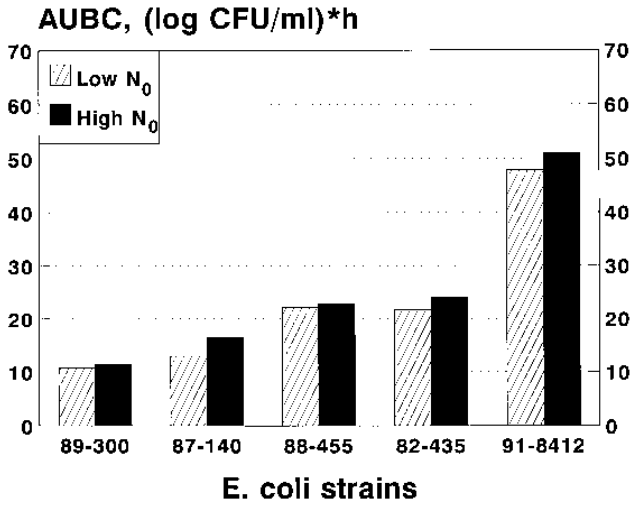

FIG. 5. Antibacterial effect of ampicillin-sulbactam determined by the AUBC for five strains of *E. coli* at high and low inocula after adjustment by the subtraction procedure (see text).

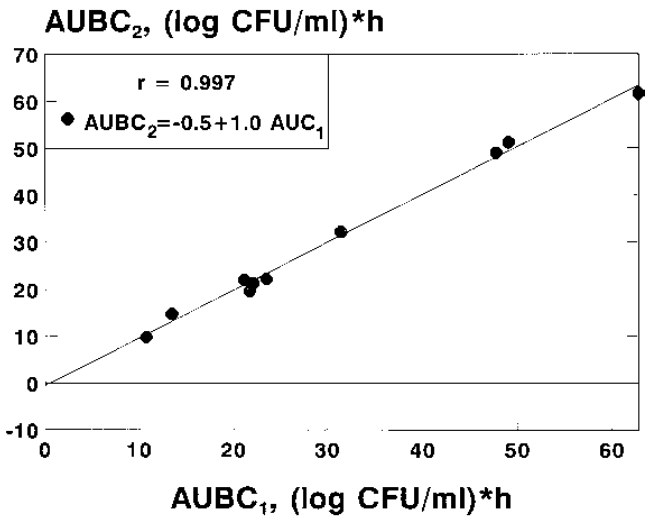

FIG. 7. Correlation between the observed ($AUBC_1$) and predicted ($AUBC_2$) values of AUBC (see text).

examine the role of inoculum size on the antibacterial action of the beta-lactam–beta-lactamase inhibitor combination.

Five clinically isolated strains of TEM-1 beta-lactamase-producing strains of *E. coli* were studied at low and high inocula with simulated single intravenous administrations of ampicillin-sulbactam (2 and 1 g, respectively). Although the shape of the killing curves was specific for each strain, the shapes were very similar at the low and high inocula for any given strain. In fact, the killing curves were just shifted along the control growth curve according to the N_0 and the time of antibiotic input (Fig. 1).

This study demonstrates the role of the correct use of parameters reflecting the inoculum-induced changes in the viable count-time curves. For instance, the difference between N_0 and N_{min} during drug exposure, log N_0 − log N_{min}, masked the inoculum effect, despite striking visual contrasts in the kinetic curves for each organism at different N_0s. Unlike log N_0 − log N_{min}, the use of an integral parameter of the bacterial killing-regrowth curve, AUBC (6, 16, 17), uncovered a pronounced effect of inoculum size for each strain (Fig. 5) and revealed the expected reduction in antibacterial effect at high inocula. Furthermore, similar slopes in the AUBC-log N_0 plots were observed with five *E. coli* strains (Fig. 6). In a previous study (6), an AUBC-related integral parameter, I_E (5), but not the killing rate, appeared to be a sensitive indicator of inoculum effect in sisomicin's action against *E. coli.*

Previous studies (1, 6, 14, 15) also document inoculum-induced changes in bacterial killing, which were more or less expected on the basis of the findings obtained at static antibiotic concentrations. In analyzing the findings of the present study, we intended to examine whether the observed reduction in the antibacterial effect at high inocula is predictable. To address this question a procedure to adjust the viable count-time curve at higher inocula has been introduced. Subtracting the difference in N_0 from each point on the killing curves obtained at higher inocula demonstrated that the killing curves

