Simulation of Human Gentamicin Pharmacokinetics in an Experimental *Enterococcus faecalis* Endocarditis Model

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Significant differences between animal and human pharmacokinetics may be responsible for the conflicting results of experimental studies. This study determined the impact of human pharmacokinetic simulation (HPS) on gentamicin activity in an *Enterococcus faecalis* endocarditis model. The decrease in bacterial counts was greater with HPS than with a dose-equivalent regimen without HPS.

Enterococcal organisms are a frequent cause of nosocomial infections (2, 3, 23, 25, 32, 35). While a synergistic combination involving a cell wall-active agent with an aminoglycoside is customarily recommended (6, 25), recent reports suggest a genuine activity of the latter alone (17, 30). Interspecies variations in metabolism and pharmacokinetic properties have been observed for the same drug (15, 20, 29). Thus, experimental models with animal native kinetics may not ensure reliable assessment for human therapeutics (11), and the choice of treatment schedule can have a dramatic influence, especially in the field of antibacterial agents (33). Notably, the elimination rate is usually faster in small animals than in humans (8, 13, 21, 31). This may explain the poor results obtained in experimental models. To circumvent this drawback, the dosage in the animal is often increased according to the clearance ratio, resulting in a similar area under the concentration-time curve (AUC), but at the expense of a considerable rise in the peak.

The purpose of the present study was to compare this latter dosage adjustment with a true simulation of the human pharmacokinetics (HPS), as previously described (1, 14, 22, 36), and to determine the impact of HPS on the antibacterial effect of gentamicin in an experimental model of enterococcal endocarditis.

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In vitro studies. MICs and MBCs of gentamicin (Schering-Plough Laboratories, Paris, France) were determined by the microdilution technique in Mueller-Hinton broth (MHB) and in MHB with serum (MHBS) for the two clinical strains of *Enterococcus faecalis* studied, HM 1061 and JH2-2 (16, 17). For both strains, MICs and MBCs were identical, equal to 8 and 4 mg/liter in MHB and MHBS, respectively (24).

In vivo studies. In vivo studies were carried out with New Zealand White female rabbits (CEGAV, St. Mars-d'Egrenne,

France) approved by the animal study committee. Experimental endocarditis was induced as previously described (9, 26) with an inoculum of 10^8 CFU of either *E. faecalis* HM 1061 o*r E. faecalis* JH2-2. For each strain, animals were randomly assigned to nine groups, including an untreated control group and eight therapeutic groups, according to a crossed design with doses of 16.6 or 33.2 mg/kg of body weight/day producing either native or human-like pharmacokinetics for 3 or 5 days. The doses administered corresponded to those required to reproduce an AUC in the rabbit similar to that obtained in humans with dosages of 3 or 6 mg/kg/day, respectively. Gentamicin was diluted in 9% sodium chloride for infusion in a marginal ear vein. Animals treated with native pharmacokinetics received the dose in a 30-min infusion. Animals treated by HPS received a similar dose of gentamicin by means of a computer-controlled pump regulated to reproduce in the rabbit the plasma pharmacokinetics observed in humans (4, 5). The intended serum drug concentration profile was based on a one-compartment simplification of human pharmacokinetics without reproduction of the initial distribution α phase after an intravenous bolus. Thus, it consisted of a single exponential decrease with a half-life of 2 h and an initial concentration of 18 or 35 mg/liter for the human-like doses of 3 or 6 mg/kg, respectively.

Gentamicin was assayed by an immunoenzymatic method (Emit 2000 gentamicin test; Dade Behring, Paris, France), with a detection threshold of 0.3 mg/liter. The intra-assay coefficient of variation was between 1.7 and 3.2%, and the interassay coefficient was between 3.7 and 4.3%. Pharmacokinetic parameters were calculated by nonlinear regression according to a two-compartment model for native kinetics and a one-compartment model for HPS kinetics, according to the expected simulation of a monoexponential decay. The pharmacokinetic parameters are indicated in Table 1 for each mode of gentamicin administration. Figure 1 shows the native and human-like plasma kinetics actually observed in rabbits and the theoretical human pharmacokinetics. Residual plasma drug concentrations after 3 and 5 days of treatment with gentamicin at 33 mg/kg/day in HPS were 0.65 ± 0.06 and 0.47 ± 0.12 mg/liter, respectively. The AUC was calculated by the least-

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TABLE 1. Pharmacokinetic results (C_{max}, C_{max}/MIC, β half-life, and AUC) for the four modes of gentamicin administration in this study

