Simulation of Amoxicillin Pharmacokinetics in Humans for the Prevention of Streptococcal Endocarditis in Rats

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The pharmacokinetic determinants of successful antibiotic prophylaxis of endocarditis are not precisely known. Differences in half-lives of antibiotics between animals and humans preclude extrapolation of animal results to human situations. To overcome this limitation, we have mimicked in rats the amoxicillin kinetics in humans following a 3-g oral dose (as often used for prophylaxis of endocarditis) by delivering the drug through a computerized pump. Rats with catheter-induced vegetations were challenged with either of two strains of antibiotic-tolerant viridans group streptococci. Antibiotics were given either through the pump (to simulate the whole kinetic profile during prophylaxis in humans) or as an intravenous bolus which imitated only the peak level of amoxicillin (18 mg/liter) in human serum. Prophylaxis by intravenous bolus was inoculum dependent and afforded a limited protection only in rats challenged with the minimum inoculum size infecting $\geq 90\%$ of untreated controls. In contrast, simulation of kinetics in humans significantly protected animals challenged with 10 to 100 times the inoculum of either of the test organisms infecting $\geq 90\%$ of untreated controls. Thus, simulation of the profiles of amoxicillin prophylaxis in human serum was more efficacious than mere imitation of the transient peak level in rats. This confirms previous studies suggesting that the duration for which the serum amoxicillin level remained detectable (not only the magnitude of the peak) was an important parameter in successful prophylaxis of endocarditis. The results also suggest that single-dose prophylaxis with 3 g of amoxicillin in humans might be more effective than predicted by conventional animal models in which only peak levels of antibiotic in human serum were simulated.

The optimal antibiotic regimen for the prophylaxis of infective endocarditis in patients at risk undergoing dental procedures is not known. Many authorities recommend a single oral dose of 3 g of amoxicillin 1 h before the procedure (9, 15), whereas the American Heart Association advocates a repeated dose of 1.5 g 6 h later (3). These recommendations are in part based on experimental data obtained from animals. In experimental models, single-dose regimens were efficacious in preventing endocarditis, but only in animals challenged with inoculum sizes corresponding to the infectious dose producing endocarditis in 90% of control animals (ID_{90}). The single dose of antibiotic failed in animals challenged with inoculum sizes larger than the ID_{90} (6, 12).

Moreover, interpretation of the dosing regimen tested with animal models is difficult (11), since elimination half-lives of antibiotics differ between animals and humans (5, 18, 19). Amoxicillin, for example, has a half-life of 20 min in rats, in comparison with 50 to 60 min in humans. Thus, it is conceivable that a single-dose regimen in humans, resulting in sustained levels in blood, might provide more effective protection than a single dose in rats.

To mimic more closely in rats the amoxicillin pharmacokinetics in humans, we developed a computer-controlled pump system which allowed us to precisely simulate in rats the concentration-versus-time curve of the antibiotic observed for humans after a single oral dose of 3 g. The efficacy of the human drug profile was tested in prevention of experimental endocarditis due to various streptococcal strains and compared with results obtained after a single intravenous (i.v.) bolus

injection of the drug, as usually used in experimental models of endocarditis.

MATERIALS AND METHODS

Determination of MICs and MBCs. Two previously described streptococcal strains (*Streptococcus sanguis* and *Streptococcus intermedius*) were used in this study (6, 12). The MICs and MBCs of amoxicillin were 0.032 and >128 mg/liter for *S. sanguis* and 0.5 and >128 mg/liter for *S. intermedius*, respectively. Thus, the tested strains were tolerant to amoxicillin as defined by an MBC/MIC ratio of >32 (13), as well as by their great ability to survive amoxicillin treatment in time-kill studies in vitro (6).

Serum amoxicillin levels in rats. Levels of amoxicillin in serum were determined for groups of three to six rats at several time points during and after antibiotic administration. Antibiotic concentrations were measured by the agar diffusion method with *Bacillus subtilis* ATCC 6633 (1). For standard curves, amoxicillin was diluted in pooled rat serum. The limit of detection of the bioassay was 0.6 mg/liter. The linearity of the standard curve was assessed by a regression coefficient of $r \geq 0.998$, and the percent coefficient of variation of the assay was 14%, as determined for six concentrations within the range of 0.6 to 20 mg/liter.

