ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1997, p. 1910–1915 Vol. 41, No. 9
0066-4804/97/$04.00+0

Activities of Vancomycin and Teicoplanin against Penicillin-Resistant Pneumococci In Vitro and In Vivo and Correlation to Pharmacokinetic Parameters in the Mouse Peritonitis Model

JENNY DAHL KNUDSEN,* KURT FUURSTED, FRANK ESPERSEN, AND NIELS FRIMODT-MØLLER

Division of Microbiology, Statens Serum Institut, DK 2300 Copenhagen S, Denmark

Received 23 December 1996/Returned for Modification 26 April 1997/Accepted 30 June 1997

The activities of glycopeptides against pneumococci were studied in vitro and in vivo. The MICs of two glycopeptides, vancomycin and teicoplanin, in different media against 10 strains of pneumococci with different susceptibilities to penicillin were determined. The MICs of teicoplanin were four times lower than those of vancomycin in Mueller-Hinton media supplemented with 5% blood, but the MICs were similar in mouse and human sera supplemented with 5% blood. The serum protein binding levels in mouse and human sera were 90% for teicoplanin in both and 25 and 35%, respectively, for vancomycin. The MICs for vancomycin and teicoplanin were only correlated in human serum ($P < 0.001$). The single doses giving protection to 50% of the animals in the mouse peritonitis model after a lethal challenge of pneumococci, the ED_{50}s, were similar for vancomycin and teicoplanin, between 0.1 and 1 mg/kg of body weight for all 10 strains. The log ED_{50}s were significantly correlated only to the log MICs of teicoplanin determined for mouse serum with 5% blood ($P = 0.01$) and to the log MICs of vancomycin determined by the E test ($P = 0.03$). Among the pharmacokinetic parameters analyzed at the ED_{50}s, the most constant parameter was the time for which the drug concentration exceeded the MIC ($T_{>MIC}$) when each drug was considered separately; however, when both drugs were considered together, the maximum concentration of drug in serum (C_{max}) varied the least. This indicates that both these parameters are of importance for predicting the effect of the drugs. We conclude that the effect of glycopeptides was not influenced by the penicillin resistance of the pneumococci, either in vitro or in vivo, and that the superior activity of teicoplanin over that of vancomycin in vitro was abolished in vivo, an effect which probably was due to the high serum protein binding of teicoplanin. Both the pharmacokinetic parameters $T_{>MIC}$ and C_{max} are important predicting the effect of glycopeptides, but the pharmacodynamics of glycopeptides are still not completely elucidated.

Streptococcus pneumoniae is one of the pathogens most frequently isolated from humans all over the world and produces its highest rate of morbidity in small children and the elderly (15, 21). It is the organism most often isolated from patients suffering from pneumonia (15). Because of the worldwide, increasing problem of antibiotic resistance in clinical isolates of pneumococci (3, 13), knowledge of the pharmacokinetic and dynamic properties of alternative drugs to penicillin is desirable. The only antibiotics active against pneumococci and without any resistance problems so far are the glycopeptides (3, 13). The activities of glycopeptides against pneumococci with various susceptibilities to penicillin have been studied in vitro and have been shown not to be correlated with susceptibility to penicillin (10). The activities in vivo of glycopeptides against penicillin-resistant pneumococci have, however, not been studied in detail. Furthermore, the correlations between the effect of treatment with glycopeptides and the various pharmacokinetic parameters have not been fully elucidated (2, 5, 11, 19, 22). In order to study the effect of glycopeptides in the mouse peritonitis model against pneumococci with different susceptibilities to penicillin, 10 isolates of pneumococci and two glycopeptides, vancomycin and teicoplanin, were used. The purpose of this study was to compare the effects of these two glycopeptides against pneumococci in vitro and in vivo and see whether penicillin resistance in pneumococci influenced the effect of glycopeptides in vivo and to study the relationships between the pharmacokinetic parameters and the effect of the drugs in the experiments.

