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Integration of Pharmacokinetics and Pharmacodynamics of Imipenem in a Human-Adapted Mouse Model

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The relationship between the pharmacokinetics and bactericidal activity of imipenem against *Pseudomonas aeruginosa* and *Escherichia coli* was investigated in a neutropenic mouse thigh infection model. To circumvent the problem of short elimination time in small animals, imipenem was administered in fractionized, decreasing doses such that the pharmacokinetic profiles as observed in humans after intravenous and intramuscular injections were approximated in mice. The human-simulated kinetic profile corresponding to an intramuscular injection of 500 mg at 12-h intervals proved to be as effective as the human-simulated profile of the same dose injected intravenously every 6 h. In contrast, the human-simulated profile corresponding to only one intravenous injection every 12 h resulted in bacterial breakthrough growth between 8 and 12 h after the onset of treatment. The results of our investigations confirm the hypothesis that the bactericidal effect of imipenem against *P. aeruginosa* and *E. coli* in vivo depends mainly on the time during which drug levels remain above the MIC rather than on the plasma peak/MIC ratio.

Integration of the in vitro activity of an antibiotic and its pharmacokinetic profile as determined in humans is a prerequisite for recommendations of dosage and dosage schedules (3, 11, 14). However, this integration is not easy, especially if maximization of the relation between effect and dose is the goal. One problem is that the target organisms are exposed to constant drug concentrations for MIC determinations in vitro, whereas drug levels are fluctuating at the site of infection in vivo. Very high concentrations of a drug shortly after its administration may be followed by concentrations below the MIC at the end of the dosage interval. Thus, to optimize the dose and the dosage schedule of a given antibiotic, we need to understand the pharmacokinetic-pharmacodynamic interactions of that particular drug.

The present study is an experimental approach to the understanding of the pharmacokinetic-pharmacodynamic interactions of imipenem against gram-negative organisms in neutropenic mice. Since a novel intramuscular (i.m.) formulation of imipenem has recently been developed (10), it was of particular interest to compare the antimicrobial effect of this drug following intravenous (i.v.) administration with its antimicrobial effect following i.m. administration. To circumvent the problem of short elimination times of antibiotics in small animals, a previously described human-adapted animal model was used (4, 5); i.e., the drug was administered such that levels and kinetics in plasma which come close to those usually observed in humans could be obtained. Thus, areas under the concentration-time curves (AUCs) equivalent to those derived from human studies were obtained by using the plasma of the study animals, and—equally important, as will be shown—AUC shapes like those derived from human studies were simulated. Neutropenic animals were used to assess the effect of the study drug per se. The bactericidal effect was quantitated over time by establishing time-kill curves of bacteria in the mouse thigh, i.e., at the site of the experimental infection.

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MATERIALS AND METHODS

Organisms. Main experiments were performed with *P. aeruginosa* ATCC 27853 (American Type Culture Collection, Rockville, Md.) and *E. coli* ATCC 25922. One clinical isolate each of *P. aeruginosa* (designated A 10) and *E. coli* (designated U 31800) from the Institute of Medical Microbiology (Bern, Switzerland) were used for confirmatory experiments. MICs and MBCs were determined in vitro by using the broth-dilution technique (9) and Mueller-Hinton broth supplemented with Ca^{2+} and Mg^{2+}, as suggested by Stratton and Reller (17).

Drugs. Imipenem was purchased from Merck & Co., Inc., Rahway, N.J. The cyclophosphamide used was a commercial product (Mead Johnson Pharmaceuticals, Evansville, Ind.). The drugs were diluted according to the recommendations of the manufacturers and further diluted with sterile pyrogen-free physiological saline.

Mice. Female ICR mice, weighing 26 to 29 g each, were obtained from Tierzucht der Universität (Tierspital, Zürich, Switzerland). Granulocytopenia was produced as previously described (7), i.e., by two intraperitoneal (i.p.) injections of cyclophosphamide (150 and 100 mg/kg of body weight) at 4 days and 1 day, respectively, before the mice were infected. In some experiments, blood smears were checked for the presence of granulocytes; the results proved that by the day of the experiment the study animals were virtually agranulocytic.

