ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1990, p. 1869–1874
0066-4804/90/101869-06$02.00/0

Vol. 34, No. 10

Antistaphylococcal Activities of Teicoplanin and Vancomycin In Vitro and in an Experimental Infection

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Received 20 December 1989/Accepted 23 July 1990

The efficacies of vancomycin and teicoplanin in an experimental *Staphylococcus aureus* infection in granulocytopenic mice were related to their activities in vitro and their pharmacokinetic profiles. In vitro teicoplanin had a higher intrinsic activity than vancomycin did; and it also had a more favorable pharmacokinetic profile, resulting in higher peak concentrations in plasma, a longer elimination half-life, and a larger area under the concentration-time curve than those of vancomycin. To predict the antibacterial efficacies of the drugs in vivo on the basis of their activities in vitro and pharmacokinetics, a mathematical model was applied. In the model the in vitro effect was expressed as the difference in growth rate between control cultures and those in the presence of the antibiotic (E_R), and the in vivo effect was expressed as the difference between numbers of CFU in control and antibiotic-treated animals (E_N). The integral of E_R against time, E_Rt, was calculated by using the concentrations found in vivo. A significant linear relationship was found between E_N and E_Rt for different dosages at the same times (4 h) after drug administration as well as for the same doses at consecutive times, although at the lowest doses of teicoplanin the observed effect was less than the predicted effect.

Teicoplanin, originally known as teichomycin A_2, is a new glycopeptide antibiotic that resembles vancomycin in both structure and mechanism of action. Both antibiotics are active in vitro against streptococci, including enterococci, and coagulase-negative as well as coagulase-positive staphylococci, including methicillin-resistant strains. Other gram-positive bacteria, such as *Corynebacterium* group JK, *Clostridium difficile*, diphtheroids, and anaerobic gram-positive cocci, are also susceptible (3–5, 8, 9, 15). Glycopeptide antibiotics interfere with bacterial cell wall synthesis in susceptible microorganisms by binding to the terminal amino acyl-D-alanyl-D-alanine sequence. As a result, they impede linkage of preformed *N*-acetylmuramylpentapeptide to the lipid carrier in the cell membrane, blocking transport of the precursor to the site of cell wall growth and thus inhibiting polymerization of the growing peptidoglycan chain (5, 8, 9). On the basis of their MICs for 50 and 90% of strains tested, the efficacies of the two agents in vitro against *Staphylococcus aureus* are considered to be similar (3–5, 13, 15). However, the pharmacokinetics of teicoplanin in humans are regarded to be more favorable than those of vancomycin because of the longer half-life of teicoplanin (13).

In the present study, the efficacies of the two antibiotics in vivo, i.e., in an experimental thigh infection, were compared with their in vitro activities against an *S. aureus* strain. To minimize interference with host factors, the experiments were performed with granulocytopenic mice.

MATERIALS AND METHODS

Antibiotics. Teicoplanin was obtained as a sodium salt (90.1% activity) from Merrell-Dow Pharmaceuticals, Lepetit Research Centre, Milan, Italy. Vancomycin hydrochloride (1,054 μg of activity per mg of powder) was obtained from Eli Lilly & Co., Utrecht, The Netherlands. Standard solutions of the antibiotics were made with phosphate-buffered saline (pH 7.2).

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Microorganism. Suspensions of *S. aureus* 42D, which was isolated from clinical material and which had a density of 3.5 × 10^9 CFU/ml, were stored at −70°C. For each experiment, one vial containing 1.5 ml of the suspension was thawed in a water bath at 37°C. The MICs, which were determined on Iso-Sensitest Agar (Oxoid Ltd., Basingstoke, United Kingdom), of teicoplanin and vancomycin for *S. aureus* 42D were 0.5 and 1 mg/liter, respectively. The strain was not sensitive to serum.

Animals. Specific-pathogen-free Swiss male mice (weight, 20 to 25 g) were obtained from Broekman, Helmond, The Netherlands.