* Corresponding author. Mailing address: Division of Microbiology, Statens Serum Institut, Artillerivej 5, DK 2300 Copenhagen S, Denmark. Phone: 45 3268 3175. Fax: 45 3268 3887.

MATERIALS AND METHODS

Bacteria, media, and antibiotics. The 10 pneumococcal strains used represented a spectrum of penicillin MICs from 0.016 to 8 μg/ml (Table 1) (14). Bacterial suspensions were prepared from fresh overnight cultures on 5% blood agar plates made from frozen stock cultures. The inoculum was prepared immediately before use by suspending colonies in sterile beef broth media and was adjusted to an optical density at 540 nm of 0.5, giving a density of approximately 10^8 CFU/ml. For each experiment the size of the inoculum was determined after making 10-fold dilutions in beef broth, of which 20 μl was plated on two 5% blood agar plates in spots in duplicate, with subsequent counting of colonies after incubation overnight at 35°C in ambient air. Beef broth, 5% blood agar plates, Mueller-Hinton agar plates with 5% horse blood, and antibiotics were produced at the Statens Serum Institut. Mucin (M-2378; Sigma Chemical Company, St. Louis, Mo.), an enzyme extract of porcine stomach, was used as an adjuvant for inoculation of the mice and was prepared as a saline stock solution of 10% (wt/vol) (14). Immediately before inoculation, the mucin solutions were diluted 1:1 with pneumococcal suspensions, giving a final mucin concentration of 5% (wt/vol).

The antibiotics used were the preparations for intravenous treatments: vancomycin, Vancocin (Eli Lilly and Company, Indianapolis, Ind.); and teicoplanin, Targocid (Astra, Södertälje, Sweden). Purity values, as given by the manufacturers, for teicoplanin and vancomycin were 82 and 93% (wt/wt), respectively. No compensation for purity was done.

MICs and MBCs. The MICs for the strains were determined by both the microtiter method and the plate dilution method (17) and by E tests (E-test; AB-Biodisk, Solne, Sweden). The microtiter method was carried out with the following media: Mueller-Hinton broth with 5% sheep blood, human serum with 5% sheep blood, and mouse serum with 5% sheep blood. The bacterial inoculum used was 50 μl per well of a 1×10^5- to 5×10^5-CFU/ml concentration suspended in the media used and 50 μl of antibiotics in twofold dilutions producing concentrations of from 4 to 0.031 μg/ml in the media, and giving a total amount per well of 100 μl. The microtiter plates (Nunc Microwell Plates 262170;

TABLE 1. MICs of penicillin, vancomycin, and teicoplanin for 10 pneumococci determined by various methods

Strain	MIC (μg/ml) of indicated drug as determined by:										
	E test with Danish blood agar			Agar dilution method with Mueller-Hinton agar and 5% horse blood		Microtiter method with:					
						Mueller-Hinton broth with 5% sheep blood		Mouse serum with 5% sheep blood		Human serum with 5% sheep blood	
	Penicillin	Vancomycin	Teicoplanin	Vancomycin	Teicoplanin	Vancomycin	Teicoplanin	Vancomycin	Teicoplanin	Vancomycin	Teicoplanin
ND-68128	8	0.38	0.032	0.125	0.031	0.125	0.031	2	2	1	1
Czech-2916	4	0.5	0.023	0.125	0.031	0.125	0.031	0.25	0.5	2	2
Iceland-1320	2	0.75	0.047	0.125	0.062	0.125	0.062	2	2	2	2
Iceland-1189	1	0.5	0.023	0.250	0.016	0.250	0.016	2	2	2	2
Iceland-625	1	0.5	0.032	0.250	0.031	0.250	0.031	0.5	2	1	2
Iceland-902	1	0.5	0.032	0.125	0.031	0.125	0.031	2	4	2	2
Iceland-999	0.5	0.5	0.016	0.250	0.031	0.250	0.031	1	2	1	1
Iceland-1064	0.25	0.5	0.016	0.125	0.016	0.125	0.016	1	2	2	2
U.S.A.-L	0.016	0.5	0.023	0.250	0.125	0.250	0.125	1	4	0.5	0.5
Denmark-493/73	0.016	0.38	0.023	0.125	0.016	0.125	0.016	1	4	0.5	0.5
50th percentile	1	0.5	0.023	0.125	0.031	0.125	0.031	1	2	1.5	2
90th percentile	4	0.5	0.032	0.250	0.062	0.250	0.062	2	4	2	2