Bacterial inocula. Broth cultures of the study organisms were grown to ~10^8 CFU/ml (estimated by turbidity, as determined in separate experiments). Before injection, the organisms were washed in physiological saline and resuspended in saline to a concentration of 10^8 CFU/ml. Two hours before starting the treatment, 0.1 ml of an ice-cold suspension of the study organisms was injected into the thighs of slightly ether-anesthetized granulocytopenic mice. In most experiments, *E. coli* was injected in one thigh and *P. aeruginosa* was injected in the opposite thigh, so that the effects on these two target organisms were thus determined at the same time in the very same animals.

Antimicrobial treatment. In the main experiments, treat-

ment started 2 h after infection. To simulate pharmacokinetics in human plasma, imipenem was injected subcutaneously (s.c.) in decreasing doses at 15-min intervals, as previously described for other drugs (5) and as is graphically shown below in Fig. 2 and 4. The highest total dose and the regimen used were chosen such that a level in plasma and a $t_{1/2}$ could be approximated similar to those observed in humans after an i.v. or i.m. administration of 500 to 1,000 mg. Subsequently, the effects of lower doses, which were obtained by twofold divisions of all individual fractional doses, were studied. The lowest total amount of drug studied over a 6-h period was 2.2 mg/kg, i.e., approximately 1/30 of the 6-h dose which results in the levels in human plasma usually aimed at in clinical work.

Quantitation of bacterial growth and killing. At various intervals, infected thighs were amputated from sacrificed animals and immediately homogenized with a Polytron tissue homogenizer (Kinematica, Lucerne, Switzerland) in 4 ml of iced saline. Homogenates were serially diluted and then plated in duplicate on tryptic soy agar (Becton Dickinson) plates. After 15 to 18 h of incubation, the numbers of CFU were counted and the number of viable organisms per thigh was calculated. With the results obtained, bacterial time-kill curves were constructed in which each data point was based on data from three to six individual animals.

Determination of drug levels in plasma. At various time intervals and immediately before the sacrifice of the animals, blood was drawn from the retro-orbital sinus into heparinized microhematocrit tubes (Clay Adams, Parsippany, N.Y.). Blood sampling was devised such that no more than two blood samples (plus a final one just prior to killing) were drawn from each mouse during the experiment. A biological assay (16) using *Bacillus subtilis* ATCC 6633 (Difco, Detroit, Mich.) was used for the determination of drug concentrations in plasma; this assay was performed on the same day as the experiment. To establish standard curves, pooled ICR mouse plasma was used as a diluent. A good correlation between inhibitory zone and log of imipenem concentration ($R > 0.99$) for the range of 1 to 32 mg/liter was found. Samples with imipenem concentrations of >32 mg/liter were thus diluted 1:4 with pooled ICR mouse plasma. The limit of imipenem detectability was 0.2 mg/liter.

Statistics. Except when otherwise mentioned, comparison of the antimicrobial efficacies of different modes of drug administration was based on experiments in which the same bacterial inoculum was injected into randomly allotted mice on the same day. Results were evaluated by using the two-tailed *t* test for unpaired samples.

RESULTS

MICs and MBCs were 2 and 8 mg/liter, respectively, for *P. aeruginosa* ATCC 27853 and 0.25 and 0.5 mg/liter, respectively, for *E. coli* ATCC 25922, as determined by standard methods (9). MICs and MBCs were 8 and >32 mg/liter for both *P. aeruginosa* and *E. coli*, as determined by using 10^7 to 10^8 organisms per ml (instead of the standard 10^5 to 10^6 CFU/ml).

The time course of imipenem's bactericidal effect on *P. aeruginosa* was first studied in vitro (Fig. 1). The killing patterns obtained by concentrations 2 and 10 times the MIC were almost identical. This finding was independent of the inoculum size. Thus, on the basis of studies with concentrations above the MIC, the bactericidal effect of imipenem proved to be poorly concentration dependent, and a ceiling effect (i.e., the maximal bactericidal effect) was already seen at concentrations two times the MIC.

