ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1991, p. 2085–2090
0066-4804/91/102085-06$02.00/0

Vol. 35, No. 10

Single Daily Dosing of Antibiotics: Importance of In Vitro Killing Rate, Serum Half-Life, and Protein Binding

GILLES POTEL,[1] NGUYEN P. CHAU,[2] BÉATRICE PANGON,[1] BRUNO FANTIN,[1] JEAN-MARIE VALLOIS,[1] FRANÇOIS FAURISSON,[1] AND CLAUDE CARBON[1]*

Institut National de la Santé et de la Recherche Médicale U13, Hôpital Claude-Bernard,[1] and Unité de Recherches Biostatistiques et Biomathématiques, Institut National de la Santé de la Recherche Médicale U263,[2] Université Paris VII, 75019 Paris, France

Received 4 March 1991/Accepted 5 August 1991

The relative importance of pharmacokinetic and pharmacodynamic parameters for the feasibility of a single daily dose (SDD) of antibiotics remains to be established. Therefore, we studied the relationship between in vitro bacteriological parameters (MIC, MBC, and killing rate [KR], defined as the reduction in the inoculum within 3 h), pharmacokinetic parameters ($t_{1/2}$ and protein binding [PB]), and the in vivo antibacterial effect of a single antibiotic dose in an experimental rabbit model of *Escherichia coli* endocarditis. Nine antibiotics were investigated: two aminoglycosides, two quinolones, and five β-lactams. For each drug, the minimal effective dose (MED) (in milligrams per kilogram) was defined as the lowest dose able to achieve a significant difference ($P < 0.05$) of CFU in the vegetations in comparison with controls 24 h after a single intravenous injection. Aminoglycosides and quinolones had the lowest MEDs, followed by β-lactams. Univariate regression analysis showed that KR was the major determinant of MED. A stepwise regression analysis showed that $t_{1/2}$ significantly improved the predictive value of KR, while PB, MIC, and MBC did not. The final equation was MED = 1,586 − 238 KR − 297 $t_{1/2}$ ($r = 0.90$, $P = 0.01$). We concluded that the pharmacodynamic parameters (especially the high KR) of aminoglycosides and quinolones explained their low MEDs and might allow SDD. In contrast, the low KR of β-lactams emphasized the critical importance of a long $t_{1/2}$, as for ceftriaxone, allowing the use of this β-lactam alone in SDD.

Single daily dosing of antibiotics appears to be a promising way to lighten therapy, reducing the work load for the nursing staff and inconvenience for the patient. For some drugs, such as aminoglycosides, efficacy and tolerance of single daily dosing have been demonstrated in different animal models (1, 11, 18) and in humans (20, 21, 28), despite their very short half-lives. This indicates that factors other than kinetic parameters play a major role in vivo in the feasibility of single daily doses. These factors include pharmacodynamic parameters, such as the dose-effect relationship (12, 19), and the presence of a postantibiotic effect (16). The conclusions of different studies suggest that some antibiotics, for example β-lactams, are mainly time-dependent, i.e., those with short half-lives are probably not suitable for single daily dosing (23), whereas others, such as aminoglycosides, are mainly concentration-dependent, i.e., single daily dosing is more effective than the same daily dose given in multiple injections (13). Thus, it appears that pharmacokinetic parameters, such as $t_{1/2}$, do not have the same influence on dosing interval, depending upon the antibiotic's bactericidal activity (i.e., time-dependent or concentration-dependent antibacterial activity). Furthermore, the killing rate (KR), determined in vitro by time-kill curves, might predict the bacteriological outcome of a treatment better than MIC or MBC. This point was underlined in a clinical study (9).

The aim of this study was to identify the bacteriological and pharmacokinetic parameters correlated with the in vivo antibacterial effect of a single intravenous (i.v.) dose of nine antibiotics from three different groups, by using an *Escherichia coli* endocarditis model in rabbits. Although this type of infection is uncommon in humans, this model provides a reliable and reproducible type of acute infection. Considered to be a rigorous test of in vivo antibiotic efficacy in a severe infection due to a gram-negative bacillus, the model appeared to be appropriate for comparing the effectiveness of different drugs (4). For each antibiotic, we determined the minimal effective dose (MED), i.e., the lowest dose allowing a significant ($P < 0.05$) in vivo bacterial reduction in vegetations. In order to identify the major elements determining the MED, the relationship between the bacteriological parameters (MIC, MBC, KR) and pharmacokinetic parameters ($t_{1/2}$, protein binding [PB]) of each antibiotic with MED was then evaluated by a stepwise regression analysis.

(This work was presented in part at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 1986 (27a) and at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Tex. 1989 (2a).

MATERIALS AND METHODS

Organisms. *E. coli* CB 1496, used to induce experimental endocarditis, was isolated from the blood of a patient with infective endocarditis. This strain was resistant to rabbit serum and was used in previous studies (3, 4, 11, 17, 23).

Antibiotics. The following nine drugs were tested: tobramycin (Eli-Lilly, Paris, France), netilmicin (Unicet-Unilabo, Paris, France), pefloxacin (Roger Bellon, Paris, France), ciprofloxacin (Bayer, Paris, France), amoxicillin (Beecham, Paris, France), ceftriaxone (Roche, Paris, France), moxalactam (Eli-Lilly, Paris, France), ceftazidime (Glaxo, Paris, France), and cefotaxime (Hoechst-Roussel, Paris, France).