Experiments in vitro. (i) Short-term-growth experiments. The thawed bacterial suspension was diluted 1:4,000 in brain heart infusion (Oxoid) medium at pH 7.5. This suspension, which contained approximately 10^6 CFU/ml, was incubated in a shaking water bath at 37°C for 1 h. The culture was then divided into 20-ml portions and stored in 50-ml bottles. Antibiotics were added at various concentrations, and 0.1-ml samples were taken at several time points during a 3-h period. After dilution of the samples in phosphate-buffered saline, 6 10-μl drops of appropriate dilutions were inoculated onto Diagnostic Sensitivity Test Agar (Oxoid) and incubated overnight at 37°C; the bacteria were then counted as CFU. A few experiments were performed at lower pH values or at a higher inoculum.

(ii) Protein binding. Binding of the antibiotics to murine plasma was determined at 37°C by equilibrium dialysis in a Dianorm Dialysis apparatus (Diachema AG, Zurich, Switzerland). The dialysis chambers, which were separated by a cellulose membrane, were filled with pooled murine plasma and a solution of the antibiotic in saline, respectively, and then placed in a rotator. Dialysis was carried out at 16 rpm. Equilibrium was reached after 6 h for vancomycin and after 16 h for teicoplanin. The concentrations of the antibiotics in the two chambers were measured as described above for the antibiotic assay. In one experiment, protein binding was determined after centrifugation at 295,000 × *g* for 3 h (2).

The concentration in the upper 0.5 ml was regarded as the free concentration.

(iii) Pharmacodynamic model. The pharmacodynamic model to assess the relationship between in vitro and in vivo activities, taking into account in vivo pharmacokinetics, has been described elsewhere (H. Mattie, A. M. van Dokkum, L. Brus-Weijer, A. M. Krul, and E. van Strijen, J. Infect. Dis., in press). In this model the in vitro effect is defined as the difference in growth rate between a control culture and a culture in the presence of a given antibiotic concentration. This effect on growth rate is derived from the effect on bacterial numbers (E_N), defined as the difference between the logarithm of the number of CFU of the control culture and that in the presence of the antibiotic. The following empirical equation was fitted to the observed values of E_N by multiple regression analysis:

$$E_N = i_1 t + i_2 t^2 + i_3 e^{-t} + i_4 \quad (1)$$

in which i_1 to i_4 are constants of the equation. In general, this equation gave a satisfactory fit to the data.

The first derivative of equation 1 gives the effect on growth rate:

$$dE_N/dt = i_1 + 2i_2 t - i_3 e^{-t} \quad (2)$$

and the second derivative ($2i_2 + i_3 e^{-t}$) is zero at the time point during the 3-h period when maximal killing was achieved. Substitution of this time into equation 2 gives the maximal effect on growth rate for a given concentration, called E_R, which was used as a single parameter of the effect of that concentration. The relation between E_R and the antibiotic concentration is given by the Hill equation (6, 14):

$$E_R = E_{R,max} \frac{C^s}{EC_{50}^s + C^s} \quad (3)$$

in which $E_{R,max}$ is the maximal value of E_R, C is the concentration, EC_{50} is the concentration at which 50% of $E_{R,max}$ is reached, and s is a parameter that determines the steepness of the concentration-effect relationship. The parameters of equation 3 were calculated by a nonlinear regression analysis (Systat 5.0; Systat Inc., Evanston, Ill.).

Experiments in vivo. (i) Infection model. Mice were rendered granulocytopenic by total body irradiation consisting of 6 Gy delivered by a 6-MV linear accelerator (SL 75/6; Philips, Eindhoven, The Netherlands). On day 5 after irradiation the effect on the leukocyte count was optimal, the number of granulocytes being less than 10% of the initial value (7).

