

## In Vitro Studies of Pharmacodynamic Properties of Vancomycin against *Staphylococcus aureus* and *Staphylococcus epidermidis*

E. LÖWDIN,\* I. ODENHOLT, AND O. CARL

Antibiotic Research Unit, Department of Infectious Diseases and Clinical Microbiology,  
University Hospital, Uppsala, Sweden

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The bactericidal activities of vancomycin against two reference strains and two clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* were studied with five different concentrations ranging from 2× to 64× the MIC. The decrease in the numbers of CFU at 24 h was at least 3 log<sub>10</sub> CFU/ml for all strains. No concentration-dependent killing was observed. The postantibiotic effect (PAE) was determined by obtaining viable counts for two of the reference strains, and the viable counts varied markedly: 1.2 h for *S. aureus* and 6.0 h for *S. epidermidis*. The determinations of the PAE, the postantibiotic sub-MIC effect (PA SME), and the sub-MIC effect (SME) for all strains were done with BioScreen C, a computerized incubator for bacteria. The PA SMEs were longer than the SMEs for all strains tested. A newly developed in vitro kinetic model was used to expose the bacteria to continuously decreasing concentrations of vancomycin. A filter prevented the loss of bacteria during the experiments. One reference strain each of *S. aureus* and *S. epidermidis* and two clinical isolates of *S. aureus* were exposed to an initial concentration of 10× the MIC of vancomycin with two different half-lives ( $t_{1/2}$ s): 1 or 5 h. The post-MIC effect (PME) was calculated as the difference in time for the bacteria to grow 1 log<sub>10</sub> CFU/ml from the numbers of CFU obtained at the time when the MIC was reached and the corresponding time for an unexposed control culture. The difference in PME between the strains was not as pronounced as that for the PAE. Furthermore, the PME was shorter when a  $t_{1/2}$  of 5 h (approximate terminal  $t_{1/2}$  in humans) was used. The PMEs at  $t_{1/2}$ s of 1 and 5 h were 6.5 and 3.6 h, respectively, for *S. aureus*. The corresponding figures for *S. epidermidis* were 10.3 and less than 6 h. The shorter PMEs achieved with a  $t_{1/2}$  of 5 h and the lack of concentration-dependent killing indicate that the time above the MIC is the parameter most important for the efficacy of vancomycin.

Vancomycin has been used for the treatment of severe infections caused by gram-positive bacteria since the 1950s. Its use has increased during the last decade due to the emergence of multiple antibiotic resistance in staphylococci, pneumococci, and enterococci. Even though vancomycin has been in clinical use for a long period of time, its pharmacodynamic properties are still incompletely known. The terminal half-life ( $t_{1/2}$ ) of vancomycin in serum is long, between 3 and 9 h, and the recommended dosing schedules are 1 g every 12 h or 500 mg every 6 h (14, 16–18). These recommended dosing regimens generally provide a trough concentration of approximately 5× the MIC for the infecting pathogen (7, 10, 20). The rate and extent of bacterial killing, the postantibiotic effect (PAE), and the postantibiotic sub-MIC effect (PA SME) (26, 27) provide a more accurate description of the antimicrobial activity than the MIC alone and thus offer a more rational basis for determining optimal dosing schedules. Another recently described pharmacodynamic parameter which may be important when determining dosing schedules for antibiotics is the post-MIC effect (PME) (22). This parameter is studied in an in vitro kinetic model, in which bacteria can be exposed to antibiotic concentrations with kinetics that mimic the kinetics of the drug in human serum. The PME represents the difference in time for the numbers of CFU to increase 1 log<sub>10</sub>/ml compared with the values obtained at the time when the antibiotic concentration has declined to the MIC and the corresponding time for a control culture grown in a test tube without antibiotic.

The aim of the present investigation was to study the rate and extent of bacterial killing, the PAE, PA SME, and PME of vancomycin against different strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

### MATERIALS AND METHODS

**Bacterial cultures and media.** The following strains were investigated: *S. aureus* ATCC 29213 (methicillin susceptible), Colindale Col. 1841 (methicillin resistant), 352-3028 (methicillin susceptible), and 344-1011 (methicillin resistant) and *S. epidermidis* ATCC 29886 (methicillin susceptible), ATCC 29887 (methicillin resistant), 928-6002 (methicillin resistant), and 931-5028 (methicillin susceptible). The strains were grown in Todd-Hewitt broth at 37°C for 6 h before the experiments to obtain an exponential growth phase.