In vitro studies. (i) Antibiotic sensitivity tests. The MIC and MBC of each antibiotic were determined by using a standard

* Corresponding author.

Mueller-Hinton broth dilution technique, with an inoculum of 10^5 CFU/ml in mid-exponential phase of growth. The MIC was defined as the lowest concentration of the drug which produced no visible growth after an 18-h incubation. The MBC was the lowest concentration which killed 99.9% of the bacteria after a 24-h incubation (24).

(ii) Killing curves. Time-kill curves were plotted for an inoculum of 10^7 *E. coli* per ml in Mueller-Hinton broth and concentrations of antibiotics equivalent to their MBCs, avoiding an antibiotic carryover with such a low concentration. Samples were removed after 1, 3, or 6 h of incubation and diluted serially. These dilutions were spread on agar plates and incubated for 24 h for colony counts. Each antibiotic was tested in triplicate and the mean value of bacterial reduction was calculated (Δlog CFU/ml in vitro).

Experimental endocarditis. (i) Rabbit model. In vivo studies were carried out with New Zealand female White rabbits (age range, 12 to 15 weeks; weight range, 2 to 3.5 kg). The animals were kept in individual cages and allowed free access to food and water throughout the experiment. Left ventricular endocarditis was induced as previously described (3, 23, 25). Twenty-four hours after a polyethylene catheter was inserted through the aortic valve, each rabbit was injected in the marginal ear vein with a 1-ml suspension containing 10^7 *E. coli* (CB 1496).

(ii) Experimental design. Treatment was initiated 48 h after infection (day 4). Each animal received a single i.v. injection of antibiotic and was killed 24 h later.

Initially, nine therapeutic regimens were tested with three drugs (in milligrams per kilogram of body weight) as follows: tobramycin, 24, 48, or 60; ceftriaxone, 200, 400, or 600; and pefloxacin, 30, 60, or 120.

In the second phase of the study, the other drugs (in milligrams per kilogram) were administered as follows, according to the results of the first phase: netilmicin, 48 or 60; ciprofloxacin, 30, 60, or 120; amoxicillin, 600 or 900; cefotaxime, 600 or 900; ceftazidime, 600 or 900; moxalactam, 400 or 600.

In order to study a possible bacterial regrowth between the 12th and 24th hour, animals were sacrificed after 12 h for the following regimens (in milligrams per kilogram): tobramycin, 48; pefloxacin, 60; and moxalactam, 600.

The control group consisted of eight untreated rabbits.

(iii) Evaluation of therapy. As detailed above, the effect of treatment was evaluated 12 or 24 h after an i.v. injection by measuring the bacterial titers in vegetations, as previously described (4, 11, 17, 23). In each case, at the time of sacrifice, the heart was removed and vegetations were excised and rapidly rinsed in sterile saline. Parts of the vegetations were weighed and homogenized with a Thomas teflon pestle tissue homogenizer, with 0.5 ml of sterile saline. Serial dilutions were spread and quantitatively cultured on Mueller-Hinton agar plates for 24 h at 37°C. CFU were counted and bacterial titers were expressed as $\log_{10}$ CFU/g of vegetations ($\log_{10}$ CFU/g). An antibiotic carryover was avoided because of the low level of the residual concentration in the diluted homogenates (10^{-2} and 10^{-4}) necessary to accurately count bacteria, since antibiotics for which carryover may be questionable were not very effective.

We were able to detect as few as 10 CFU/ml. Because of this sensitivity limit, vegetations found to be sterile were considered to contain 10 CFU/ml of homogenate, and the value integrated into the calculation of the mean took into account the weight of vegetations. Part of each vegetation was frozen until antibiotic assays were run. In order to analyze the in vivo antibacterial effect and its relationship with pharmacokinetics, residual concentrations in vegetations were assayed at the time of sacrifice.

Antibiotic concentrations were measured by the agar diffusion method. After being weighed and homogenized in 0.3 ml of 0.1 M phosphate buffer, vegetations were centrifuged and supernatant samples were taken for the microbiological assay, as previously described (17). Each sample was tested in duplicate. The following strains were used: *Bacillus subtilis* ATCC 6633 for tobramycin and netilmicin; *E. coli* 1976-712 (provided by Bayer Laboratories) for pefloxacin and ciprofloxacin; *E. coli* ATCC 10536 for moxalactam; and *E. coli* ATCC 25922 for ceftriaxone, cefotaxime, ceftazidime, and amoxicillin. The limits of sensitivity and the range of linearity of measurable concentrations were as follows (in micrograms per milliliter): 0.07 to 2.5 for netilmicin and tobramycin; 0.07 to 5 for pefloxacin and 0.01 to 0.3 for ciprofloxacin; 0.6 to 10 for moxalactam; and 0.02 to 1.25 for ceftriaxone, cefotaxime, ceftazidime, and amoxicillin. For each drug, the level of concentration (when detectable) in the vegetations was within the range of linearity.