Total daily dose (mg/kg)	Pharmacokinetics	C_{max} (mg/liter)	C_{max}/MIC	β half-life (h)	AUC (mg/h/liter)
16.6	HPS (3 mg/kg/day)	22.4 ± 6.8	1.4	2.03 ± 0.46	64.0 ± 17.4
	Native	64.4 ± 6.6	4	0.98 ± 0.19	77.6 ± 5.2
33.2	HPS (6 mg/kg/day)	48.7 ± 4.0	3	1.63 ± 0.29	115.70 ± 30.8
	Native	90.1 ± 8.0	5.6	0.97 ± 0.12	109.2 ± 7.5

squares regression method. Animals were sacrificed (100 mg of thiopental) at the end of 3 or 5 days of treatment for bacterial count in the vegetation. Quantitation was performed after a 24-h culture on Trypticase soy agar plates of serial dilutions of a homogenate of the vegetations. The limit of detection was 2 $\log_{10}$ CFU/g of vegetation. No significant carryover effect was expected, because the gentamicin concentrations anticipated in serum and vegetation were far less than the MIC.

Experimental groups were compared by analysis of variance plus a Bonferroni test for intergroup comparisons (Statview; Abacus Concepts). A notable difference was observed for the HM 1061 strain (Table 2), depending on whether human kinetics was simulated or not. Gentamicin administration with
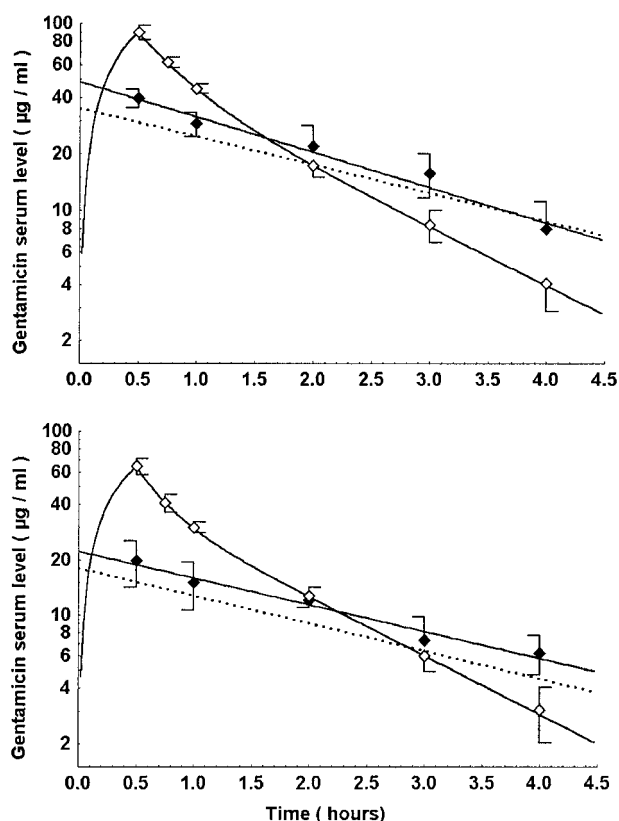

FIG. 1. Pharmacokinetic profiles for 3 (lower panel) and 6 (upper panel) mg of gentamicin/kg/day in humans (dashed line) and the corresponding doses reproducing equivalent AUCs (16 and 33 mg/kg) in the rabbit according to a 30-min infusion with native pharmacokinetics (open symbols) or with human pharmacokinetic simulation of an intravenous bolus (solid symbols).

native kinetics did not reduce the bacterial count below 7 $\log_{10}$ CFU/g of vegetation, regardless of the dose and treatment period. However, animals receiving gentamicin with HPS had a bacterial count of less than 4 $\log_{10}$ CFU/g of vegetation, regardless of the dose and treatment period.

For strain JH2-2 (Table 2), the antibacterial effect of gentamicin was on the whole less marked than for HM 1061 in groups receiving the antibiotic in HPS, but the effects were comparable in groups with native kinetics. However, administration of gentamicin with HPS was followed by a greater reduction of bacterial counts (0.9 to 3.7 $\log_{10}$ CFU/g of vegetation) than in corresponding groups receiving the same dose without simulation.

In experimental endocarditis (Table 2), a lower bacterial count was observed in animal groups receiving gentamicin with HPS versus native kinetics.