On that day a 0.1-ml sample of a 1:50 dilution in brain heart infusion medium of the stock suspension of *S. aureus* 42D, containing approximately 7×10^7 CFU/ml, was injected into one thigh muscle per mouse. The infection was allowed to develop for 1 h. Then, the antibiotic, at a dosage of 40 mg/kg, or saline (controls) was injected subcutaneously. At 0, 30, 60, 90, 120, 180, and 240 min, animals were sacrificed by exposure to 100% CO_2. The thigh muscle was isolated from the femur and homogenized for 30 s in 5 ml of phosphate-buffered saline in a tissue homogenizer (Ystrall type X-1020; International Laboratoriums Apparate GmbH, Dottingen, Federal Republic of Germany). Tenfold dilutions of the muscle homogenate were made in phosphate-buffered saline, and appropriate dilutions were plated to obtain bacterial counts, as described above for the short-term growth experiments in vitro. As a parameter of the antibacterial efficacy in vivo, E_N, i.e., the difference between log CFU per thigh in controls and log CFU per thigh in animals treated with vancomycin or teicoplanin, was used. In a second series of experiments, the effects of different dosages of the antibiotics (10, 20, 40, and 80 mg/kg) were assessed at one time point, i.e., 4 h after administration.

(ii) Pharmacokinetics. To determine drug concentrations in plasma, vancomycin or teicoplanin was administered subcutaneously at a dose of 40 mg/kg. At 0, 10, 20, 30, 60, 90, 120, 180, and 240 min, blood was collected via cardiac puncture in heparinized syringes after the animals were killed by exposure to 100% CO_2. Samples were centrifuged at 1,500 × *g* for 10 min to obtain plasma for assay of the antibiotic concentration. The following biexponential equation was fitted to the plasma concentrations (C_p):

$$C_p = D \times C_1 (e^{-k_{el}t} - e^{-k_a t}) \quad (4)$$

in which D is the dose, C_1 is a constant of the equation, and k_a and k_{el} are the apparent absorption and elimination rate constants, respectively. Fitting was performed graphically by calculating k_{el} from the descending part of the concentration-time course by log-linear regression analysis, and k_a was established by feathering.

(iii) Antibiotic assay. Concentrations of vancomycin and teicoplanin in plasma were determined microbiologically by an agar diffusion method, with *Bacillus subtilis* ATCC 6633 used as the indicator organism and antibiotic medium 5 (pH 7.9; Difco Laboratories, Detroit, Mich.) used as the assay medium (10, 12, 13, 15). Antibiotic standards were obtained from twofold dilutions in pooled murine plasma. The standard range for both antibiotics was 80 to 2.5 mg/liter. Plasma samples were analyzed undiluted and in twofold and fourfold dilutions in pooled murine plasma. All assays were performed at least in duplicate. A limited number of samples was also assayed by using *S. aureus* 42D. The inocula were similar in all assays.

For the assay in saline, the standard was prepared in saline. The levels of detection were 5 mg/liter for teicoplanin and 2.5 mg/liter for vancomycin. The coefficient of variation was generally 5 to 10%.

Quantitative comparison of in vivo and in vitro efficacies. The in vivo and in vitro efficacies were compared quantitatively on the basis of a calculated parameter, the integral of E_R against time ($E_R t$) (Mattie et al., in press). To calculate $E_R t$, the concentrations observed in vivo were substituted into equation 3, resulting in presumed in vivo values of E_R versus time. The integral of this plot, $E_R t$, represented values of E_N, as defined above. The observed antibiotic effect on the number of CFU in vivo was postulated to be correlated to $E_R t$.