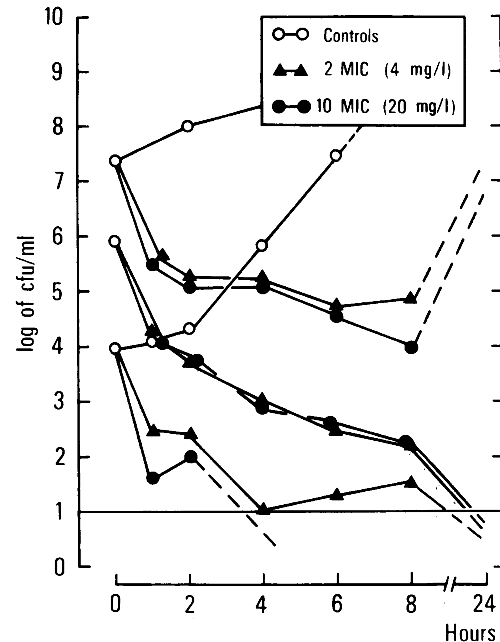

FIG. 1. Time-kill curves of three different inocula of *P. aeruginosa* ATCC 27853 exposed to concentrations of imipenem 2 and 10 times the MIC in vitro.

Human adaption of imipenem pharmacokinetics and efficacy against *P. aeruginosa*. To approximate in mice the imipenem pharmacokinetics found in humans, a total amount of drug was fractionized in decreasing doses which were injected s.c. at 15-min intervals. The method of fractionization of the total dose is shown in the bottom panel of Fig. 2. The resulting levels in plasma were compared with the levels and kinetics in plasma obtained when the same total amount of drug was given as a single s.c. bolus injection (middle panel of Fig. 2). Bolus injection resulted in very high peak levels in plasma by 15 min after the injection and was followed by the typical elimination pattern in mice, with a half-life of approximately 17 min. By 2 h after the bolus injection, the concentrations of imipenem in plasma had fallen below the MIC for *P. aeruginosa* ATCC 27853, and no detectable levels in plasma could be found after 3 h. In contrast, the fractionized dosing of the same total amount of drug (140 mg/kg) resulted in the peak levels in plasma usually observed after a bolus injection of 500 to 1,000 mg in normal volunteers, and it was followed by a $t_{1/2}$ in plasma (65 min) which was again similar to that found in humans. Human-simulated elimination kinetics of imipenem resulted in levels in plasma which fell below the MIC of *P. aeruginosa* after only 5 h. The bactericidal efficacy of the two modes of imipenem dosing against *P. aeruginosa* was striking (Fig. 2, top panel). By 2 h after the onset of treatment, the high spiking levels in plasma after the bolus injection (exceeding the MIC for *P. aeruginosa* by a factor of 50) were no more bactericidal than the lower levels in plasma after fractionized dosing. In addition, bacterial breakthrough growth was observed by 4 and 6 h after the bolus injection, whereas the sustained levels in plasma after fractionized dosing prevented early breakthrough growth and the sustained albeit low levels in plasma even continued the bactericidal activity. By 6 h after the onset of