A/S Nunc, Roskilde, Denmark) were incubated in ambient air at 35°C for approximately 20 h; the MIC was the lowest concentration at which no visible growth was observed. The MBCs were determined by plating 50 μl from wells without visible growth with Mueller-Hinton broth and 5% sheep blood and by comparing the results with those from controls. The MBCs were determined as the lowest concentrations that reduced the inoculum by 99.9%. The plate dilution method was performed with Mueller-Hinton broth agar supplemented with 5% horse blood and antibiotics in twofold dilutions producing concentrations from 128 to 0.004 μg/ml. Inocula of 10^4 CFU per spot were applied to the plates with a multipoint inoculator (A400; Denley Instruments Ltd., Sussex, United Kingdom). The plates were incubated overnight, and the MIC was defined as the lowest concentration at which one or no colonies could be observed.

Serum protein binding. This binding was done by a method modified from the ultrafiltration method described by Craig and Suh (6). After 2 h of incubation of the human serum and mouse sera in ambient air at 35°C, the pH was adjusted to 7.0 to 7.5 by bubbling with CO_2. Beef broth and H_2O were included as controls. The glycopeptides in final concentrations of 150, 100, and 50 μg/ml were dissolved in the sera or controls and incubated for 2 h. The samples were divided and one-half were centrifuged in tubes with filters with a cutoff at approximately 30,000 Da (Centricon 30, no. 4208; Amicon, Beverly, Mass.) in a fixed-angle rotor at 3,000 × *g* for 20 min. The antibiotic concentrations in both halves, the uncentrifuged and the centrifuged parts, were determined by the agar cup method (as described for serum concentrations; see below). A standard curve was produced by using antibiotic concentrations dissolved in the same sera or media. Thus, the protein-bound fraction could be calculated.

Mouse peritonitis model. Outbred, female, *ssc*:CF1 mice (age, approximately 8 weeks; weight, 30 ± 2 g) were used throughout the study. The mice were kept in cages of five to a cage, and they had free access to chow and water. Inoculation was performed by intraperitoneal injection of an inoculum of 0.5 ml of a pneumococcal suspension via a 25-gauge syringe. The inoculum contained 1×10^6 to 5×10^6 CFU/ml, with 5% (wt/vol) mucin in beef broth. Treatments of mice in groups of five with different doses of vancomycin or teicoplanin were performed as single subcutaneous injections in the neck region at a volume of 0.5 ml per dose 1 h after inoculation. Mice were observed for 6 days.

Determination of the ED_{50}. The ED_{50}, the single dose giving protection to 50% of the mice, for each combination of drug and pneumococcus was determined from two trials and was calculated from the Hill equation (GraphPad Prism; GraphPad Software, Inc., San Diego, Calif.). In a first trial, five mice in each of five groups were given the drug in 10-fold dilutions producing doses of from 100 to 0.1 mg/kg of body weight. In a second trial, doses determined in the first trial to produce from no effect to full effect were used. For each strain, a group of mice treated with saline was included as a control for the lethality of the infection.