RESULTS

Short-term growth experiments. The in vitro growth pattern of *S. aureus* 42D in the presence of vancomycin or teicoplanin is shown in Fig. 1. Higher concentrations than those shown in Fig. 1 did not lead to higher killing rates. The maximal effect on growth rate, E_R, was calculated as described above. The concentration-dependent values of E_R for both antibiotics are shown in Fig. 2. The calculated value of $E_{R,max}$ was the same for the two antibiotics, namely 1.7 log units/h. The respective values of EC_{50}, which was calculated according to equation 3, were 0.76 mg/liter for vancomycin and 0.22 mg/liter for teicoplanin. There was a significant difference in the values of s, which represents the steepness of the concentration-effect curve (8.5 for vancomycin and 1.63 for teicoplanin; $P < 0.001$). At pHs of 6.5 and 5.5, the effect of vancomycin was not different from that at

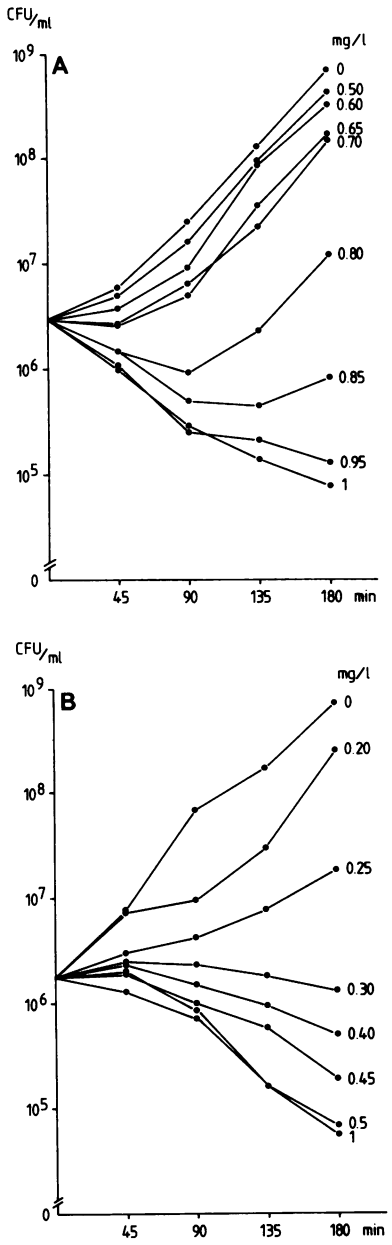

FIG. 1. Numbers of CFU of *S. aureus* in vitro in the presence of various concentrations of vancomycin (A) or teicoplanin (B) during a 3-h exposure.

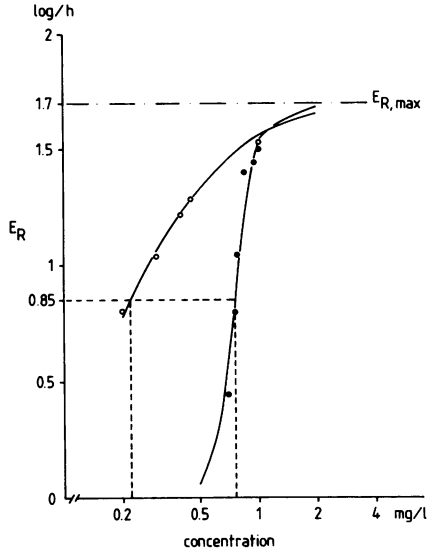

FIG. 2. Effect on growth rate (E_R) of vancomycin (●) or teicoplanin (○). Values of E_R were determined at the time during the 3-h exposure when maximal killing was achieved. The vertical dotted lines indicate the respective EC_{50}s.

pH 7.5, but the EC_{50} of teicoplanin decreased by 22 and 56%, respectively, reflecting an increased potency at a lower pH. A higher inoculum decreased the effects of both antibiotics; at 10^7 and 10^8 CFU the potency of vancomycin was reduced by 19 and 99%, respectively, and that of teicoplanin was reduced by 90 and 94%, respectively.

Experiments in vivo. The in vivo growth curves and the corresponding E_N-versus-time curves for the two antibiotics at a dose of 40 mg/kg were remarkably similar (Fig. 3). This finding was confirmed by the results with other dosages of vancomycin or teicoplanin at 4 h after drug administration (Fig. 4). These results indicate that, in terms of dose, vancomycin and teicoplanin are about equally potent in vivo.