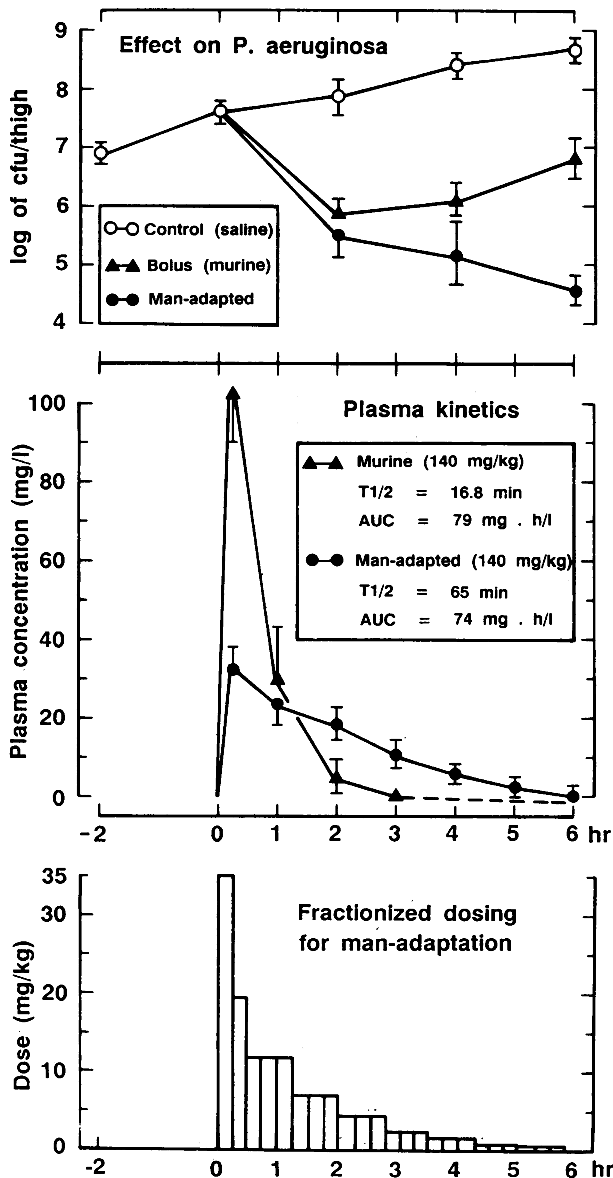

FIG. 2. Imipenem pharmacokinetics after simulated human i.v. dosing and efficacy against *P. aeruginosa* ATCC 27853 in neutropenic mice. Murine kinetics after a single bolus was compared with human-adapted pharmacokinetics (middle panel) as obtained by fractionization of the total 6-h dose (bottom panel). Kinetics of imipenem in plasma in the very same mice on the same day and its efficacy in time-kill curves (top panel) were determined. Each symbol represents the geometric mean of CFU from three to six mice ± 1 standard deviation.