Production of endocarditis and installation of the infusion pump device. Sterile aortic vegetations were produced in female Wistar rats (body weight, 180 to 200 g) by a previously described method (6, 12). In brief, a polyethylene catheter was placed through the right carotid artery across the aortic valve and secured with a silk ligature. At the same time, a sterile silastic catheter (Dow Corning Corp., Midland, Mich.) was inserted through the jugular vein into the superior vena cava. The line was tunnelled subcutaneously and brought to the skin

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of the interscapular region. The external portion of this cannula was connected to a programmable infusion pump (Pump 44; Harvard Apparatus, Inc., South Natick, Mass.) through a flowthrough swivel, which permitted the animal to move in the cage (16). Bacterial endocarditis was induced 24 h after catheterization by i.v. challenge with various sizes of bacterial inocula.

Prophylaxis of experimental endocarditis by simulation of kinetics in humans versus an i.v. bolus. Groups of rats were challenged with bacterial inocula of *S. sanguis* or *S. intermedius* ranging from the ID₉₀ to 100 times the ID₉₀. The ID₉₀s were 10⁶ CFU for *S. sanguis* and 10⁵ CFU for *S. intermedius*. Before bacterial challenge, groups of rats were allocated to one of the following prophylactic regimens. One group (group a) received an i.v. bolus of 40 mg of amoxicillin per kg of body weight 30 min before bacterial challenge. This regimen resulted in drug levels, at the time of bacterial challenge, similar to those achieved in humans following an oral dose of 3 g of amoxicillin (see Fig. 1) (14). A second group of animals (group b) was connected to the infusion pump, which was set to deliver the antibiotic at a variable flow rate in order to simulate the kinetics of amoxicillin in humans following a 3-g oral dose of the drug (14). To further mimic the situation in humans, in whom amoxicillin prophylaxis is given 1 h before medicosurgical procedures that induce bacteremia (3, 9, 15), the pump was started 1 h before bacterial challenge. A third (control) group of rats received i.v. saline.

Evaluation of prophylaxis. Animals were killed 48 to 72 h after bacterial challenge. Aortic vegetations were excised, weighed, homogenized in 1 ml of saline, serially diluted, and plated on penicillinase-containing blood agar plates. Viable bacteria were counted after 48 h of incubation at 37°C, and the results were expressed as log₁₀ CFU per gram of vegetation. The dilution technique permitted the detection of ≥10² CFU per g of vegetation.

Statistical analysis. The chi-square test with Yates' correction was used for statistical comparison. The Bonferroni correction was used for multiple group comparisons.

RESULTS

Amoxicillin levels in serum following single i.v. bolus versus simulation of antibiotic kinetics in humans. Figure 1 shows the different serum profiles generated by the simulation of kinetics in humans and by the i.v. bolus of amoxicillin. At the time of bacterial challenge, serum drug levels ± standard deviations were similar (i.e., 16 ± 5 and 18 ± 0.3 mg/liter, respectively). However, in accordance with the different elimination times in humans and rats, the duration for which antibiotics remained detectable in serum after bacterial challenge was twice as long after simulation of kinetics in humans as it was after the i.v. bolus (i.e., 9 and 4.5 h, respectively).

Efficacies of the two different modes of administration of amoxicillin in the prevention of experimental endocarditis. The two pharmacokinetic profiles resulted in distinct prophylactic efficacies. As shown in Fig. 2, the effect of the i.v. bolus was inoculum dependent and provided only limited protection in animals challenged with the smaller bacterial inoculum, i.e., the ID₉₀. In contrast, simulation of amoxicillin kinetics in humans (resulting in a serum antibiotic level which was detectable for twice as long as it was after bolus injection) was much more effective and significantly protected rats challenged with 10- to 100-times-larger inocula. Therefore, the duration for which antibiotics remained detectable appeared to be an important parameter predicting successful endocarditis prophylaxis in these experiments.