Pharmacokinetics. Concentrations of drugs in plasma after a subcutaneous injection of 40 mg of vancomycin and teicoplanin per kg, which were measured with *B. subtilis* ATCC 6633 as the indicator organism, were used to calculate the pharmacokinetic parameters (Fig. 5). The course of the concentration in plasma, which was computed according to equation 4, is expressed by the following equations: C_p (vancomycin) = $D \times 2.25(e^{-1.7t} - e^{-10t})$ and C_p (teicoplanin) = $D \times 4.25(e^{-0.25t} - e^{-2.5t})$. The elimination rate constants for vancomycin and teicoplanin corresponded to elimination half-lives of 25 min and 2 h and 46 min, respectively. Thus, at the same subcutaneous dose, higher and much more sustained levels were obtained in plasma with teicoplanin than with vancomycin. The areas under the concentration-versus-time curve from 0 to 4 h were 43.6 mg · h/liter for vancomycin and 615.4 mg · h/liter for teicoplanin. Protein binding in murine plasma was 10% for vancomycin and 84% for teicoplanin.

Antibiotic assay. There was no difference in the concentrations of vancomycin obtained in plasma when *B. subtilis* ATCC 6633 and *S. aureus* 42D were used as indicator organisms. For teicoplanin, however, the concentrations obtained in plasma with *S. aureus* 42D as the indicator organism were only 65% of the values obtained with *B. subtilis* ATCC 6633.

Quantitative comparison of in vivo and in vitro efficacies. In Fig. 6 the observed values of E_N for both antibiotics are plotted against the values of $E_R t$, which were calculated for different doses at 4 h as well as at consecutive time points for the dose of 40 mg/kg. For the calculation of $E_R t$ for teicoplanin, a correction factor of 0.65 was applied to the equations for C_p, because of the above-mentioned findings with the assay procedure, and protein binding was also taken into account. For the dose of 40 mg/kg, the observed values of E_N at all time points showed a significant linear relation with the predicted values of $E_R t$ for vancomycin as well as teicoplanin ($P < 0.001$). The slopes of this relation were similar for the two antibiotics, namely, 0.26 ± 0.02 (standard error of the mean) for vancomycin and 0.20 ± 0.03 (standard error of the mean) for teicoplanin. For the other dosages of