Pharmacokinetic parameters. Three infected animals were assigned to each antibiotic to determine the pharmacokinetics of each drug at the MED. A catheter was inserted into the left femoral artery to take serum samples 0.5, 1, 2, 4, 6, 8, and 24 h after the i.v. bolus. Concentrations of each antibiotic in serum were determined by using a microbiological assay, with strains other than those used to determine the concentrations in the vegetations. A *B. subtilis* strain (HN 841269) was used for all antibiotics except for moxalactam (*Proteus mirabilis* HN 841267) and amoxicillin (*Micrococcus* sp.). When necessary, a proper dilution was performed in order to detect a concentration within the range of linearity. $t_{1/2}$ was then calculated by using a monocompartmental model.

PB in the rabbit serum was taken from the literature for each drug studied.

Statistical evaluation. Bacterial titers in vegetations were expressed as means ± standard deviations. Statistical evaluation was performed by nonparametric tests (Kruskal-Wallis test followed by Mann-Whitney U test). For each drug, the MED was defined as the lowest dosage able to induce a significant bacterial reduction in vegetations compared with that in controls. Because of the size of each group and the level of significance desired ($P < 0.05$), this effect corresponded approximately to a 2 $\log_{10}$ CFU drop per g of vegetations in comparison with the amount in controls. The bacteriological (MIC, MBC, KR) and pharmacokinetic ($t_{1/2}$, PB) parameters were correlated with in vivo efficacy (MED) by using univariate and stepwise multilinear regression analysis, in order to take into account the interdependence between these parameters (8).

RESULTS

In vitro antibiotic sensitivity. (i) MIC-MBC. The nine drugs were active against the *E. coli* strain studied (Table 1). The aminoglycoside MBCs were four times their MICs. For the other compounds, the MBCs were twice (or equal to) the MICs.

(ii) Killing curves. The rate at which *E. coli* CB 1496 in standard broth culture was killed by the drugs at concentrations equal to their MBCs is shown in Fig. 1. From these curves, the KR for each drug can be defined as the Δlog CFU/ml between the initial inoculum and viable bacteria after 1, 3, or 6 h of incubation in the MBC. KR 1 h was not significant for β-lactams while there was a significant drop

TABLE 1. In vitro susceptibility of *E. coli* CB 1496 to the antibiotics studied

Antibiotic	MIC (μg/ml)	MBC (μg/ml)
Aminoglycosides		
Tobramycin	1	4
Netilmicin	1	4
Quinolones		
Pefloxacin	0.25	0.5
Ciprofloxacin	0.125	0.125
β-lactams		
Ceftriaxone	0.06	0.06
Moxalactam	0.5	1
Ceftazidime	0.125	0.125
Cefotaxime	0.25	0.5
Amoxicillin	4	8

for quinolones and aminoglycosides (KR 1 h to 3 and 5 h, respectively). At 6 h, the culture was sterile for aminoglycosides. For these reasons, the KR 3 h appeared to be the most discriminating interval for comparing drugs, since it allowed the most accurate measure of a significant antibacterial effect.

Experimental endocarditis. Results of the different regimens used are shown in Table 2. The lowest MEDs were obtained with aminoglycosides (48 mg/kg) and quinolones (60 mg/kg), followed by ceftriaxone (400 mg/kg), moxalactam (600 mg/kg), and ceftazidime (900 mg/kg). Aminoglycosides were the only antibiotics able to sterilize more than 50% of the vegetations. This result was achieved with a 60-mg/kg dose. In contrast, neither quinolones (120 mg/kg) nor β-lactams (400 to 900 mg/kg) were able to do so. Increasing the aminoglycoside dose from 48 to 60 mg/kg induced an increased in vivo bactericidal effect, whereas for quinolones, no advantage was observed when the dosage was increased from 60 to 120 mg/kg. Most important, for β-lactams, no MED could be obtained despite very high doses of cefotaxime (900 mg/kg) and amoxicillin (900 mg/kg). Ceftriaxone, moxalactam, and ceftazidime were the only β-lactam compounds able to reach their MEDs (400, 600, and 900 mg/kg, respectively). Increasing the ceftriaxone dose from 400 to 600 mg/kg did not improve in vivo efficacy.

For aminoglycosides, the residual concentration in vegetations was approximately equal to the MIC (0.9 ± 0.2 and 1.1 ± 0.3 μg/g of vegetation for tobramycin and netilmicin, respectively). In contrast, for quinolones, residual concentrations were 14- to 24-fold the MIC (3.6 ± 1 and 3.0 ± 5.4 μg/g of vegetation for pefloxacin and ciprofloxacin, respectively). Of the β-lactams, ceftriaxone and ceftazidime exhibited the highest residual concentrations in terms of multiples of their MICs (7.9 ± 3.1 and 4.5 ± 2.5 μg/g of vegetation; 131- and 36-fold their respective MICs). For amoxicillin, the residual concentration was approximately twice the MIC (6.8 ± 1.6 μg/g of vegetation; 1.7-fold the MIC). For cefotaxime and moxalactam, residual concentrations could not be determined by our method. They corresponded to levels lower than the MICs (<0.14 μg/g of vegetation) for cefotaxime and possibly below the MIC for moxalactam (<4.2 μg/g of vegetation), for a mean vegetation weight of 50 mg.