**Determination of vancomycin concentrations.** Vancomycin was provided as a reference powder from Eli Lilly Sweden AB, Stockholm, Sweden. The drug concentrations were assayed by using a commercial fluorescence polarization immunoassay (TD<sub>x</sub>FL<sub>x</sub>; Abbott Laboratories, North Chicago, Ill.). The within-run coefficient of variation (CV) for vancomycin at a concentration of 7 µg/ml was 2.45%, and the within-run CV for vancomycin at a concentration of 75 mg/ml was 1.97%. The between-day CVs at these concentrations were 2.98 and 1.58%, respectively.

**Determination of MICs.** The MICs for all strains were determined by the macrodilution technique in 2 ml of Todd-Hewitt broth by using twofold dilutions with an inoculum of approximately 10<sup>5</sup> CFU/ml. The MIC was defined as the lowest concentration of the antibiotic allowing no visible growth after 20 h of incubation (8). In addition, the MICs were also determined for all strains in the BioScreen C (Lab Systems, Helsinki, Finland) (21) and were defined as the lowest concentration of the antibiotic that prevented growth for 20 h, as measured by determining the optical density (OD). The lowest detectable OD for *S. aureus* and *S. epidermidis* corresponded to approximately 5 × 10<sup>5</sup> CFU/ml. Determinations of MICs were performed at least in duplicate on separate occasions.

**Determination of killing.** All strains, at an inoculum of approximately 5 × 10<sup>5</sup> CFU/ml, were incubated in tubes with vancomycin at concentrations of 2, 4, 8, 16, and 64× the MIC for 24 h. A growth control was also included. The tubes were incubated at 37°C. Samples were drawn at 0, 3, 6, 9, 12, and 24 h and, if necessary, were diluted in phosphate-buffered saline and spread onto agar plates, the plates were incubated at 37°C, and the colonies were counted after 24 h. Determina-

\* Corresponding author. Mailing address: Department of Infectious Diseases and Clinical Microbiology, University Hospital, S-751 85 Uppsala, Sweden. Phone: (46)-18-665647. Fax: (46)-18-665650.

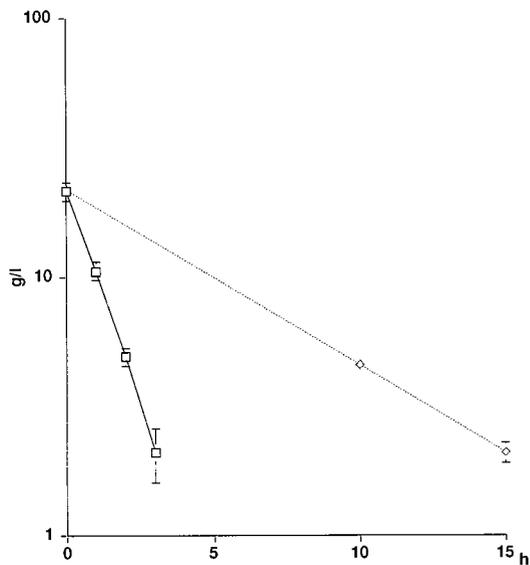


FIG. 1. Concentration-time curves from the experiments with an initial concentration of  $10\times$  the MIC of vancomycin and  $t_{1/2}$ s of 1 h ( $\square$ ) and 5 h ( $\diamond$ ). Values are the means of six experiments. Bars indicate standard deviations.

tions of the numbers of CFU were based on plates with 10 to 500 colonies per plate. All experiments were performed in triplicate.

**Induction of the PA phase.** The cultures in the exponential growth phase were diluted to obtain a bacterial density of approximately  $5 \times 10^7$  CFU/ml. Four-milliliter aliquots were then exposed to  $10\times$  the MIC of vancomycin for 2 h in 10-ml, round-bottom glass tubes. Control cultures were left untreated. The exposed cultures and the controls were then washed twice by centrifugation at  $1,500 \times g$  for 10 min and were diluted in fresh broth to obtain a bacterial density of approximately  $10^5$  CFU/ml.

**Determination and definition of the PAE with viable counts.** A PA phase was induced as described above with *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 29886, and the cultures were then incubated at  $37^\circ\text{C}$ . Samples were drawn every 1 to 3 h for 11 h, and at 24 h samples were cultured as described above. The experiments were performed in triplicate for both strains.