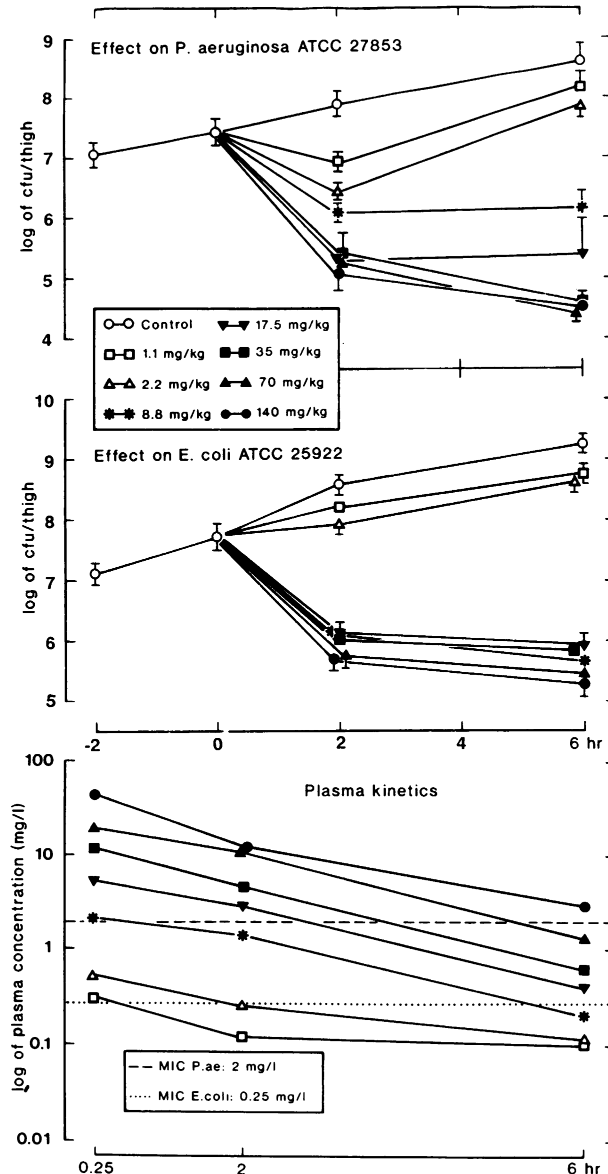

FIG. 3. Plasma kinetics of various doses of imipenem (bottom panel) and efficacy against *P. aeruginosa* and *E. coli* (upper panels) determined in time-kill curves in neutropenic mice. Plasma kinetics was human adapted by fractionized imipenem dosing as in Fig. 2. Inserted box gives interpretations of symbols used in all three panels. Symbols represent geometric means of CFU from four mice ± standard deviation. Data were compiled from three different experiments. The coefficients of variation were greatest at 15 min after the onset of treatment (0.1 to 0.28) but were similar for high and low doses of imipenem. At 6 h of treatment, coefficients of variation were invariably <2.

treatment, the isodose point—i.e., the point in time when equal total amounts of drug had been injected in both groups of mice comparatively treated—was reached. By that time, the superiority of the human-adapted pharmacokinetic profile (i.e., the fractionized mode of dosing) over the murine profile (i.e., the bolus injection) was highly significant ($P < 0.01$).

Relationship between imipenem dose and bactericidal efficacy. The bactericidal efficacies against *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 of various doses of imipenem were investigated. Human-adapted pharmacokinetics (fractionized dosing) was used, as described above. Thigh-infected granulocytopenic mice were comparatively treated with various total amounts of drug which were injected over a treatment period of 6 h, and bacterial time-kill curves were constructed from the data from the infected thighs. Results are shown in Fig. 3. Human-adapted i.v. (simulated i.v.) dosing of various amounts of drug (1.1 to 140 mg/kg) resulted in linear pharmacokinetics; i.e., a direct

correlation was found between dose and concentrations in plasma as determined at 15, 120, and 360 min after the onset of treatment (Fig. 3, bottom panel). Aggregate doses of 140 and 17.5 mg/kg for *P. aeruginosa* and *E. coli*, respectively, were needed to maintain drug levels in plasma above the MIC up to 6 h. Only one-fourth of that dose (35 mg/kg) was needed to obtain maximal bactericidal efficacy against *P. aeruginosa*, although levels in plasma fell below the MIC by approximately 4 h after the onset of treatment with this particular dose (as calculated from the data shown in Fig. 3). Similarly, with *E. coli*, maximal bactericidal efficacy was obtained with sustained drug levels in plasma near the MIC, and much higher levels in plasma (super-MICs) added poorly to the overall bactericidal efficacy by 2 and 6 h. Thus, results including those obtained by 2 h after the onset of treatment again demonstrated that high-spiking concentrations of imipenem in plasma were no more efficacious against *P. aeruginosa* and *E. coli* than smaller concentrations, providing that the concentration of drug was maintained above the MIC for at least 70 to 80% of the treatment period.

Comparison of human-adapted i.v. and i.m. dosing of imipenem and efficacy against *P. aeruginosa* and *E. coli*. Additional experiments, in which identical aggregate doses of imipenem were used to compare the efficacies of simulated i.v. and i.m. injections in humans, were performed. Since in humans the sustained-release i.m. formulation of imipenem (500 mg) results in peak levels in plasma of approximately 10 mg/liter followed by a $t_{1/2}$ of 2.5 to 2.6 h (10), we chose the dose (70 mg/kg) and the dose fractionization in our mice that could simulate the human i.m. pharmacokinetic profile (Fig. 4). In a comparatively treated group of mice, an identical total amount (70 mg/kg) of drug was used to simulate human i.v. kinetics. The effect of such dosing on *P. aeruginosa* ATCC 27853 and *E. coli* ATTC 25922 in randomized neutropenic mice was comparatively investigated (Fig. 4). By 2 and 6 h of treatment, the simulated i.v. treatment was no more efficacious than the simulated i.m. treatment against either *P. aeruginosa* or *E. coli*. Beyond 6 h, however, breakthrough growth occurred after simulated i.v. dosing, unless the treatment was continued with a second simulated i.v. injection at 6 h after the onset of treatment. By 12 h of treatment, the isodose point was reached for the simulated single i.m. injection and the simulated single i.v. (every 12 h) administrations. In contrast, twice the total amount of drug had been used for the simulated i.v. (every 6 h) treatment. Because of breakthrough growth, the regimen of a single simulated i.v. injection was clearly ($P < 0.01$) less efficacious than the regimen of a single simulated i.m. injection. In addition, by 12 h of treatment, no difference regarding overall bactericidal efficacy between the regimen of simulated i.v. injection every 6 h and the regimen of a single simulated i.m. injection was found. In conclusion, sustained supra-MIC levels of imipenem were more important than the magnitude by which MICs were exceeded.