The pharmacokinetic parameters. The pharmacokinetic parameters were determined from healthy mice that were bled in groups of two at different time intervals after treatments with different doses of one of the two drugs. The mice were bled after anesthesia with CO_2 at time points 10, 20, 30, 60, 90, 120, 180, and 240 min; bleeding at the last time point was only for teicoplanin-treated mice. After collection of blood samples, the blood was centrifuged at 1,630 × *g* for 10 min, and the serum was stored at −80°C until analyses, which were performed in duplicate. The cup plate or the disk diffusion bioassay method was used for measuring the glycopeptide concentration in mouse serum. A nonhemolytic streptococcus species (strain EB-68; Statens Serum Institut) was used for the bioassay, and the lowest measured value was 1 μg/ml for both drugs. For standard curves, vancomycin and teicoplanin were diluted in pooled normal mouse serum. The variation coefficients for standard concentrations were less than 4.2 and 4.0% for vancomycin and teicoplanin, respectively. The serum half-life, $t_{1/2}$, was calculated as $-\log 2/\beta$ where β is the slope of the serum elimination regression line (time versus $\log_{10}$ serum concentration). The time for which the serum concentration was above the MIC, $T_{>MIC}$, the peak concentration, C_{max}, and the area under the serum concentration-time curve, AUC, could be calculated by extrapolations for different antibiotic doses and the different strains. The AUCs were calculated with 0.001 μg/ml as the baseline by the trapezoidal method. The relationships between the pharmacokinetic parameters of importance and the effect were evaluated by the same principle as that previously used for evaluating those between penicillin and the same pneumococcal strains (14). In principle, the pharmacodynamics were studied by correlating the values of the different pharmacokinetic parameters with a defined effect for a spectrum of strains. The parameter that had the lowest variation for different strains while producing the same effect must be assumed to be the parameter of importance. In brief, values of the different pharmacokinetic parameters at the given doses and the ED_{50}s for the different strains (with ED_{50} defined as the dose at which half the mice survived after a lethal challenge) were evaluated.

Statistical methods. The Hill equation with variable slope (GraphPad Prism; GraphPad Software, Inc.) was used to calculate the ED_{50}s. Spearman's rank correlation test was used in the correlation tests between MICs and in the analysis of the importance of pharmacokinetic parameters. Values of *P* less than 0.05 were considered significant.

RESULTS

MICs and MBCs. The MICs for the 10 strains determined by three different methods are shown in Table 1. The MBCs were the same as the MICs found by the microdilution method using Mueller-Hinton broth supplemented with 5% sheep blood. The MICs determined on Mueller-Hinton agar with 5% horse blood correlated significantly with the MICs determined in Mueller-Hinton broth with 5% sheep blood for each of the drugs (Spearman's rho = 1; *P*s < 0.001), but there was no correlation between the MICs for vancomycin and for teicoplanin in Mueller-Hinton media with blood. Significant correlations between MICs for vancomycin and for teicoplanin were only found for the determinations in human serum with 5% sheep blood by the microtiter method (Spearman's rho = 0.90; $P < 0.001$). No other significant correlations between MICs for vancomycin and for teicoplanin were found, but it should be borne in mind that these MICs varied at only four dilution steps (Table 1).

Serum protein binding. For the concentrations tested, the protein-bound fractions of vancomycin and teicoplanin were 20 to 28% and 90 to 94%, respectively, in mouse serum; the

TABLE 2. ED_{50}s for the 10 pneumococcal strains and the two glycopeptides

Strain	Vancomycin		Teicoplanin	
	No. of mice	ED_{50} (mg/kg) (95% CI)	No. of mice	ED_{50} (mg/kg) (95% CI)
ND-68128	47	0.30 (0.24–0.37)	50	0.10*[a]
Czech-2916	50	0.56 (0.48–0.65)	50	0.10 (0.10–0.10)
Iceland-1320	75	0.65 (0.62–0.69)	115	0.12 (0.10–0.14)
Iceland-1189	50	0.60 (0.58–0.61)	50	0.46*
Iceland-625	50	0.41 (0.36–0.49)	50	0.10 (0.10–0.10)
Iceland-902	50	0.74 (0.66–0.82)	50	0.46*
Iceland-999	50	0.54 (0.46–0.64)	50	0.36*
Iceland-1064	50	0.61 (0.60–0.63)	50	0.27 (0.19–0.36)
U.S.A.-L	50	0.93 (0.82–1.05)	50	0.46*
Denmark-493/73	50	0.31 (0.21–0.45)	50	0.38 (0.36–0.39)
50th percentile		0.58		0.32
90th percentile		0.74		0.46

[a] *, the 95% CI could not be determined, often because no or only one value was obtained between no and full antibiotic effect due to the relative steepness of the curves.

protein-bound fraction in human serum was 25 to 41% for vancomycin and 89 to 95% for teicoplanin. The bound fractions in H_2O and in beef broth were found to be 2 to 7% and 0 to 12%, respectively, probably representing the loss of drug in the filters.