In order to analyze the kinetics of the in vivo bacterial killing between 12 and 24 h, animals receiving the MED (for one drug from each group of antibiotic) were sacrificed at 12 h. For the three antibiotics tested, bacterial titers in vegetations were comparable at 12 and 24 h: 6.5 ± 1.7 versus 6.1 ± 1.9 log CFU/g of vegetation for tobramycin (48 mg/kg), 7.2 ± 0.7 versus 6.4 ± 0.6 log CFU/g of vegetation for pefloxacin (60 mg/kg), and 6.8 ± 1.5 versus 6.7 ± 1.6 log CFU/g of vegetation for moxalactam (600 mg/kg).

Predictive value of microbiological and pharmacokinetic parameters. MEDs, KR 3 h, serum half-lives, and PB for the nine antibiotics studied are listed in Table 3. Aminoglycosides had short half-lives (0.8 and 0.7 h for tobramycin and netilmicin, respectively), whereas quinolones exhibited longer ones (1.6 and 1.8 h for pefloxacin and ciprofloxacin, respectively). Of the β-lactams, ceftriaxone had the longest half-life (3.0 h), followed by ceftazidime (1.2 h).

Data from the literature indicate that aminoglycosides and quinolones are weakly bound (range, 0 to 25%). Ceftriaxone and cefotaxime are strongly bound, ceftazidime is weakly bound, and levels of moxalactam and amoxicillin fixation are intermediate in rabbits.

Univariate regression analysis showed that neither MIC and MBC nor $t_{1/2}$ and PB significantly correlated with MED for the seven molecules with an achievable MED. The only parameter which fit with MED was KR 3 h ($F = 15.0$; $r = 0.86$; $P = 0.01$). A stepwise regression analysis showed that neither $t_{1/2}$ nor PB significantly improved the predictive value of KR 3 h. Nevertheless, when the maximum doses used with cefotaxime and amoxicillin were integrated for the calculation of the stepwise regression analysis, then $t_{1/2}$ significantly improved the predictive value of KR, and the equation derived was MED = 1586 − 238 KR 3 h − 297 $t_{1/2}$, where MED is measured in milligrams per kilogram, KR 3 h is measured as Δ log CFU/3 h, and $t_{1/2}$ is measured in hours. Predicted MEDs, calculated from this equation were plotted versus observed MEDs (or highest doses used) in Fig. 2. This graph clearly distinguished 2 groups of drugs: aminoglycosides and quinolones with the lowest MEDs and β-lac-

TABLE 2. In vivo results in vegetations from *E. coli* CB 1496-infected rabbits 24 h after a single i.v. injection of antibiotic

Antibiotics	Dose[a] (mg/kg)	$\log_{10}$ CFU/g of vegetation (mean ± SD)	Animals with sterile vegetation (no./total)
Tobramycin	24 ($n = 5$)	8.3 ± 0.3	0/5
	48 ($n = 8$)	6.1 ± 1.9[b]	1/8
	60 ($n = 7$)	4.0 ± 2.2[b]	5/7
Netilmicin	48 ($n = 6$)	5.4 ± 0.7[b]	3/6
	60 ($n = 7$)	4.0 ± 1.6[b]	4/7
Pefloxacin	30 ($n = 6$)	7.4 ± 0.6	0/6
	60 ($n = 8$)	6.4 ± 0.6[b]	0/8
	120 ($n = 7$)	5.8 ± 1.9[b]	0/7
Ciprofloxacin	30 ($n = 8$)	6.9 ± 1.2	0/8
	60 ($n = 9$)	5.0 ± 1.9[b]	0/9
	120 ($n = 9$)	5.6 ± 1.7[b]	0/9
Ceftriaxone	200 ($n = 6$)	7.8 ± 1.2	0/6
	400 ($n = 9$)	6.9 ± 1.7[c]	0/9
	600 ($n = 9$)	6.4 ± 2.2[c]	1/9
Moxalactam	400 ($n = 6$)	8.0 ± 1.6	0/6
	600 ($n = 7$)	6.7 ± 1.6[c]	0/7
Cefotaxime	600 ($n = 6$)	7.6 ± 1.9	0/6
	900 ($n = 6$)	8.2 ± 1.9	0/6
Ceftazidime	600 ($n = 7$)	7.3 ± 1.0	0/7
	900 ($n = 7$)	6.8 ± 1.1[c]	0/7
Amoxicillin	600 ($n = 7$)	8.5 ± 1.1	0/7
	900 ($n = 6$)	7.7 ± 1.2	0/6
Controls	($n = 8$)	8.8 ± 1.0	0/8

[a] The lowest dose exhibiting a significant antibacterial effect in comparison with controls corresponded to the MED.
[b] $P < 0.01$ in comparison with controls.
[c] $P < 0.05$ in comparison with controls.