An interesting consistent finding was that the simulated second i.v. dose of imipenem given at 6 h was merely bacteriostatic. This phenomenon has previously been observed in similar studies focusing on other antibiotics. Its mechanisms are poorly understood.

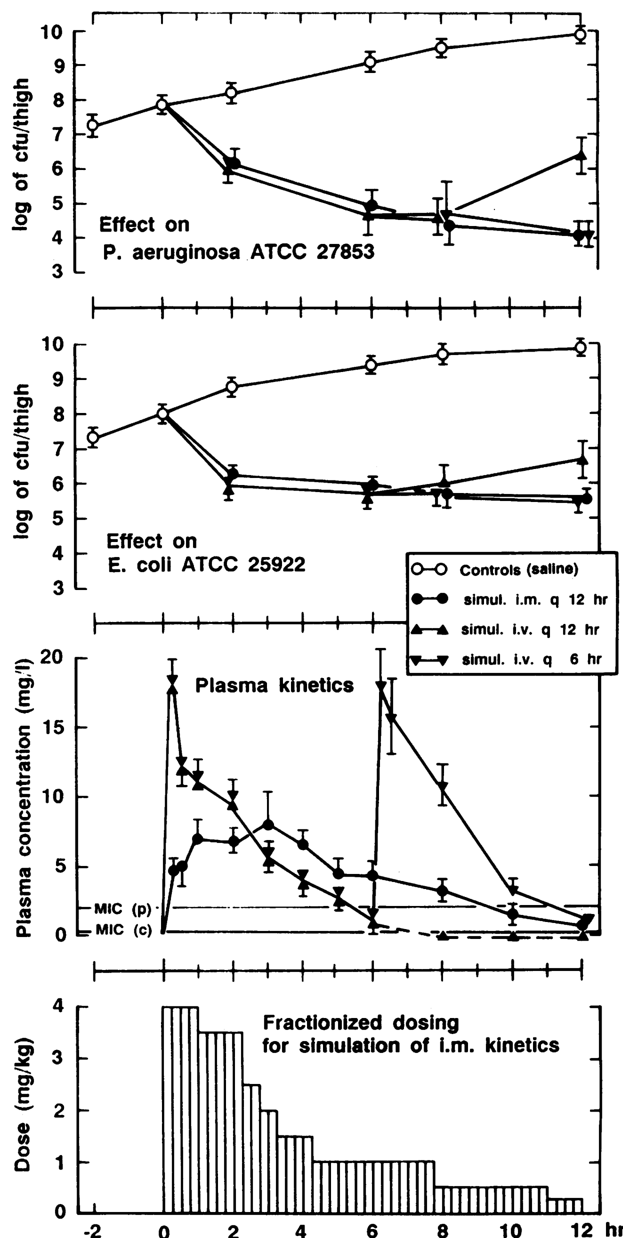

FIG. 4. Imipenem pharmacokinetics after simulated human i.m. and i.v. dosing and efficacy against *P. aeruginosa* and *E. coli* in neutropenic mice. The total 12-h dose (70 mg/kg) was fractionized to simulate human i.m. and i.v. dosing as shown in the bottom panel and in analogy to Fig. 2, respectively. To simulate two courses of i.v. dosing, a total of 140 mg (70 mg every 6 h) was used. Symbols represent mean values (geometric means of CFU) from three to six animals ± 1 standard deviation. MIC (p), MIC for *P. aeruginosa*; MIC (c), MIC for *E. coli*.