The ED_{50}s. The ED_{50}s for both glycopeptides and the 10 strains are given in Table 2. Because of the relatively small difference between doses giving no effect and doses giving full effect, less than one log unit, it was often difficult to obtain values of survival rate between 0 and 100%. This is the reason for some missing confidence limit values, especially for teicoplanin (Table 2). For teicoplanin the only significant correlation was found between log ED_{50}s and log MICs obtained in mouse serum with 5% sheep blood (Spearman's rho = 0.72; $P = 0.02$). For vancomycin, the only significant correlation was found between the log ED_{50}s and log MICs determined by the E test (Spearman's rho = 0.69; $P = 0.03$). In Fig. 1, the relationships between the MICs and the ED_{50} values for the two drugs and the 10 strains are shown. In Fig. 1a the MICs determined by the E test are used, in Fig. 1b the MICs determined in mouse serum with 5% sheep blood are used.

Pharmacokinetics. $t_{1/2}$s for the two drugs in healthy mice were determined to be 32 min (95% confidence interval, [CI], 25 to 45 min) for vancomycin and 151 min (95% CI, 91 to 435 min) for teicoplanin. The C_{max} was found to occur at 30 min for vancomycin and at 60 min for teicoplanin.

Pharmacodynamics. The values of various pharmacokinetic parameters for doses at the ED_{50} level are given in Table 3. These figures were calculated from the observed pharmacokinetic data for the drugs in mice. In Table 3, the variation of the pharmacokinetic parameters at the ED_{50} level is given as the ratio between the highest and lowest values for each one of the drugs individually and for both together. In Table 3, the parameter most constant at the ED_{50} level of the drugs was $T_{>MIC}$ when both glycopeptides were evaluated separately; when both of the drugs were evaluated together, it was C_{max}. Only small variations in the parameters for vancomycin were obtained (Table 3).