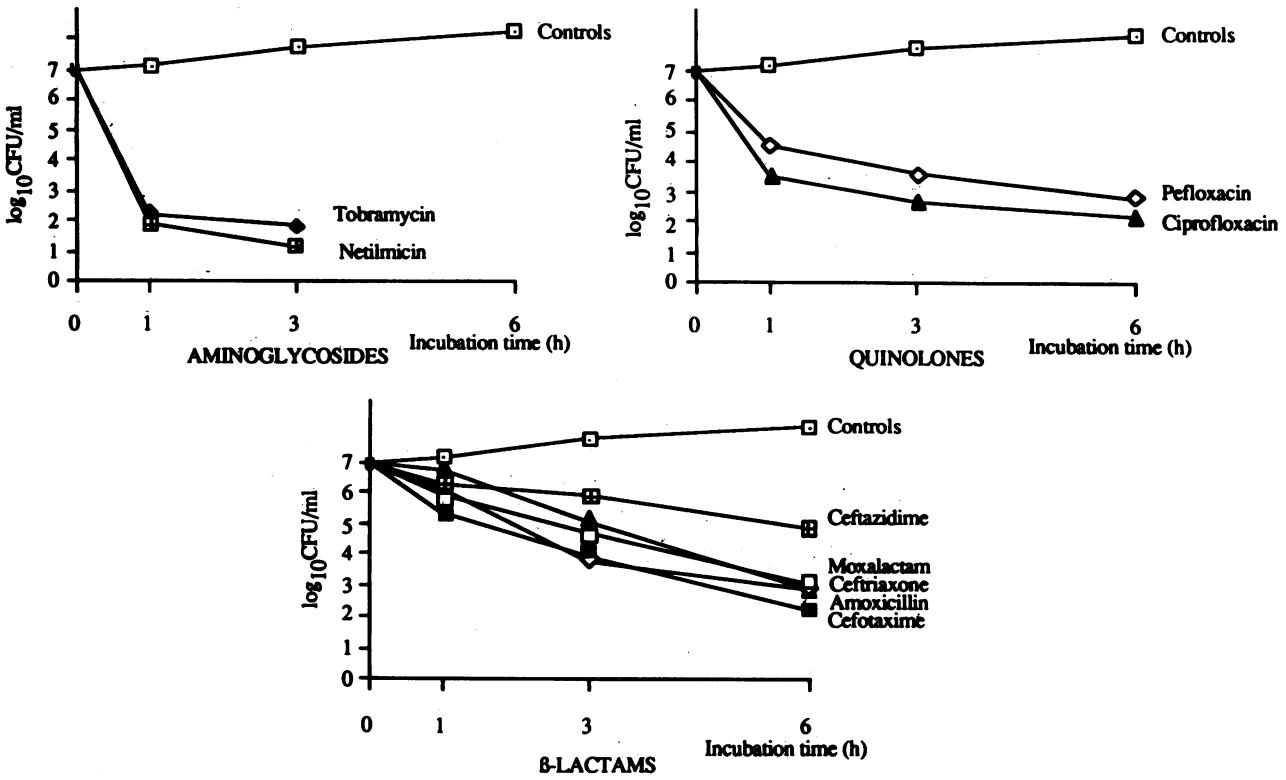

FIG. 1. In vitro killing of *E. coli* incubated with each antibiotic at a concentration equal to its MBC.

tam compounds. Ceftriaxone appeared to be intermediate between those two groups.

DISCUSSION

This model of experimental endocarditis was previously used, among other purposes, to evaluate the relationship between the bacterial titer and the antibiotic level in the infected site (3, 4, 17, 23) when repeated doses of antibiotics were given. For this study, we decided to use a single dose of each drug in order to simplify the approach of the relationship between the rapidity of in vitro killing, in vitro susceptibility tests, pharmacokinetic parameters, and in vivo efficacy in the perspective of a single daily dose. For the analysis of the data, we decided to consider residual antibiotic concentrations in vegetations in order to be able to interpret the in vivo results in terms of MIC multiples at the site of infection and to analyze the possibility of a postantibiotic effect. This approach was probably more accurate than the analysis of the residual levels in serum, because of the uncertainty of the similarity between concentrations in serum and vegetations 24 h after such a high dosage. Furthermore, a monocompartmental model was used to calculate the $t_{1/2}$ of the drugs. This simplified approach probably underestimated the terminal half-lives of most of the antibiotics tested and vegetation levels 24 h after injection.

TABLE 3. MED in vitro bactericidal activity (KR 3 h) and pharmacokinetic parameters ($t_{1/2}$, PB) for nine antibiotics in an *E. coli* endocarditis model in rabbits

Antibiotic	MED (mg/kg)	KR 3 h[a] (Δlog CFU/ml/3 h)	$t_{1/2}$[a] (h)	PB[b] (%)
Tobramycin	48	5.1 ± 0.6	0.8 ± 0.1	0 (6)
Netilmicin	48	5.7 ± 0.2	0.7 ± 0.1	15 (2)
Pefloxacin	60	3.3 ± 0.3	1.6 ± 0.2	25 (3)
Ciprofloxacin	60	4.3 ± 0.2	1.8 ± 0.2	25 (22)
Ceftriaxone	400	1.9 ± 0.2	3.0 ± 0.3	98 (15)
Moxalactam	600	2.3 ± 0.2	0.8 ± 0.2	60 (6)
Ceftazidime	900	1.1 ± 0.5	1.2 ± 0.2	14 (6)
Amoxicillin[c]	>900	3.2 ± 0.3	0.5 ± 0.1	17 (6)
Cefotaxime[c]	>900	3.1 ± 0.6	0.5 ± 0.1	93 (14)

[a] KR 3 h represents the mean reduction of the bacterial inoculum (Δlog CFU/ml) after 3 h of in vitro incubation at a concentration of each drug equal to its MBC. Values for KR 3 h and $t_{1/2}$s are expressed as means ± standard deviations.

[b] Numbers in parentheses indicates the references from which the data were taken.

[c] MED was not reached with the indicated dose.