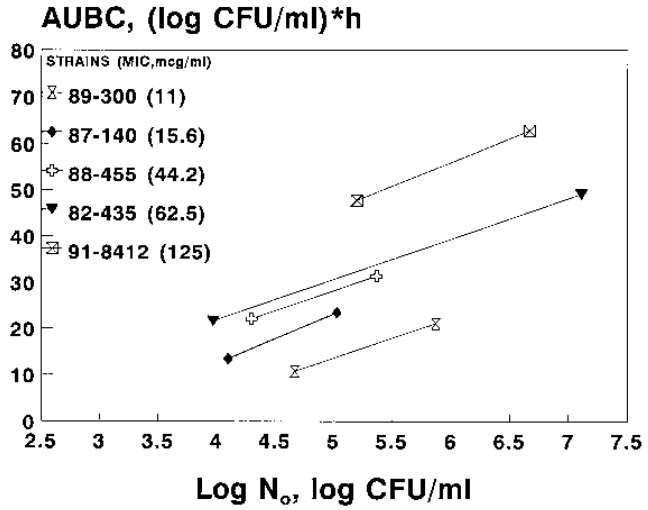

FIG. 6. Slopes reflecting the relation of AUBC to initial inoculum size for five strains of *E. coli.*

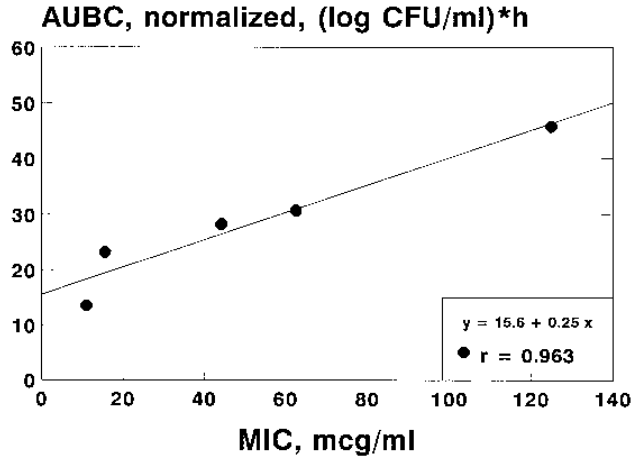

FIG. 8. Correlation between antibacterial effect of ampicillin-sulbactam against *E. coli* in the in vitro pharmacokinetic-pharmacodynamic model and the MIC. The effect is expressed as AUBC normalized to the N_0 (log N_0 = 5 log CFU/ml).

at the high and low inocula were virtually superimposable (Fig. 4). This suggests that there are minimal or no qualitative differences in the antimicrobial action of ampicillin-sulbactam at the different inocula. This was confirmed by finding similar AUBC values by using these adjusted data, although the adjusted AUBC appeared to be slightly higher than the AUBC obtained with low inocula (Fig. 5). Hence, a very weak if any net effect of the inoculum has been demonstrated.

It is remarkable that the difference of $\log N_0 - \log N_{min}$ which masked the observed inoculum effect might be a reliable indicator of the net effect of the inoculum, since similar values for this difference were obtained at different N_0s (Fig. 2). However, the use of the adjusted AUBCs to test the hypothesis of the net effect may result in even more reliable conclusions since any integral measure of the effect is more reliable than any point estimate. For example, the estimates of N_{min} and the respective differences in $\log N_0$ and $\log N_{min}$ may be insufficiently precise if the time-kill curve has a diffused minimum (e.g., for strains 89-300 and 87-140) and/or N_{min} is close to the limit of detection (for strains 89-300, 87-140, and 82-435) (Fig. 1).

Due to the similar slopes of the AUBC-$\log N_0$ plots (Fig. 6) the antibacterial action at different inocula may be predicted by a simple equation. The predicted AUBCs for the five strains studied correlated with the observed AUBCs (Fig. 7). However, this equation may be applied only if there is no pronounced net effect of inoculum size. In another study, visual inspection of the killing-regrowth curves demonstrating the antibacterial action of piperacillin, moxalactam, and their combination against *S. aureus* at two different inocula (1) showed that only moxalactam met this requirement. Similarly, an almost negligible net effect of inoculum was found with aztreonam against *E. coli* and *Pseudomonas aeruginosa* (15), but a pronounced net effect was seen with gentamicin and ceftriaxone against the same organisms and with aminosidine and cephalothin action against *Staphylococcus aureus*, *E. coli*, and *Salmonella typhi* (14). Thus, a preliminary inspection of the killing-regrowth curves obtained at different inocula by using visual inspection or the subtraction procedure is necessary for the appropriate use of the proposed equation.

These precautions are probably less critical when interpolating the AUBC to compile data obtained at different inocula. Such a procedure was used in this study to relate the N_0-normalized AUBC to the MIC. The respective correlation demonstrated that the antibacterial effect of ampicillin-sulbactam in an in vitro pharmacokinetic-pharmacodynamic model is predictable by the MIC determined at a fixed ratio of ampicillin to sulbactam at 2:1 (Fig. 8), but not by MICs determined at a fixed concentration (4 μg/ml) of sulbactam (7).