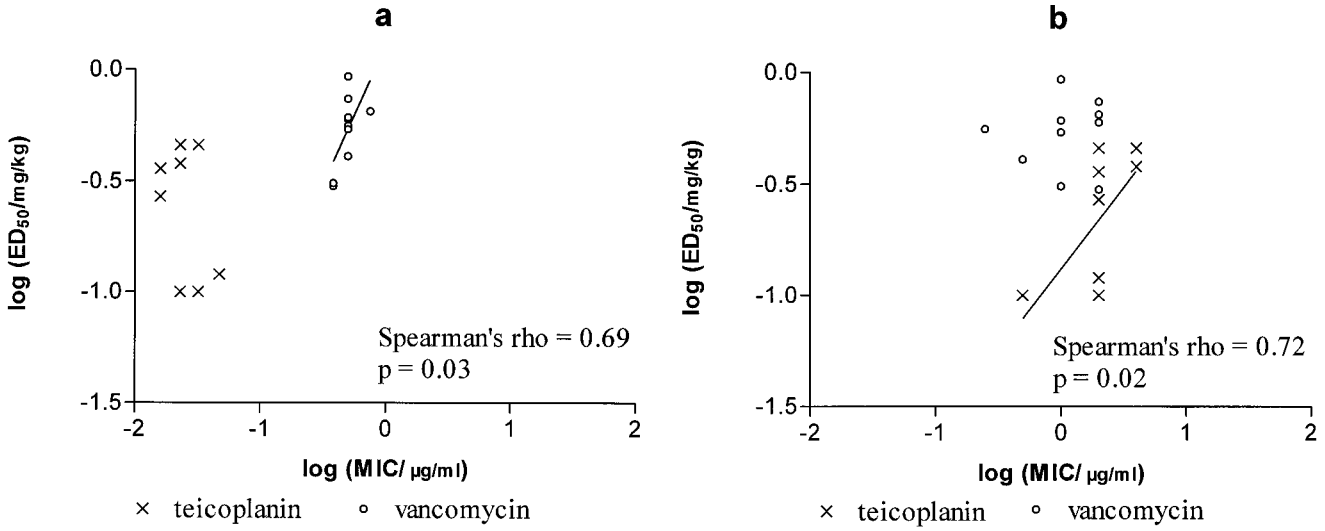

FIG. 1. The correlations between MICs and ED_{50}s for the 10 pneumococci treated with vancomycin and teicoplanin. (a) The MICs determined by the E test are given, and a significant correlation between log MIC and log ED_{50} was found for vancomycin. (b) The MICs determined with mouse serum as the medium by the microdilution method. A significant correlation between log MIC and log ED_{50} was found for teicoplanin.

TABLE 3. The pharmacokinetic parameters at the ED_{50} levels for the 10 strains and the two glycopeptides

Strain	Glycopeptide	C_{max} (μg/ml)	$C_{max\text{-}free}$[a] (μg/ml)	C_{max}/MIC[b]	$C_{max\text{-}free}$/MIC[b]	C_{max}/MIC_{MS}[c]	$T_{>MIC}$[d] (min)	$T_{>MIC\text{-}free}$[d] (min)	AUC[e] (min × μg/ml)	AUC/MIC[f] (min)
ND-68128	Vancomycin	0.48	0.36	3.84	2.88	0.24	69	54	76	608
	Teicoplanin	0.30	0.03	9.68	0.97	0.15	517	7	195	6,290
Czech-2916	Vancomycin	0.90	0.68	7.20	5.40	3.60	100	85	155	1,240
	Teicoplanin	0.30	0.03	9.68	0.97	0.60	517	7	195	6,290
Iceland-1320	Vancomycin	1.04	0.78	8.32	6.24	0.52	107	92	183	1,464
	Teicoplanin	0.36	0.04	5.81	0.58	0.18	400	124	241	3,887
Iceland-1189	Vancomycin	0.96	0.72	3.84	2.88	0.48	68	53	167	668
	Teicoplanin	1.38	0.14	86.25	8.63	0.69	1,005	486	1,125	70,313
Iceland-625	Vancomycin	0.66	0.50	2.64	1.98	1.32	49	34	109	436
	Teicoplanin	0.30	0.03	9.68	0.97	0.15	517	7	195	6,290
Iceland-902	Vancomycin	1.18	0.89	9.44	7.08	0.59	113	99	211	1,688
	Teicoplanin	1.38	0.14	44.52	4.45	0.35	856	337	1,125	36,290
Iceland-999	Vancomycin	0.86	0.65	3.44	2.58	0.86	63	48	147	588
	Teicoplanin	1.08	0.11	34.84	3.48	0.54	802	282	851	27,452
Iceland-1064	Vancomycin	0.98	0.74	7.84	5.88	0.98	104	89	171	1,368
	Teicoplanin	0.81	0.08	50.63	5.06	0.41	887	367	61	38,125
U.S.A.-L	Vancomycin	1.49	1.12	5.96	4.47	1.49	90	76	276	1,104
	Teicoplanin	1.38	0.14	11.04	1.10	0.35	541	22	1,125	9,000
Denmark-493/73	Vancomycin	0.50	0.38	3.97	2.98	0.50	71	56	79	632
	Teicoplanin	1.14	0.11	71.25	7.13	0.29	963	443	905	565,625
Ratio (highest/lowest)	Both	5	37	33	15	24	21	69	18	1,297
Ratio (highest/lowest)	Vancomycin	3	3	4	4	15	2	3	4	3
Ratio (highest/lowest)	Teicoplanin	5	5	15	15	5	3	69	18	145