The PAE was defined as described by Craig and Gudmundsson (5) as  $T - C$ , where  $T$  is the time required for the viable counts of the exposed bacteria to increase by  $1 \log_{10}$  above the counts observed immediately after washing and  $C$  is the corresponding time for the unexposed controls.

**Determination and calculation of the PAE, PA SME, and SME in BioScreen C.** Experiments were performed in triplicate with *S. aureus* ATCC 29213 and

*S. epidermidis* ATCC 29886 and once each with *S. aureus* Colindale Col. 1841, 352-3028, and 344-1011, and *S. epidermidis* ATCC 29887, 928-6002, and 931-5028. A PA phase was induced as described above. For measurement of PAE and PA SME, 360- $\mu\text{l}$  volumes of broth with no antibiotic or with a concentration of 0.1 to  $0.9\times$  the MIC of vancomycin were placed in wells with a volume of 400  $\mu\text{l}$ . Forty microliters of the cultures in the PA phase was then added, and the wells were incubated in the BioScreen C for 20 h at  $37^\circ\text{C}$ . To study the SME, previously untreated controls were similarly exposed to 0.0 to  $0.9\times$  the MIC of vancomycin. The absorbance (OD) was measured every 10 min during the experiment. The results were processed in the BioScreen C and were printed out as growth curves. At least three different dilutions of the control cultures were made in order to obtain a control with an inoculum as close as possible to that of the cultures exposed to the antibiotic. The PAE was defined as the difference in time for the exposed and the unexposed cultures to grow to a chosen point ( $A_{50}$ ) on the absorbance curve.  $A_{50}$  was defined as 50% of the maximum absorbance of the control cultures. PA SME and SME were likewise defined as the difference in time for the exposed culture and the control culture to reach an  $A_{50}$  (21).

**In vitro kinetic model.** A recently described in vitro kinetic model was used (22). It consists of a spinner flask with a  $0.45\text{-}\mu\text{m}$ -pore-size filter membrane and a prefilter fitted between the upper and the bottom parts. A magnetic stirrer ensures the homogeneity of the culture and prevents membrane pore blockage. The original model contains a stirrer with a magnet covered in Teflon. However, in preliminary experiments with this model, when the staphylococci were exposed to a constant concentration of vancomycin, regrowth occurred, even though the concentration was  $10\times$  the MIC. Staphylococci are known to adhere to Teflon (19, 28, 31), and we suspected that the bacteria were adhering to the surface of the stirrer. A new stirrer in which the magnet was encased in glass was therefore constructed (AB Wiklunds Glas Instrument, Stockholm, Sweden). Subsequent experiments with this stirrer and  $10\times$  the MIC of vancomycin showed no regrowth of bacteria during 24 h.

In one of the side arms of the culture vessel, a silicon membrane was inserted to enable repeated sampling. The other arm was connected with a thin plastic tube to a vessel containing fresh medium. The medium was removed from the culture flask, through the filter, at a constant rate with a pump. Fresh sterile medium was sucked into the flask at the same rate by the negative pressure that built up inside the culture vessel. The antibiotic was added to the vessel and was eliminated at a constant rate according to the first-order kinetics  $C = C_0 \cdot e^{-kt}$ , where  $C_0$  is the initial antibiotic level,  $C$  is the antibiotic level at time  $t$ ,  $k$  is the rate of elimination, and  $t$  is the time that has elapsed since the addition of antibiotic. The apparatus was placed in a thermostatic room at  $37^\circ\text{C}$  during the experiments. The culture vessel was sterilized by autoclaving between every experiment.

**Definition and determination of PME.** The PME was defined as the difference between the time needed for the numbers of CFU in the culture vessel to increase  $1 \log_{10}/\text{ml}$  from the numbers obtained at the time when the antibiotic concentration had declined to the MIC and the corresponding time for a control culture grown in a test tube without antibiotic (22).