Our major in vivo observation was that aminoglycosides and quinolones had the lowest MEDs. The explanations for these results were probably different for these two classes of antibiotics. The low MEDs of aminoglycosides could be explained by their high KR in vitro, despite very short half-lives. The low MEDs of quinolones could be explained by their high KR in vitro and prolonged serum half-lives. Furthermore, quinolones provided high residual concentrations in vegetations at the time of sacrifice, probably because of their long half-lives. They have also been shown to have good tissue penetration (3). It must be stressed that for these two classes of antibiotics, the postantibiotic effect could be excluded from the analysis, since residual concentrations in vegetations were around the MIC for aminoglycosides (5), and because of the absence of sterile vegetations for the quinolones. The different drugs tested in these two groups of antibiotics exhibited very similar MED values. Neverthe-

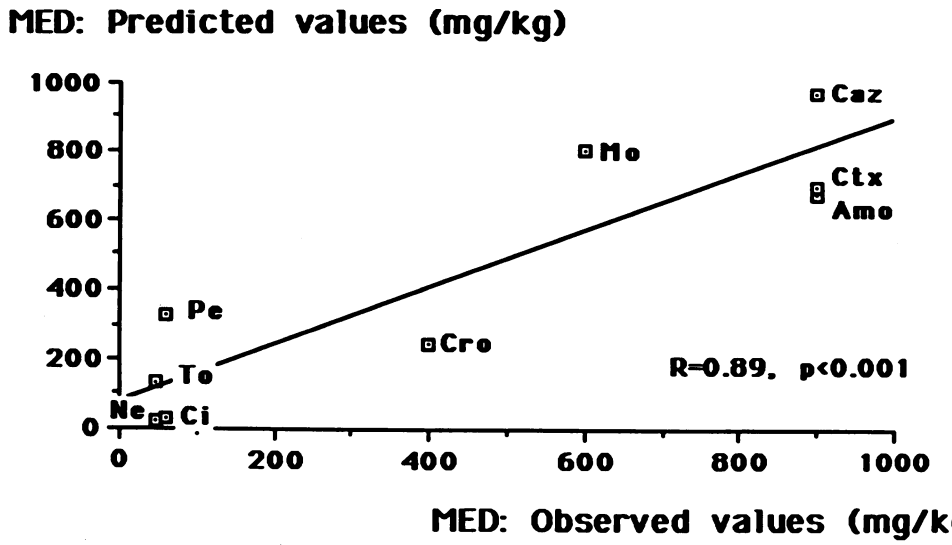

FIG. 2. Observed values of MEDs plotted versus values derived from the stepwise regression analysis. Abbreviations: To, tobramycin; Ne, netilmicin; Pe, pefloxacin; Ci, ciprofloxacin; Cro, ceftriaxone; Mo, moxalactam; Caz, ceftazidime; Ctx, cefotaxime; Amo, amoxicillin.

less, if the dose dependence of the aminoglycosides appeared clearly in this model (60 versus 48 mg/kg; see Results), then the prolonged $t_{1/2}$ was probably the major determinant of the MED of quinolones (120 versus 60 mg/kg; see Results).

On the other hand, among β-lactams, a great dispersion of MEDs was observed. Ceftriaxone exhibited the lowest MED, probably due to its very long half-life, allowing very high residual concentrations in vegetations (>100-fold the MIC). In contrast, MEDs could not be obtained for cefotaxime and amoxicillin. Their very short half-lives probably explained residual concentrations in vegetations around or below their MICs. For ceftazidime, a serum half-life intermediate between other β-lactams and ceftriaxone and a high residual concentration in vegetations (>30-fold the MIC) allowed it to reach the MED. Moxalactam exhibited a lower MED than ceftazidime did, despite a shorter half-life and a lower residual concentration in vegetations, probably because of its higher KR in vitro (KR 3 h, 2.3 log CFU/ml).

Although we studied nine antibiotics from three different classes, with different pharmacodynamic and pharmacokinetic properties, it was possible to correlate the in vivo antibacterial effect (expressed as MEDs) with 2 parameters: KR in vitro (expressed as KR 3 h) and serum half-life ($t_{1/2}$). Our results do not agree with those of Frimodt-Moller et al. (12), who found a significant correlation between the MIC and the 50% effective dose in a mouse model of *Streptococcus pneumoniae*-induced peritonitis, but these authors studied only cephalosporins with a large range of MICs (0.02 to 25 μg/ml). In the study by Legett et al. (19), the MIC was found to be an excellent predictor of the relative in vivo potency of three β-lactams and two aminoglycosides. It must be stressed that the pharmacokinetics of these drugs in mice were quite similar; nevertheless, the authors suggested that different results might be obtained with drugs that display markedly different elimination rates, as observed in the rabbit model.

The in vitro KR expressed as KR 3 h was the parameter which best fit with the MED. A correlation between the in vitro KR and the in vivo antibacterial effect in gram-negative infections has already been described, with tobramycin versus two strains of *Enterobacter cloacae* in the same experimental model (26) and with moxalactam versus several gram-negative bacilli in human meningitis (10). Moreover, the predictive value of in vitro killing of aminoglycosides (gentamicin, tobramycin, and amikacin) has been observed with the same model, by using a *Serratia marcescens* strain (27).