[a] $C_{max\text{-}free}$, the non-protein-bound fraction of the drug in mouse serum (75% for vancomycin and 10% for teicoplanin).
[b] The MIC used was from the microtiter method with Mueller-Hinton broth and 5% sheep blood.
[c] The MIC used was from the microtiter method with mouse serum and 5% sheep blood as the medium.
[d] $T_{>MIC\text{-}free}$, the time for which the non-protein-bound fraction of the serum concentrations was above the MIC. MICs were determined in Mueller-Hinton broth with 5% sheep blood.
[e] The baseline for the AUC was at 0.001 μg/ml.
[f] The MICs from the determinations in Mueller-Hinton broth with 5% sheep blood were used.

DISCUSSION

Both vancomycin and teicoplanin proved to have excellent activities against pneumococci in this study, both in vitro with MICs below 0.5 μg/ml and especially in vivo with ED_{50}s below 1 mg/kg. For comparison, susceptible pneumococci show ED_{50}s for penicillin in the range of 0.5 to 2 mg/kg and for macrolides in the range of 2 to 6 mg/kg in this animal model (7, 14). The effects of teicoplanin and vancomycin were not influenced by the penicillin resistance of the strains of pneumococci, either in vitro or in vivo, since no correlations were found between the MICs of penicillin and the MICs or ED_{50}s of the glycopeptides (Table 1 and Table 2). Although both glycopeptides and β-lactams inhibit cell wall synthesis, the mechanism of action of glycopeptides is different from that of β-lactams. The changes in the penicillin-binding proteins in the pneumococci that make penicillin less effective seem to have no effect on the binding of glycopeptides to their target. The MICs of glycopeptides were in the same range for all 10 pneumococcal strains, but depended on the media used for testing. The MBCs did not differ from the MICs. The MICs in different media reflected the difference in protein binding of the two drugs; the superiority of teicoplanin in low-protein media, e.g., low MICs in Mueller-Hinton media supplemented with 5% blood, disappeared when the MIC determinations were performed with mouse or human sera (Table 1). The MICs for vancomycin and teicoplanin were rather similar in human and mouse sera, and the ED_{50}s for mice were at the same level (less than a twofold difference between the 50th and 90th percentiles; Table 2). This has also been found by others, who have shown that the effective doses were similar in animal studies in spite of the superiority of teicoplanin in traditional MIC testing of the bacterial pathogens (4, 19). In studies of time-kill experiments with glycopeptides it has been found that the addition of serum proteins made the drugs inactive relative to the protein-bound fractions (1, 5). Surprisingly, a study of the activity of teicoplanin relative to protein binding using serum bactericidal tests on samples from humans treated with teicoplanin indicated that the protein-bound fraction of teicoplanin also was active (9). Although differences in endpoints for effect must be taken into account, our study seems to indicate the opposite; i.e., that the protein-bound fraction of teicoplanin is inactive both in vitro and in vivo.

The data in this study, the MICs determined by using National Committee for Clinical Laboratory Standards guide-

lines, the protein binding in human and mouse sera, and the serum elimination rates in mice were comparable to those found by others (1, 10, 18–20).

Considering the evaluation of the pharmacodynamics of the glycopeptides, we determined various pharmacokinetic parameters at the values of the ED_{50}s (Table 3). The most important parameters under these conditions will be those that vary the least (14). It should be kept in mind, however, that the small variation in the MICs of both drugs over only three or four twofold dilutions may make this type of evaluation of the pharmacodynamics less optimal, and further studies clearly need to be done. We have studied two drugs with very different pharmacokinetics, and therefore it may be best to study the pharmacodynamics for each drug separately. The most constant pharmacokinetic parameters were $T_{>MIC}$, which only varied by two- to threefold for both drugs, and C_{max}, which varied by three- to fivefold; the ratios between the pharmacokinetic parameters and the MICs showed greater variation (Table 3). The variations for vancomycin were small for all parameters (Table 3). When both drugs were included, the variation in the parameters was least for C_{max} (Table 3). The pharmacokinetic parameter predictive of an effect must be expected to be universal within a class of antibiotics and to be unaffected by the organisms; however, the evaluation of drugs in the same class of antibiotics with different pharmacokinetic profiles needs to be done separately.