*S. aureus* ATCC 29213, 344-1011, and 352-3038 and *S. epidermidis* ATCC 29886 were exposed in the kinetic model to an initial concentration of  $10\times$  the MIC of vancomycin with a  $t_{1/2}$  of 1 or 5 h. Samples for bacterial counts and antibiotic concentrations were drawn at appropriate intervals during the experiments. The samples were cultured as described above. Samples from the waste

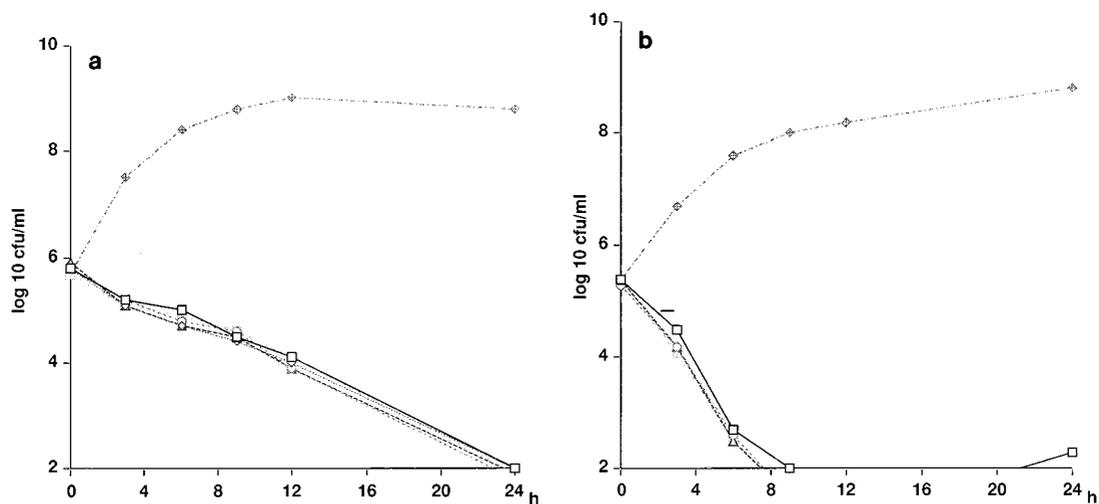


FIG. 2. (a) Killing curves for *S. aureus* ATCC 29213 exposed to 2, 4, 8, 16, and  $64\times$  the MIC of vancomycin. (b) Killing curves for *S. epidermidis* ATCC 29886 exposed to 2, 4, 8, 16, and  $64\times$  the MIC of vancomycin. Values are the means of three experiments. Standard deviations were less than 0.4 for all measurements.

TABLE 1. Reduction in bacterial numbers at 3 and 9 h with a concentration of 8× the MIC of vancomycin

Strain	Mean (range) reduction (log <sub>10</sub> CFU/ml) at the following times <sup>a</sup> :	
	3 h	9 h
<i>S. aureus</i> ATCC 29213	0.5 (0.4–0.6)	1.2 (1.2)
<i>S. aureus</i> Colindale Col. 1841	1.2 (1.0–1.7)	2.9 (2.5–3.1)
<i>S. aureus</i> 352-3028	0.4 (0.3–0.5)	2.7 (2.3–3.1)
<i>S. aureus</i> 344-1011	0.5 (0.4–0.5)	2.0 (1.8–2.3)
<i>S. epidermidis</i> ATCC 29886	1.1 (0.9–1.4)	3.3 (2.8–3.6)
<i>S. epidermidis</i> ATCC 29887	0.9 (0.7–1.0)	3.3 (3.1–3.4)
<i>S. epidermidis</i> 931-5028	0.2 (0.0–0.3)	2.2 (2.0–2.4)
<i>S. epidermidis</i> 928-6002	0.7 (0.6–0.8)	3.4 (3.1–3.9)

<sup>a</sup> Values are the means of three experiments. Results below the limit of detection were given a value of 2 log<sub>10</sub> CFU/ml.

were also drawn at intervals during the experiments to ensure that there was no filter leakage. The experiments were performed in triplicate with the reference strains and once each with the clinical isolates.

## RESULTS

**MICs.** The MICs were 2 mg/liter for all strains except *S. aureus* Colindale Col. 1841 and *S. epidermidis* ATCC 29887, for which the MICs were 4 mg/liter. Identical results were obtained by the macrodilution method and with BioScreen C.

**Antibiotic concentration.** The concentrations of vancomycin obtained in the in vitro kinetic model showed little variation between experiments (Fig. 1).

**Killing curves.** No concentration-dependent killing of any of the strains tested was detected (Fig. 2a and b). The reductions in the numbers of log<sub>10</sub> CFU per milliliter at 3 and 9 h with a concentration of 8× the MIC of vancomycin are listed in Table 1. The killing rate was low for all strains. However at 24 h, a reduction of >3 log CFU/ml was achieved for all strains with all concentrations. The only exception was *S. epidermidis* ATCC 29886 exposed to 2× the MIC, which showed a reduction of >3 log CFU/ml after 9 h, but regrowth occurred at between 12 and 24 h in two of three experiments (Fig. 2b).