FIG. 1. Serum amoxicillin levels following either an i.v. bolus of 40 mg of antibiotic per kg or simulation in rats of kinetics in humans. Curve a (●) represents the drug profile following the i.v. bolus, and curve b (▲) represents the drug levels generated by simulation of kinetics in humans. Curve c (■), shown for comparison, represents the pharmacokinetics produced by a single oral dose of 3 g of amoxicillin in humans (14). Error bars indicate standard deviations.

DISCUSSION

To mimic in animals drug kinetics in humans may be important for interpretation of antibiotic efficacy. It is known

FIG. 2. Prophylaxis of experimental endocarditis by either an i.v. bolus of amoxicillin or simulation of drug kinetics in humans. The upper panel depicts the experiments performed with *S. sanguis*, and the lower panel depicts those performed with *S. intermedius*. The columns represent the percentage of infected vegetations. The treatment groups (a represents i.v. bolus prophylaxis and b represents simulation of kinetics in humans) and the number of animals per group are indicated at the bottom of the columns. An asterisk indicates a *P* value of <0.05 compared with controls, and a cross indicates a *P* value of <0.05 compared with the i.v. bolus group.

that the efficacy of beta-lactam antibiotics is related not only to the dose or the area under the curve following drug injection, but also to the elimination rate (5, 18). In the present study, we were able to mimic in rats the pharmacokinetic profiles observed in human serum after a single oral dose of 3 g of amoxicillin (14) by using a computer-controlled pump system to deliver the drug.

The simulation of pharmacokinetics in humans provided detectable drug levels for twice as long as the bolus injection (9 versus 4.5 h). As a result, animals in which antibiotic levels in humans were simulated were significantly protected against inoculum sizes up to 100 times greater than those against which rats receiving single-bolus injections were protected. These observations suggest that the prolonged presence of amoxicillin in the blood might be more important than the mere peak level in serum in predicting the prophylactic efficacy of the drug. As a matter of fact, this hypothesis has been recently confirmed by a series of experiments which showed that neither the peak levels in serum nor the area under the curve values of amoxicillin correlated with successful prophylaxis of experimental streptococcal endocarditis (4). The only predictive parameter appeared to be the duration during which serum drug levels were able to inhibit bacterial growth (as measured by inhibitory titers in serum), which closely correlated with blood amoxicillin levels above the MIC, the so-called time above MIC. Such results were also observed by others (2).

The present results were obtained with amoxicillin-tolerant bacteria, which were poorly killed by the antibiotic in vitro (6). Therefore, it is likely that prolonged amoxicillin levels would also protect rats challenged with nontolerant streptococci (which are more rapidly killed by the drug), as suggested by earlier experimental evidence (6). The mechanism(s) by which prolonged levels of amoxicillin afford successful protection against tolerant bacteria remains speculative. Considering the great ability of the test organism to survive amoxicillin treatment in vitro, direct drug-induced killing of bacteria attached to the damaged valves is very unlikely. Nevertheless, it is conceivable that in such nonbactericidal conditions, inhibition of bacterial growth by nonbactericidal antibiotics might allow the time for alternative host defense mechanisms (such as, for instance, the platelet microbicidal protein[s] [20]) to clear the bacteria from the vegetations (21). If true, such an alternative defense mechanism(s) might be of major importance for prophylaxis (and presumably also for therapy) of endocarditis, because two-thirds of streptococcal isolates from patients with infective endocarditis proved to be tolerant to beta-lactam antibiotics and other cell wall inhibitors when tested carefully (7, 10).

The practical implications of the present results are twofold. First, they underline the importance of considering the differential pharmacokinetics between humans and animals for the interpretation of results obtained in animals. Second, they support the current recommendations of some authorities which advocate a single dose of 3 g of amoxicillin as standard oral prophylaxis before dental procedures in patients at risk. However, while simulation of single-dose prophylaxis with 3 g of amoxicillin was optimal in our system, one might gain yet another level of safety by the addition of a second dose of antibiotic given 6 h after the first, as recommended by the American Heart Association (3). In view of two recent studies with humans which suggest that the protective efficacy of prophylaxis with a single dose of amoxicillin might only be approximately 50%, the prolongation of adequate levels in serum with such a second dose appears to be justified (8, 17).

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