From the evaluation of our results, we can conclude, that both the parameters $T_{>MIC}$ and C_{max} are of major importance for predicting the effect of single-dose glycopeptide treatments of pneumococci in mice. Animal studies focusing on the important pharmacokinetic parameter for prediction of the effect of glycopeptides are few (5, 16, 19). In a rabbit model of endocarditis with *Streptococcus sanguis*, doses of 6.25 or 10 mg/kg of teicoplanin twice a day (b.i.d.) were given both with and without gentamicin at 1 mg/kg b.i.d. for 5 days (16). In terms of culture positivity of daily drawn blood samples or weight and number of CFU in vegetations 8 h after the last treatments, there was no difference between high and low doses and there was synergy between gentamicin and teicoplanin (16). In another study of teicoplanin in the rabbit endocarditis model with *S. sanguis* and *Staphylococcus aureus*, increasing effect was found with increasing b.i.d. doses from 4.5 to 18 mg/kg against *S. sanguis* alone (5). The sterilization of *S. aureus* vegetations was better in a regimen in which teicoplanin was given intramuscularly than in one in which the same dose was given intravenously; i.e., the intravenous regimen gave the higher C_{max} levels but lower trough levels in serum and also lower mean concentrations in the vegetations (5). From these studies no pharmacokinetic parameters can easily be selected as the most important; however, the importance of the trough levels indicates that a drug concentration above a certain level is important. A study of the prediction of the killing effect in vivo from a knowledge of the killing effect in vitro for both vancomycin and teicoplanin against *S. aureus* showed that the effect in vivo was correlated to the killing in vitro (19). Since the killing effect in vitro is concentration independent above a certain level (one to two times the MIC), the study indicates that $T_{>MIC}$ is the most important (19). Studies of different dosing regimens in different in vitro models simulating human kinetics have not been able to elucidate the pharmacodynamics of the glycopeptides completely. In a study using four different dosing regimens of vancomycin against *S. aureus*, i.e. a regimen giving one peak concentration of 48 μg/ml, two peaks of 30 μg/ml with 12 h in between, or constant levels of either 16 or 8 μg/ml, all four regimens induced a similar decrease in the CFUs during the 24-h treatment period (8). No correlation with the pharmacokinetic parameters could be found, as only the maximum kill rate was observed. The pharmacokinetics following treatments of humans with glycopeptide have been well described, but efficacy has almost always only been correlated to peak and trough levels (2, 11, 22). For humans a study of conventional versus continuous dosing of vancomycin in response to documented or suspected gram-positive infection was performed as a crossover study of 10 patients receiving each regimen for two days (12). Serum samples from the patients were used to determine serum bactericidal titers against two strains of *S. aureus* (12). No difference in the outcome of the patients was seen, and a large interpatient variability of concentration was found. The mean values of the pharmacokinetic parameters in the regimens were almost identical, and no prediction of effect on the basis of a pharmacokinetic parameter could be made.

In conclusion we showed that vancomycin and teicoplanin were effective against pneumococci independently of their resistance to penicillin, both in vitro and in vivo. When the higher degree of protein binding of teicoplanin was taken into account, the two glycopeptides showed similar efficacies. The prediction of the effect of teicoplanin in mice was best if the MICs were determined with mouse serum as the medium. The E test was the best MIC method to predict the effect of vancomycin in the mice. We found that the parameters $T_{>MIC}$ and C_{max} are both of major importance for predicting the effect of the single-dose glycopeptide treatments of pneumococci in mice but that the pharmacodynamics should preferably be evaluated with multidosing regimens.

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