DISCUSSION

Our experimental study clearly demonstrates the impact of pharmacokinetics on the pharmacodynamics of imipenem against two different gram-negative target organisms. The length of time during which imipenem was maintained in plasma at concentrations above or near the MIC was significantly more important than the magnitude by which levels in plasma exceeded the MIC. Although a short postantibiotic effect was found with imipenem against low bacterial inocula of *P. aeruginosa* in vitro (1, 2, 12) and in neutropenic mice (8, 19), this effect was not strong or long enough to cover more than 1 to 2 h when concentrations were below the MIC. Thus, at least for the two target organisms focused on in the

present study, levels in blood below the MIC reduced the overall antimicrobial activity of imipenem because of early breakthrough growth when levels in plasma were below the MIC. This finding assumes even greater importance when one considers that a postantibiotic effect with imipenem has not consistently been found for all strains of *P. aeruginosa* (19).

Exceedingly high (super-MIC) concentrations of imipenem killed a given inoculum of target organisms no faster and no more completely than did lower (supra-MIC) concentrations. This ceiling effect was found in vitro as well as in vivo, and it was consistent for *P. aeruginosa* as well as for *E. coli*. The ceiling effect of antimicrobial activity was observed at concentrations close to the MIC.

The concentration-response pattern of imipenem against gram-negative organisms—i.e., poor concentration-dependent killing and high time-dependent killing—is similar to the one observed with other beta-lactam drugs (3, 4, 6, 18). This pattern of activity makes it very difficult to assess and compare the antimicrobial efficacies of different dosage schedules in small animals. The paramount difficulty is the interpretation of the results obtained in small animals with respect to humans. We and others have previously stressed that misleading results may be obtained if dosing intervals similar to those used clinically are employed with small animals (4, 5, 13). Imipenem, similar to all other antibiotics, is eliminated four times faster in mice than in humans. This pharmacokinetic difference cannot be compensated for by simply injecting higher doses of a drug into mice to obtain AUCs quantitatively similar to those observed with humans. Since it is not only the dose of the drug (visualized by the AUC) that affects antimicrobial efficacy but, in particular, the pharmacokinetic time profile (the shape of the AUC), human pharmacokinetic profiles must be approximated in experimental animals.

In the present study, such argumentation is well illustrated by a comparison of the efficacies obtained by the different shapes of murine and human-simulated AUCs of imipenem. The exceedingly high peak concentrations in the first 2 hours after bolus injections were clearly no more bactericidal than the broader albeit much lower concentrations indicated by the shape of the human-simulated, otherwise identical AUC. Moreover, the bolus injection was clearly less effective at preventing bacterial breakthrough growth than was the regimen indicated by the shape of the human-simulated AUC. In our view, these findings demonstrate once more the limits of conventional models of small-animal infection for the study of dosage intervals in antimicrobial research.

Our experimental mouse model proved to be suitable for simulating the human kinetics of imipenem after both i.v. and i.m. dosing. This was achieved by varying the fractionization of identical total amounts of imipenem. Although the shapes of the AUCs were different, the AUCs per se derived from both modes of simulated dosing were comparable. In addition, our study design allowed us to compare the effects of a single simulated human i.v. and a single simulated human i.m. injection of imipenem. Thus, the effect of a very high peak/MIC ratio as seen after simulated i.v. injection could be compared with the effect of lower peak/MIC ratios but covering a longer time above the MIC (i.m. injection). This comparison demonstrated again the superiority of a broad AUC of imipenem (which prevented early breakthrough growth) over a needlessly high-spiking concentration in plasma followed by a much longer sub-MIC time. Moreover, with two simulated i.v. doses resulting in the same aggregate time of supra-MIC levels as a single simulated i.m. dose, no better bactericidal activity could be obtained against either *P. aeruginosa* or *E. coli*.

We conclude that the human-simulated i.m. dosing of imipenem every 12 h is as effective as giving double the amount of total drug in two simulated i.v. doses. Although the animal model used was human adapted, it is clear that the results of our study cannot directly be extrapolated to humans. The ultimate proof of imipenem's efficacy after i.m. administration must come from clinical trials.

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