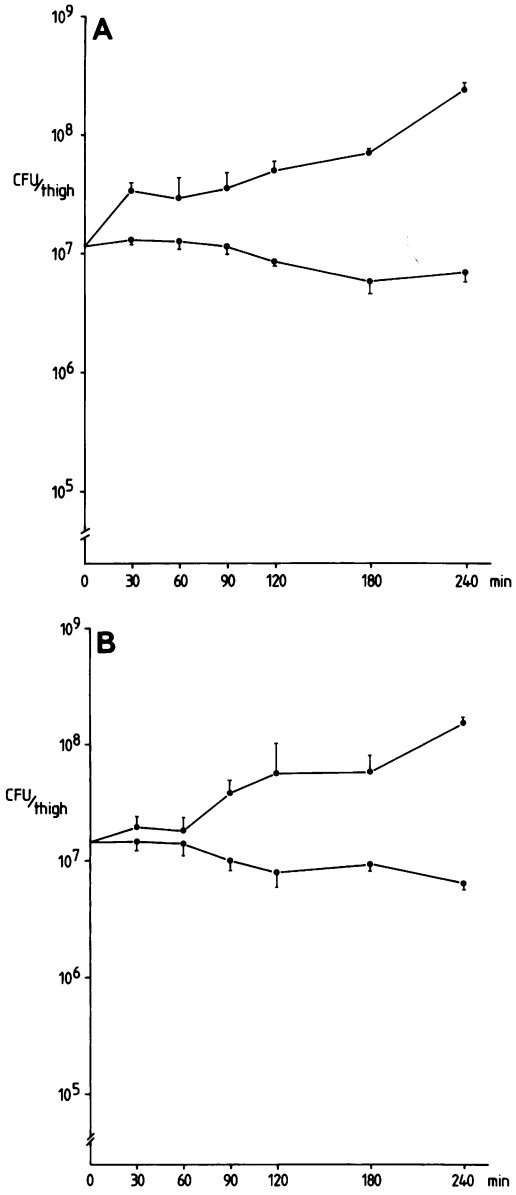

FIG. 3. Numbers of *S. aureus* (± standard error of the mean) in an experimental thigh infection in granulocytopenic mice treated with 40 mg of vancomycin (A) or teicoplanin (B) per kg and in untreated animals (top lines in each panel).

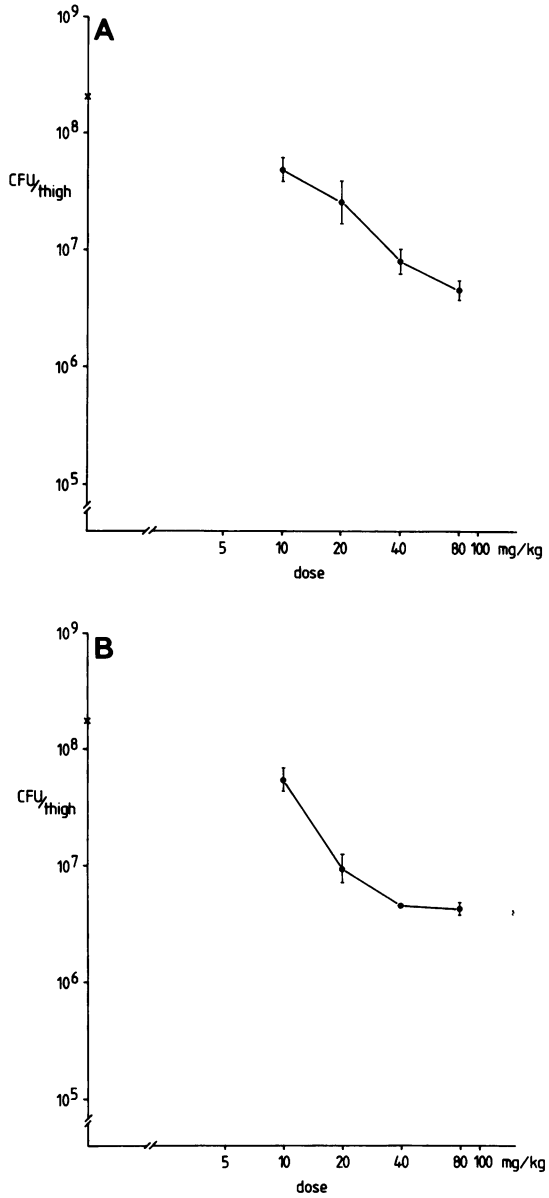

FIG. 4. Numbers of *S. aureus* (± standard error of the mean) in an experimental thigh infection 4 h after administration of various dosages of vancomycin (A) or teicoplanin (B).

vancomycin, there was also a highly significant linear relation between the calculated value of $E_R t$ and the observed values of E_N at the time point of 4 h ($P < 0.001$), and this relation was not significantly different from that found for the dose of 40 mg/kg at consecutive time points. For teicoplanin, the results at 4 h were different for dosages lower than 40 mg/kg; the calculated value of $E_R t$ was the maximal value for all dosages, but the actual observations showed a significant increase in $E_R t$ with dose ($P < 0.001$).

DISCUSSION

The results of this study show that vancomycin and teicoplanin were about equally effective in a 4-h experimental *S. aureus* thigh infection in granulocytopenic mice, although teicoplanin had higher intrinsic activity in vitro, as indicated by the lower MIC and the lower EC_{50}, and a much more favorable pharmacokinetic profile, resulting in higher peak concentrations in plasma, a longer half-life, and a 14-fold-higher area under the concentration-time curve. These potential advantages did not lead to better efficacy in the experimental infection.