**PAEs.** There was a good correlation between the PAEs determined by obtaining viable counts and those obtained in BioScreen C. The PAE for *S. aureus* ATCC 29213 was 1.2 h (range, 0.6 to 1.9 h) by the viable counting method and 1.6 h (range, 1.3 to 2.0 h) in BioScreen C. For *S. epidermidis* ATCC

29886 the corresponding figures were 6.0 h (range, 5.7 to 6.5 h) and 4.8 h (range, 4.3 to 5.4 h). The PAEs for all strains in BioScreen C are presented in Table 2.

**PA SMEs and SMEs.** The PA SMEs and SMEs in BioScreen C for the different strains are presented in Tables 2 and 3. The PA SMEs were very long for all strains tested, while the SMEs were shorter. Long SMEs were seen, however, when *S. aureus* Colindale Col. 1841 and *S. epidermidis* ATCC 29887, 928-6002, and 931-5028 were challenged with vancomycin at sub-MICs greater than 0.4 to 0.5× the MIC. No regrowth could then be demonstrated for 20 h in BioScreen C, indicating that the true MICs for these strains were lower than those determined with twofold serial dilutions.

**PMEs.** The PME for *S. aureus* ATCC 29213 when  $t_{1/2}$ s of vancomycin of 1 h and 5 h were simulated in the kinetic model were 6.5 h (range, 5.1 to 8.1 h) and 3.6 h (range, 2.6 to 4.6 h), respectively (Fig. 3a and b). The PME for *S. aureus* 344-1011 and 352-3038 were 5.6 and 6.3 h, respectively, with a  $t_{1/2}$  of 1 h and 3.6 and 3.8 h, respectively, with a  $t_{1/2}$  of 5 h.

For *S. epidermidis* ATCC 29886 the PME with a  $t_{1/2}$  of 1 h was 10.3 h (range, 8.8 to 11.4 h). When a  $t_{1/2}$  of 5 h was used, the bacterial density declined to below the level of detection (10<sup>2</sup> CFU/ml) and was not measurable at the time when the concentration had declined to the MIC (Fig. 4a and b). The exact length of the PME therefore could not be calculated. However, in two of the three experiments the numbers of CFU per milliliter were above 10<sup>3</sup> before the MIC was reached, at approximately 15.5 h, or at 21 h; hence, the PME in these experiments was less than 6 h.

## DISCUSSION

The MICs and the minimum bactericidal concentrations of antibiotics have been the major parameters used to quantify the in vitro antimicrobial activities of antibiotics against infecting pathogens, and together with the pharmacokinetic properties of the drug they are still the major factors determining antibiotic dosing regimens. Since the MICs are determined after 20 to 24 h of incubation with a constant antibiotic concentration, they represent the net result of a series of events that may have taken place during this time. Furthermore, the MIC does not provide any information about the activities of increased drug concentrations, the antibacterial effects that may persist after elimination of the drug, or the influence of sub-MICs. Pharmacodynamic parameters such as the rate of

TABLE 2. PAE and PA SME after exposure to 10× the MIC of vancomycin for 2 h for *S. aureus* and *S. epidermidis* strains studied in BioScreen C

Strain	PAE (h)	PA SME (h) with the following multiples of the MIC:					
		0.1	0.2	0.3	0.4	0.5	0.6
<i>S. aureus</i> ATCC 29213 <sup>a</sup>	1.6 (1.3–2.0)	3.1 (2.4–3.6)	5.3 (4.6–6.1)	8.2 (7.3–9.6)	10.0 (9.1–11.0)	13.3 (12.4–14.7)	NC <sup>b</sup> (13.9–>20)
<i>S. epidermidis</i> ATCC 29886 <sup>a</sup>	4.8 (4.3–5.4)	9.2 (7.4–10.1)	NC (11.3–>12)				
<i>S. aureus</i>							
1841	2.0	7.3	10.0	11.6	>13.0		
3028	0.7	2.3	4.6	10.7	>14.6		
1011	1.8	3.3	5.7	7.6	11.1	>16	
<i>S. epidermidis</i>							
ATCC 29887	2.6	10.0	>11				
6002	2.1	6.0	10.3	>10.3			
5028	1.4	10.0	13.8	>14.0			

<sup>a</sup> For these strains, values are means (ranges).