Despite the fact that the MEDs of amoxicillin and cefotaxime were not reached, the maximum doses used (900 mg/kg) were integrated in the stepwise regression analysis, allowing the role of a short $t_{1/2}$ to be exhibited as a predictive parameter for a high MED.

The half-lives of the drugs seem to play a key role only for molecules with a low KR, i.e., β-lactams, for which the in vivo effect depends on the duration of concentrations above the MIC (19, 29). It is noteworthy that with the same model, a 4-day regimen of 30 mg/kg i.v. per day of ceftriaxone caused the sterilization of most vegetations, emphasizing the importance of the duration of bacterium-antibiotic contact in vivo for the efficacy of β-lactams (17). In their study, Frimodt-Moller et al. did not find a correlation between the 50% effective dose and $t_{1/2}$, but it has to be stressed that their studies were performed with mice, in which the $t_{1/2}$s of all drugs are particularly short. That is probably the reason why the influence of the $t_{1/2}$ did not appear clearly in their study, except for with ceftriaxone. For quinolones, a prolonged half-life and a high KR combine favorable pharmacodynamic and pharmacokinetic parameters.

The influence of PB on the MED level did not appear clearly in this study. Nevertheless, it has been noted that aminoglycosides have the lowest serum PB, associated with the best KR in vitro and a homogeneous distribution throughout the vegetations (7). Moreover, an increase in the $t_{1/2}$ must be emphasized for strongly bound compounds such as ceftriaxone. In this study, the $t_{1/2}$ found for ceftriaxone at 400 mg/kg was similar to that observed with lower doses (3.0 versus 3.5 h) (11), indicating that this high dose was not responsible for significant modifications of the kinetics of the drug.

Finally, several factors may contribute to the feasibility of single daily dose. Of them, the KR in vitro might be an important one. For single-drug therapy, aminoglycosides, which associate a high KR with a dose-dependent antimicro-

bial activity, are the most suitable, followed by quinolones and long-acting cephalosporins. Of course, this experimental design did not take into account toxicological parameters, which must be investigated when considering the feasibility of a single daily dose in humans. These results were obtained with an animal model by using *E. coli*. Further investigations are warranted with other bacterial strains, i.e., other gram-negative bacilli or gram-positive cocci. The in vivo relevance of the in vitro KR also remains to be established in other types of experimental infections and in humans.

ACKNOWLEDGMENTS

We thank M. O. Hervy and N. Muller for helpful secretarial assistance.

This work was supported by grants from Institut National de la Santé et de la Recherche Médicale U13, Hôpital Claude-Bernard, Paris, France.

REFERENCES

1. **Bennett, W. M., C. E. Plamp, D. N. Gilbert, R. A. Parker, and G. A. Porter.** 1979. The influence of dosage regimen on experimental gentamicin nephrotoxicity: dissociation of peak serum levels from renal failure. J. Infect. Dis. **140**:576–580.
2. **Brion, N., J. Barge, I. Godefroy, F. Dromer, C. Dubois, A. Contrepois, and C. Carbon.** 1984. Gentamicin, netilmicin, dibekacin, and amikacin nephrotoxicity and its relationship to tubular reabsorption in rabbits. Antimicrob. Agents Chemother. **25**:168–172.

2a. **Chau, N. P., G. Potel, B. Fantin, J. M. Vallois, and C. Carbon.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 942.