This discrepancy was further explored by applying a mathematical model, which served as a relative predictor of the in vivo antibacterial efficacy, based on in vitro activity and pharmacokinetics. Parameters of in vitro activity and pharmacokinetic parameters were used to compute the integral of the effect of the antibiotic on the growth rate in vitro against time ($E_R t$). Indeed, for vancomycin, a significant linear relationship was found between the observed effect on numbers of bacteria (E_N) and calculated values of $E_R t$. This relationship was found for different dosages of vancomycin 4

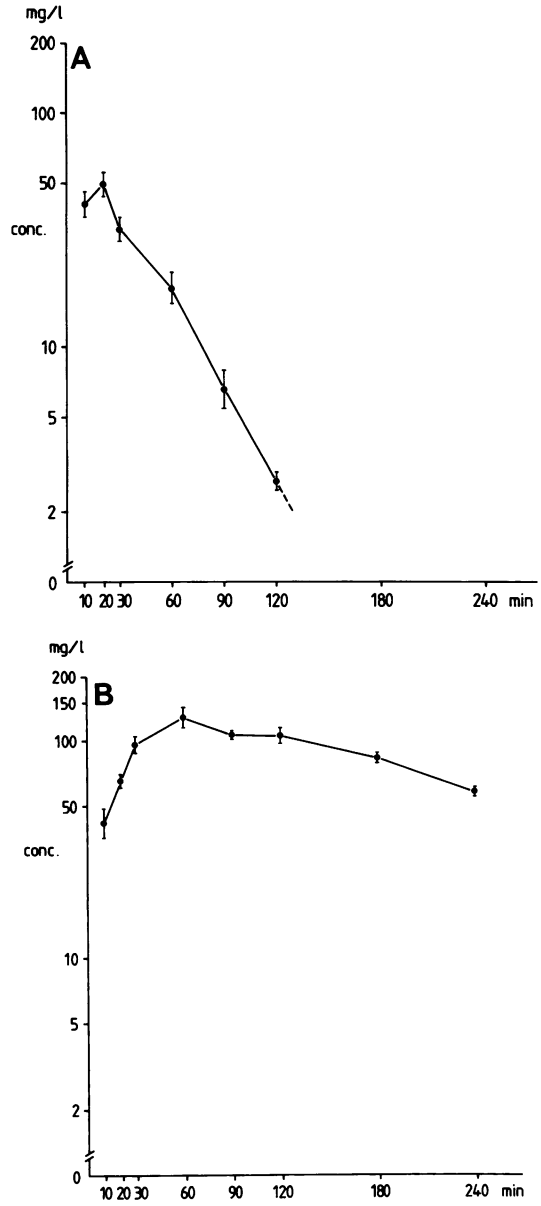

FIG. 5. Concentrations (± standard error of the mean) of vancomycin (A) or teicoplanin (B) in plasma after subcutaneous administration of 40 mg/kg.

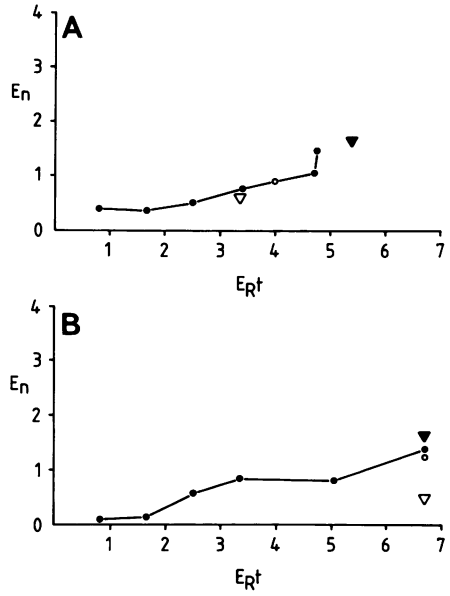

FIG. 6. Observed values of the effect on numbers of *S. aureus* (E_N) in vivo for vancomycin (A) and teicoplanin (B) plotted against calculated values of $E_R t$ (see text) for a dose of 40 mg/kg (●) at consecutive time points (connected points) and for doses of 10 mg/kg (▽), 20 mg/kg (○), and 80 mg/kg (▼) at 4 h after administration.