<sup>b</sup> NC, not calculated.

TABLE 3. SME of vancomycin for *S. aureus* and *S. epidermidis* strains studies in BioScreen C

Strain	SME (h) at the following multiples of the MIC:					
	0.1	0.2	0.3	0.4	0.5	0.6
<i>S. aureus</i> ATCC 29213 <sup>a</sup>	0.1 (0.0–0.3)	0.3 (0.1–0.4)	0.6 (0.3–1.0)	1.9 (1.1–2.9)	5.9 (3.7–9.0)	NC <sup>b</sup> (7.0–15.8)
<i>S. epidermidis</i> ATCC 29886 <sup>a</sup>	0.1 (0.0–0.3)	0.3 (0.0–0.6)	0.8 (0.0–1.3)	1.8 (1.0–2.3)	3.4 (2.0–4.3)	5.9 (3.3–7.7)
<i>S. aureus</i>						
1841	0.1	0.9	10.0	>13.1	>13	>13
3028	0.1	1.0	1.7	2.7	5.6	8.1
1011	0.0	0.0	0.3	1.3	4.6	10.7
<i>S. epidermidis</i>						
ATCC 29887	4.3	5.6	6.7	10.7	>10.9	>10.9
6002	3.3	3.3	4.6	7.0	>9.9	>9.9
5028	0.1	0.9	2.9	>14.1	>14.1	>14.1

<sup>a</sup> For these strains, values are means (ranges).

<sup>b</sup> NC, not calculated.

bacterial killing, the PAE, and the PA SME may therefore provide a more accurate description of the antimicrobial activity than the MIC alone (3, 5, 25–27, 34).

Small and Chambers (33) reported a slow rate of killing of *S. aureus* exposed to 4× the MIC of vancomycin for 24 h. At 4 h a reduction of 1.1 log<sub>10</sub> CFU/ml was seen, and at 24 h no further significant change in the numbers of CFU was observed. Also, in our experiments the rate of killing was slow for all strains; however, killing continued beyond 4 h and there was a reduction of >3 log<sub>10</sub> CFU/ml at 24 h for all strains of *S. aureus*. Our results showed no concentration-dependent killing against any of the strains of *S. aureus* and *S. epidermidis* that were studied, which is in agreement with the results of several previous studies (1, 6, 9, 15, 30). However, Greenberg and Benes (12) compared the efficacies of oxacillin, vancomycin, and teicoplanin against different strains of *S. aureus* incubated with 50% bovine serum and reported a significantly increased rate and amount of killing when the concentration of vancomycin was increased from 20 to 80 mg/liter, even though the free drug concentration still exceeded the MIC with the lower

dose. Peetermans et al. (29) have shown concentration-dependent killing for concentrations below the MIC but not for higher concentrations.

The PAEs found in the present study were relatively short (0.7 to 2.6 h) for all strains except *S. epidermidis* ATCC 29886 (range, 4.3 to 6.5 h). Our results are in accordance with those reported previously (4, 15, 23). In contrast to the PAEs, the PA SMEs were very long for all strains tested. We have shown earlier (27) that vancomycin exhibits very long PA SMEs against strains of *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Peetermans et al. (29) showed an initial killing when *S. aureus* was challenged with vancomycin concentrations just below the MIC; this may explain the long SMEs seen in the present study with concentrations close to the MIC.

In the in vivo situation, when intermittent antibiotic dosing is used, antibiotic levels in serum and tissues will gradually decrease and the microorganisms will often be exposed to both supra- and sub-MICs during the dosing interval. The killing of bacteria subjected to various drug concentrations may therefore provide more meaningful information about optimal dos-