These data suggest that the net effect of inoculum size on the antibacterial action of ampicillin-sulbactam is low. The calculations of AUBC and the adjustment and normalization procedure described here might be used to predict the inoculum effect on the actions of beta-lactam agents.

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REFERENCES

1. **Blaser, J.** 1991. Interactions of antimicrobial combinations in vitro: the relativity of synergism. Scand. J. Infect. Dis. **74**(Suppl.)**:**71–79.
2. **Blaser, J., B. B. Stone, and S. H. Zinner.** 1985. Two compartment kinetic model with multiple artificial capillary units. J. Antimicrob. Chemother. **15**(Suppl. A)**:**131–137.
3. **Blaser, J., B. B. Stone, and S. H. Zinner.** 1985. Efficacy of intermittent versus continuous administration of netilmicin in a two-compartment in vitro model. Antimicrob. Agents Chemother. **27:**343–349.
4. **Blaser, J., and S. H. Zinner.** 1987. In vitro models for the study of antibiotic activities. Prog. Drug Res **31:**349–381. Birkhauser Verlag, Basel.
5. **Firsov, A. A., V. M. Chernykh, and S. M. Navashin.** 1991. Quantitative analysis of antimicrobial effect kinetics in an in vitro dynamic model. Antimicrob. Agents Chemother. **34:**1312–1317.
6. **Firsov, A. A., A. D. Nazarov, and V. M. Chernykh.** 1989. Pharmacokinetic approaches to rational antibiotic therapy. *In* Series: Advances in Science and Engineering, vol. 17. VINITI Publishers, Moscow, Russia. (In Russian.)
7. **Firsov, A. A., D. Savarino, M. Ruble, D. Gilbert, B. Manzano, A. A. Medeiros, and S. H. Zinner.** 1996. Predictors of the effect of ampicillin-sulbactam against TEM-1 β-lactamase-producing *Escherichia coli* in an in vitro dynamic model: enzyme activity versus MIC. Antimicrob. Agents Chemother. **40:**734–738.
8. **Garrod, L. P., H. P. Lambert, F. O'Grady, and P. M. Waterworth.** 1981. Antibiotic and chemotherapy, 5th ed., p. 464–504. Churchill Livingstone, Edinburgh, United Kingdom.
9. **Gavan, T. L., and A. L. Barry.** 1980. Microdilution test procedures, p. 459–462. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
10. **Grasso, S.** 1985. Historical review of in-vitro models. J. Antimicrob. Chemother. **15**(Suppl. A)**:**99–102.
11. **Greenwood, D.** 1985. Models in the assessment of antimicrobial agents, p. 323–339. *In* Scientific basis of antimicrobial chemotherapy, 38th Symposium of the Society for General Microbiology. Society for General Microbiology, Cambridge, United Kingdom.
12. **Kinishi, T.** 1993. Pharmacokinetic profile of antimicrobial agents. II-1. Penicillins, p. 43. *In* H.-P. Kuemmerle, T. Murakawa, and C. H. Nightingale (ed.), Pharmacokinetics of antimicrobial agents: Principles. Methods. Application. Ecomed Verlags GmbH & Co., Landsberg/Lech, Germany.
13. **Murakawa, T.** 1993. IV. Pharmacokinetics and therapeutic efficacy. IV-1. In vitro/in vivo kinetic models for evaluating efficacy of antimicrobial agents, p. 165–177. *In* H.-P. Kuemmerle, T. Murakawa, and C. H. Nightingale (ed.), Pharmacokinetics of antimicrobial agents: Principles. Methods. Application. Ecomed Verlags GmbH & Co., Landsberg/Lech, Germany.
14. **Sanfilippo, A., and L. Mazzoleni.** 1974. Antibacterial activity of aminosidine and of beta-lactamic antibiotics. Farm. Ed. Prat. **29:**21–26.
15. **Satta, G., G. Cornaglia, G. Foddis, and R. Pompei.** 1988. Evaluation of ceftriaxone and other antibiotics against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* under in vitro conditions simulating those of serious infections. Antimicrob. Agents Chemother. **32:**552–560.
16. **Tisdale, J. E., M. T. Pasko, and J. M. Mylotte.** 1989. Antipseudomonal activity of simulated infusions of gentamicin alone or with piperacillin assessed by serum bactericidal rate and area under the killing curve. Antimicrob. Agents Chemother. **33:**1500–1505.
17. **White, C. A., and R. D. Toothaker.** 1985. Influence of ampicillin elimination half-life on in vitro bactericidal effect. J. Antimicrob. Chemother. **15**(Suppl. A)**:**257–260.