h after administration and for a dose of 40 mg/kg at different times after administration. The slope of this relationship, 0.26, was less than unity, but there was a priori no reason to expect that the quantitative effects on growth rates in vivo and in vitro would be identical. It was, however, also less than the value of about 0.60 found in an earlier study with respect to the effects of four cephalosporins on gram-negative rods (Mattie et al., in press). Apparently, the extrapolation from in vitro efficacy to in vivo efficacy may be quantitatively different for different kinds of antibiotics (11). Nevertheless, taking this into account, the mathematical model that we used can serve as a reliable predictor of the antibacterial efficacy of vancomycin in vivo. For teicoplanin, the relation between the observed and predicted values was different, since for all dosages used a maximal effect at 4 h was predicted, while for dosages lower than 40 mg/kg the observed values had not yet reached the maximum value. Interestingly, at the dose of 40 mg/kg, the observed values at earlier time points were linearly related to the predicted values, with a slope of 0.20, i.e., very similar to the slope for vancomycin. Whatever the reason for the discrepancy between vancomycin and teicoplanin, it indicates that it is not warranted to predict therapeutic equivalency between vancomycin and teicoplanin in human disease on the basis of pharmacokinetic parameters and activity in vitro only. The reason for the unexpected behavior of teicoplanin is not clear. In vitro, the different shapes of the concentration-effect curves of the two antibiotics (Fig. 2) indicate that the two drugs have different modes of action, maybe resulting in different relative efficacies in vivo. Another explanation could be that teicoplanin is a complex of five closely related glycopeptide antibiotics (5). It is not inconceivable that the different components of teicoplanin may exert different effects on different test organisms; moreover, differences in the pharmacokinetics of the distinct components of teicoplanin may contribute to the discrepancy between the observed and predicted efficacies. However, since in our calculations we took the precaution of expressing concentrations in plasma in terms of activity against the infective microorganism, the inhomogeneity of teicoplanin does not offer a complete explanation for the discrepancy between predictions and observations. Other possible differences between the in vitro and in vivo situations, namely, pH and inoculum size, are not good explanations either, since a lower pH would enhance the efficacy of teicoplanin and the inoculum effect would also influence the efficacy of vancomycin.

The therapeutic equivalency of vancomycin and teicoplanin was also found by other investigators in a bacterial endocarditis rabbit model (1). In contrast, a higher level of protection was reported for teicoplanin compared with vancomycin (expressed as 50% effective dose) in an intraperitoneal infection in mice (13).

In humans, the pharmacokinetic pattern of teicoplanin also differs from that of vancomycin, as indicated by its longer elimination half-life and larger area under the concentration-time curve (10). If one applies the pertinent equations to pharmacokinetics in humans, this leads to the conclusion that the dosage schedule generally applied for vancomycin (1 g every 12 h) is much more than necessary; even dosages of 250 mg every 12 h would lead to a practically maximal result. In view of the apparent toxicity of vancomycin, this is not an unimportant consequence. In the case of teicoplanin, a dosage schedule leading to maximal total concentrations of 20 mg/liter every 24 h would lead to about 95% of the maximal effect, even if one does not take into account that in the animal experiments the results were not as good as expected.

ACKNOWLEDGMENTS

We thank Merrell-Dow Pharmaceuticals for providing teicoplanin and Eli Lilly & Co for providing vancomycin. We gratefully acknowledge the technical assistance of E. van Strijen, B. A. Razab-Sekh, and A. Matze. We thank M. L. van Ogtrop for advice and critical discussion of the results.

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