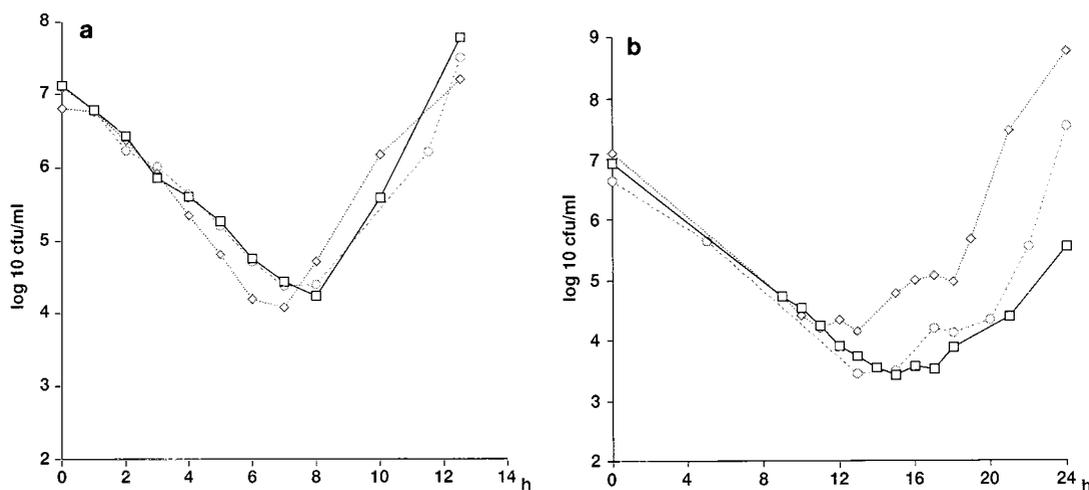


FIG. 3. (a) Killing and regrowth of *S. aureus* ATCC 2913 exposed to 10× the MIC of vancomycin with a  $t_{1/2}$  of 1 h. Three separate experiments were performed on different occasions. The concentration declined to the MIC at approximately 3.3 h, and the time for a control to grow 1 log<sub>10</sub> CFU/ml was 1.4 h. The PME was 6.5 h (range, 5.8 to 8.1 h). (b) Killing and regrowth of *S. aureus* ATCC 2913 exposed to 10× the MIC of vancomycin with a  $t_{1/2}$  of 5 h. Three separate experiments were performed on different occasions. The concentration declined to the MIC at approximately 15.3 h, and the time for a control to grow 1 log<sub>10</sub> CFU/ml was 1.4 h. The PME was 3.6 h (range, 2.6 to 4.6 h).

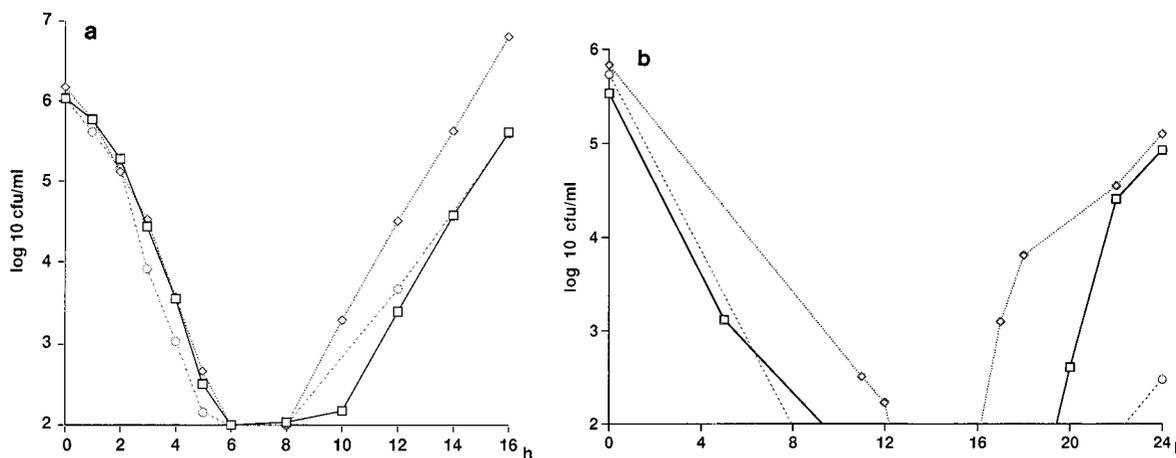


FIG. 4. (a) Killing and regrowth of *S. epidermidis* ATCC 29886 exposed to 10 $\times$  the MIC of vancomycin with a  $t_{1/2}$  of 1 h. Three separate experiments were performed on different occasions. Values below the detection limit were given a value of 2. The concentration declined to the MIC at approximately 3.3 h, and the time for a control to grow 1 log<sub>10</sub> CFU/ml was 1.2 h. The PME was 10.3 h (range, 8.8 to 11.4 h). (b) Killing and regrowth of *S. epidermidis* ATCC 29886 to 10 $\times$  the MIC of vancomycin with a  $t_{1/2}$  of 5 h. Three separate experiments were performed on different occasions. Values below the detection limit were given a value of 2. The concentration declined to the MIC at approximately 15.3 h, and the PME could not be calculated because the bacterial density declined to below the level of detection.

ing strategies than determinations obtained with static concentrations. To obtain conditions in vitro that stimulate the antibiotic pharmacokinetics in vivo, different kinetic models have been developed (2, 11, 12a, 24, 32).