In an enterococcal endocarditis model, Vazquez et al. observed no reduction of bacteria with a daily dose of 6 mg/kg administered in two intramuscular injections during 3 days of treatment (34). In the study by Sullam et al., the bacterial count was reduced by 1 log CFU/g after 4.5 days of treatment (30), whereas in that of Lefort et al., the reduction was 1.3 log after 5 days of treatment (17). Our results are in complete agreement with these findings. Other studies in which this aminoglycoside was evaluated in the treatment of experimental endocarditis with HPS are not informative about its intrinsic activity in monotherapy, because it was associated with ampicillin (11, 18), teicoplanin (18), or penicillin (19).

In our study, pairwise comparison (same antibiotic and same treatment period) of groups with an equivalent AUC showed that the antibacterial effect was definitely improved with HPS. The comparison clearly indicates that an increase in the total daily dose (native kinetics) to compensate for faster elimination of gentamicin was not adequate for reliable prediction of the effects of the drug obtained in vivo with pharmacokinetics equivalent to that of humans (HPS). All pharmacokinetic parameters need to be taken into account.

Drug half-life is markedly shorter for small laboratory animals than for humans: the plasma half-lives of gentamicin are 22 min in the mouse (13), 0.6 h in the rat (31), 1 h in the rabbit (8), and 2 h in humans (21). Thus, it is not possible, on the basis of the native kinetic characteristics of the animal, to reproduce both an AUC and a maximum concentration of drug in serum (C_{max}) equivalent to those of humans. For gentamicin, administration of the dose used in humans (3 mg/kg/day) produces an equivalent C_{max}, whereas the AUC is much lower according to the clearance ratio.

The impact of these kinetic differences on the efficacy of

TABLE 2. Mean antibacterial effect of gentamicin infusion in vivo with native pharmacokinetics or HPS after 3 or 5 days of treatment against *E. faecalis* HM 1061 and JH2-2 in an experimental rabbit endocarditis model

Expt group		Mean ± SD $\log_{10}$ CFU/g of vegetation (no. of animals)[a]			
		HM 1061		JH2–2	
Dose (mg/kg/day)	Pharmacokinetics	3 days	5 days	3 days	5 days
0	Controls	9.1 ± 0.4 (13)	9.1 ± 0.4 (13)	8.5 ± 0.5 (9)	8.5 ± 0.5 (9)
16.6	HPS (3 mg/kg/day)	3.8 ± 0.6 (7)*†	3.1 ± 0.7 (5)*†	6.0 ± 0.8 (7)*†	3.4 ± 1.4 (6)*†
16.6	Native	8.7 ± 0.6 (5)	8.1 ± 0.8 (5)†	8.2 ± 0.4 (6)	7.1 ± 0.3 (6)
33.2	HPS (6 mg/kg/day)	3.5 ± 0.2 (6)*†	2.9 ± 0.1 (5)*†	6.5 ± 0.4 (5)†	4.8 ± 1.0 (6)†
33.2	Native	7.7 ± 0.7 (6)†	7.1 ± 0.6 (5)†	7.4 ± 0.4 (5)	6.2 ± 1.5 (6)†

[a] * and †, $P < 0.0001$ (Bonferroni's test) versus homologue native kinetics and versus controls, respectively.

aminoglycosides was already suggested in the studies of Potel et al. performed before and after the development of a model with HPS. Bacteriological results differed depending on the use of HPS or not (5, 27, 28). Similar discrepancies concerning aminoglycoside activity against gram-negative strains have been reported in comparisons of animal and human pharmacokinetics (7). In our study, the pharmacokinetic parameters of animals receiving gentamicin with HPS were comparable to those for humans (AUC, $C_{\max}$, and half-life) and in agreement with the results obtained by Gavalda et al. with a similar simulation model (12). Without HPS, the peak level increased and the half-life decreased. As a result, bacterial killing was greater with HPS. Most experimental studies in the past have designated maximal concentration and the AUC as good predictors for aminoglycoside activity. However, our study emphasizes the essential impact of half-life, which was obviously neglected in previous investigations based on a single species, with all animals sharing the same half-life. Furthermore, the positive impact of serum half-life on the duration of the postantibiotic effect has been previously documented (10), possibly explaining in part the results observed in this work.

In summary, this study clearly shows the importance of using an infectious animal model simulating human pharmacokinetics. This approach appears to be more reliable than others for assessment of the activity of antibiotics in the context of experimental infection and extrapolation of the results to human therapeutics.

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