3. **Contrepois, A., C. Daldoss, B. Pangon, J. J. Garaud, M. Kecir, C. Sarrazin, J. M. Vallois, and C. Carbon.** 1984. Pefloxacin in rabbits: protein binding, extravascular diffusion, urinary excretion and bactericidal effect in experimental endocarditis. J. Antimicrob. Chemother. **14**:51–57.
4. **Contrepois, A., J. M. Vallois, J. J. Garaud, B. Pangon, J. Mohler, A. Meulemans, and C. Carbon.** 1986. Kinetics and bactericidal effects of gentamicin and moxalactam in experimental *E. coli* endocarditis. J. Antimicrob. Chemother. **17**:227–231.
5. **Craig, W. A., and S. Gudmundsson.** 1986. The postantibiotic effect, p. 515–536. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
6. **Craig, W. A., and B. Suh.** 1986. Protein binding and the antimicrobial effects, p. 477–514. *In* V. Lorian (ed), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
7. **Cremieux, A. C., B. Mazière, J. M. Vallois, M. Ottaviani, A. Azancot, H. Raffoul, A. Bouvet, J. J. Pocidalo, and C. Carbon.** 1989. Evaluation of antibiotic diffusion into cardiac vegetations by quantitative autoradiography. J. Infect. Dis. **159**:938–944.
8. **Draper, N. R., and H. Smith.** 1981. Applied regression analysis, p. 294–352. John Wiley & Sons, Inc., New York.
9. **Eng, R. H., C. E. Cherubin, J. C. Pechere, and T. R. Beam.** 1987. Treatment failures of cefotaxime and latamoxef in meningitis caused by *Enterobacter* and *Serratia* spp. J. Antimicrob. Chemother. **20**:903–911.
10. **Eng, R. H. K., C. Cherubin, S. M. Smith, and F. Buccini.** 1984. Examination of gram-negative bacilli from meningitis patients who failed or relapsed on moxalactam therapy. Antimicrob. Agents Chemother. **26**:850–856.
11. **Fantin, B., B. Pangon, G. Potel, J.-M. Vallois, F. Caron, A. Bure, and C. Carbon.** 1989. Ceftriaxone-netilmicin combination in single-daily-dose treatment of experimental *Escherichia coli* endocarditis. Antimicrob. Agents Chemother. **33**:767–770.
12. **Frimodt-Moller, N., M. W. Bentzon, and V. F. Thomsen.** 1986. Experimental infection with *Streptococcus pneumoniae* in mice: correlation of in vitro activity and pharmacokinetic parameters with in vivo effect for 14 cephalosporins. J. Infect. Dis. **154**:511–517.
13. **Gerber, A. U., H. P. Brugger, C. Feller, T. Stritzko, and B. Stalder.** 1986. Antibiotic therapy of infections due to *Pseudomonas aeruginosa* in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. J. Infect. Dis. **153**:90–97.
14. **Gerding, D. L., L. Van Etta, and L. R. Peterson.** 1982. Role of serum protein binding and multiple antibiotic doses in the extravascular distribution of ceftizoxime and cefotaxime. Antimicrob. Agents Chemother. **22**:844–847.
15. **Henning, C., and S. E. Holm.** 1981. Penetration of 14C-Ro 13-9904 into tissue cage fluid in rabbits. Chemotherapy (Basel) **27**(Suppl. 1):32–36.
16. **Ingerman, M. J., P. G. Pitsakis, A. F. Rosenberg, and M. E. Levison.** 1986. The importance of pharmacodynamics in determining the dosing interval in therapy for experimental *Pseudomonas aeruginosa* endocarditis in the rat. J. Infect. Dis. **153**:707–714.
17. **Joly, V., B. Pangon, J. M. Vallois, L. Abel, N. Brion, A. Bure, N. P. Chau, A. Contrepois, and C. Carbon.** 1987. Value of antibiotic levels in serum and cardiac vegetations for predicting antibacterial effect of ceftriaxone in experimental *Escherichia coli* endocarditis. Antimicrob. Agents Chemother. **31**:1632–1639.
18. **Kapusnik, J. E., G. J. Hackbarth, H. F. Chambers, T. Carpenter, and M. A. Sande.** 1988. Single, large, daily dosing vs intermittent dosing of tobramycin for treating experimental *Pseudomonas* pneumonia. J. Infect. Dis. **158**:7–12.
19. **Legett, J. E., B. Fantin, S. Ebert, K. Totsuka, B. Vogelman, W. Calame, H. Mattie, and W. A. Craig.** 1989. Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh-infection models. J. Infect. Dis. **159**:281–292.
20. **Maller, R., B. Isaksson, L. Nilsson, and L. Soren.** 1988. A study of amikacin given once vs twice daily in serious infections. J. Antimicrob. Chemother. **22**:75–79.
21. **Moore, R. D., P. S. Lietman, and C. R. Smith.** 1987. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. J. Infect. Dis. **155**:93–99.
22. **Nichol, K. L., L. R. Peterson, C. E. Fasching, and D. N. Gerding.** 1984. Ciprofloxacin penetration into extravascular spaces in a rabbit model. Antimicrob. Agents Chemother. **26**:935–936.
23. **Pangon, B., V. Joly, J.-M. Vallois, L. Abel, A. Buré, N. Brion, A. Contrepois, and C. Carbon.** 1987. Comparative efficacy of cefotiam, cefmenoxime, and ceftriaxone in experimental endocarditis and correlation with pharmacokinetics and in vitro efficacy. Antimicrob. Agents Chemother. **31**:518–522.
24. **Pearson, R. D., R. T. Steibigel, H. T. Davis, and S. W. Chapman.** 1980. Method of reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18**:699–708.
25. **Perlman, B. B., and L. R. Freedman.** 1971. Experimental endocarditis. II. Staphylococcal infection of the aortic valve following placement of a polyethylene catheter in the left side of the heart. Yale J. Biol. Med. **44**:206–213.
26. **Potel, G., J. Caillon, D. Bugnon, H. B. Drugeon, and D. Baron.** 1988. Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 373.
27. **Potel, G., J. Caillon, B. Fantin, J. Raza, F. Le Gallou, J.-Y. Lepage, P. Le Conte, D. Bugnon, D. Baron, and H. B. Drugeon.** 1991. Impact of dosage schedule on the efficacy of gentamicin, tobramycin, or amikacin in an experimental model of *Serratia marcescens* endocarditis: in vitro-in vivo correlation. Antimicrob. Agents Chemother. **35**:111–116.

27a. **Potel, G., B. Pangon, B. Fantin, J. M. Vallois, F. Faurisson, and C. Carbon.** 1986. Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 575.

28. **Powell, S. H., W. L. Tompson, M. A. Luthe, R. C. Stern, D. A. Grossniklaus, D. D. Bloxham, D. L. Hroden, M. R. Jacobs, A. O. Discenna, H. A. Cash, and J. D. Klinger.** 1983. Once-daily vs continuous aminoglycoside dosing: efficacy and toxicity in animal and clinical studies of gentamicin, netilmicin and tobramycin. J. Infect. Dis. **147**:918–931.
29. **Vogelman, B., S. Gudmundsson, J. Legett, J. Turnidge, S. Ebert, and W. A. Craig.** 1988. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. J. Infect. Dis. **158**:831–847.