In a recent study with an in vitro kinetic model in which bacteria are exposed to fluctuating antibiotic concentrations, a new parameter, the PME, was introduced (22). The PME was calculated as the difference in time for the bacteria to grow 1 log<sub>10</sub> CFU/ml from the numbers obtained at the time when the drug concentration declined to the MIC and the corresponding time for a control culture not exposed to antibiotic. Beta-hemolytic streptococci were challenged with benzylpenicillin with different  $t_{1/2}$ s, and the PMEs were calculated. The PME seemed to be dependent on the  $t_{1/2}$  of benzylpenicillin, and longer PMEs were obtained with a  $t_{1/2}$  of 1 h than with one of 3 h (22).

Vancomycin interferes with cell wall synthesis in susceptible bacteria by binding to the terminal aminoacyl D-alanyl-D-alanine sequence, which is different from the interference caused by benzylpenicillin, which binds to penicillin-binding proteins. It also has a longer terminal  $t_{1/2}$  in humans (approximately 3 to 9 h) whereas benzylpenicillin has a terminal  $t_{1/2}$  of approximately 1 h (14, 16–18). Since the PA SMEs were shown to be very long for vancomycin against staphylococci, we found it to be of interest to investigate the effects of different  $t_{1/2}$ s of vancomycin on the length of the PME.

Vancomycin and teicoplanin both bind firmly to peptidoglycan. It has been suggested that the regrowth of staphylococci exposed to vancomycin and teicoplanin may occur if inadequately inhibited bacteria synthesize new peptidoglycan to override the antibacterial effect (13). The PAE, PA SME, and PME would then reflect the time that the amount of peptidoglycan is kept below a critical level needed for bacterial growth. The PMEs were shorter for both *S. aureus* and *S. epidermidis* with the longer  $t_{1/2}$  of 5 h, with which regrowth started when the drug concentration declined to the MIC (Fig. 3b and 4b). In these experiments the concentration declines slowly and remains close to the MIC for a longer period of time, during which synthesis of new peptidoglycan could be allowed. The level of peptidoglycan necessary for regrowth could therefore be reached when the drug concentration declined to the MIC and the following sub-MICs would not prevent cell divi-

sion. In contrast, when a  $t_{1/2}$  of 1 h was used the MIC was already reached after 3.5 h and the killing continued even 3 to 4 h after this time point. One explanation for this could be that the amount of peptidoglycan at this time was not yet sufficient for the initiation of regrowth.

In conclusion, we found no concentration-dependent killing of vancomycin against the strains of *S. aureus* and *S. epidermidis* tested in this study, relatively short PAEs, but very long PA SMEs. The PA SMEs were calculated from the results of experiments performed with a very short exposure (2 h) to supra-MICs and seem to overestimate the effects of subinhibitory concentrations. When a short  $t_{1/2}$  was used, the PME exceeded the PAE probably due to the effects of subinhibitory concentrations. However, the PME was shorter when the  $t_{1/2}$  was extended to 5 h (simulating the terminal  $t_{1/2}$  in humans), and regrowth started when the drug concentration declined to the MIC. It is still unclear which serum concentration-time profile in relation to MIC leads to the optimal efficacy for glycopeptides. When Duffull et al. (6) used four different vancomycin dosing regimens against *S. aureus* in an in vitro kinetic model, they found that killing was not dependent on either the peak concentration or the area under the curve. Maintaining a constant concentration above the MIC was equally effective even with an area under the curve that was half of that obtained by the other three regimens. Our findings also indicate that the time that the concentration in serum exceeds the MIC is the parameter most important for the efficacy of this drug, hence suggesting that dosing schedules with vancomycin should maintain the concentration above the MIC for most of the dosing interval and that high peak concentrations are not needed. However, to further define the optimal dosing of vancomycin, studies with other species and multiple dosing regimens are needed.

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