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NOTES

Pronounced Postantibiotic Effect of Quinupristin-Dalfopristin in Static Cultures of *Staphylococcus aureus*: an Effect Not Seen with Other Antibiotics

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Quinupristin-dalfopristin produced postantibiotic effects (PAEs) on exponentially growing (log phase) and nongrowing (lag phase) cultures of 0.4 to 6.9 h. PAEs of the other antibiotics tested were 0.5 to 1.9 h, determined with exponentially growing cultures. None of these antibiotics show a PAE on lag-phase bacteria.

The period of bacterial growth suppression seen after the removal of an antibiotic from a culture medium has been termed postantibiotic effect (PAE).

Quinupristin-dalfopristin (QID) produced an excellent PAE against *Staphylococcus aureus* in a recent study by Pankuch et al. (9). PAE measurements are traditionally performed with bacteria when they are in the logarithmic growth phase (3, 7, 8). However, in vivo bacteria often grow less efficiently, because of substrate limitation in biofilms on medical implants or infections like endocarditis or osteomyelitis. In comparison to the growth rate in vitro, the growth rate in vivo can be lengthened from 0.4 to 24 h (6). A change in the growth rate may influence the pharmacodynamic properties like the PAE of an antibiotic. Because the PAE is often used to find the optimal dosing schedule of an antibiotic, a decrease in the PAE on bacteria with a long generation time could lead to an insufficient dosing schedule. In our study, we compared the PAE of nongrowing cultures (lag phase) and exponentially growing cultures (log phase) by using QID, vancomycin (VAN), gentamicin (GEN), moxifloxacin (MOX), cefotaxime (CTX), ceftriaxone (CRO), and roxithromycin (ROX).

Four strains of *S. aureus*—one strain of methicillin-susceptible *S. aureus* (MSSA) and three strains of epidemic multiresistant methicillin-resistant *S. aureus* (MRSA) (various macrolide-lincosamide-streptogramin B [MLS] phenotypes)—were used in this study (10): *S. aureus* 306/94 (methicillin susceptible, erythromycin susceptible), *S. aureus* 1159/93 (methicillin resistant, erythromycin susceptible), *S. aureus* 1000/93 (methicillin resistant, inducibly macrolide resistant), and *S. aureus* 134/94 (methicillin resistant, constitutively macrolide resistant).

PAE was determined by the viable plate count method as described by Craig and Gudmundsson (5) with Iso-Sensitest broth (Oxoid, Wesel, Germany) as the test medium.

Bacteria were grown in Iso-Sensitest broth for 16 h at 37°C with shaking. Antibiotics were added to each culture to get a final concentration of 1, 2, and 4 times the MIC. Because of the multiresistance of the three MRSA strains, only the MICs of

TABLE 1. PAEs for exponentially growing (log-phase) and nongrowing (lag-phase) bacterial cultures of *S. aureus* 306/94 (MSSA)

Growth phase	Concn of antibiotic	Exposure time (h)	PAE (h) (range) of:						
			QID	VAN	MOX	GEN	ROX	CTX	CRO
Log phase[a]	1× MIC	1	2.5 (2.0–3.0)	0.5 (0.4–0.8)	0.8 (0.7–1.1)	1.3 (1.0–1.5)	1.2 (1.1–1.4)	0.6 (0.4–0.9)	0.5 (0.5–0.6)
	2× MIC	1	4.0 (3.7–4.3)	0.7 (0.5–0.9)	1.6 (1.1–1.9)	2.2 (1.8–2.8)	1.5 (1.4–1.6)	0.7 (0.5–1.0)	0.7 (0.5–0.8)
	4× MIC	1	5.0 (4.6–5.3)	0.8 (0.6–0.1)	1.9 (2.1–1.7)	2.5 (2.0–3.0)	1.7 (1.4–1.9)	0.9 (0.5–1.1)	0.6 (0.4–0.8)
Lag phase[b]	1× MIC	1	0.5 (0.5–0.6)	0.0	0.0	0.0	0.1 (0.0–0.1)	0.0	0.1 (0.0–0.1)
	2× MIC	1	0.7 (0.6–0.7)	0.0	0.1 (0.1)	0.1 (0.1)	0.3 (0.2–0.5)	0.1 (0.0–0.1)	0.0
	4× MIC	1	1.5 (1.1–1.7)	0.1 (0.1)	0.2 (0.0–0.2)	0.1 (0.0–0.1)	0.4 (0.2–0.5)	0.0	0.1 (0.0–0.1)
Lag phase[b]	1× MIC	4	1.9 (1.7–2.1)	0.0	0.1 (0.0–0.1)	0.2 (0.1–0.2)	0.4 (0.3–0.5)	0.1 (0.0–0.2)	0.1 (0.0–0.1)
	2× MIC	4	5.7 (5.5–6.0)	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.2 (0.1–0.3)	0.5 (0.4–0.6)	0.1 (0.1)	0.1 (0.0–0.1)
	4× MIC	4	6.9 (6.7–7.1)	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.0–0.2)	0.4 (0.3–0.4)	0.0	0.1 (0.0–0.2)

[a] Experiments were done in triplicate for all antibiotics.
[b] Experiments with QID were done in triplicate, and the experiments with the other antibiotics were done in duplicate.

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TABLE 2. PAEs for exponentially growing (log phase) and nongrowing (lag phase) bacterial cultures of MRSA strains

Growth phase	*S. aureus* strain	Exposure time (h)	PAE (h) (range) of:					
			QID			VAN		
			1× MIC	2× MIC	4× MIC	1× MIC	2× MIC	4× MIC
Log phase[a]	1159/93	1	2.8 (2.5–3.3)	3.5 (3.4–3.8)	4.8 (4.4–5.1)	1.1 (0.9–1.3)	1.2 (0.8–1.5)	1.2 (1.0–1.4)
	1000/93	1	2.3 (2.2–2.7)	2.8 (2.5–3.2)	4.1 (3.8–4.2)	0.8 (0.6–1.0)	0.8 (0.7–0.9)	0.9 (0.8–1.0)
	134/94	1	1.3 (1.0–1.7)	2.5 (2.4–2.7)	3.8 (3.6–4.0)	0.9 (0.7–1.1)	1.0 (0.8–1.1)	1.2 (0.8–1.4)
Lag phase[b]	1159/93	1	0.6 (0.5–0.7)	0.8 (0.7–0.9)	1.2 (1.0–1.5)	0.1 (0.0–0.1)	0.2 (0.1–0.2)	0.2 (0.1–0.2)
	1000/93	1	0.5 (0.4–0.5)	0.6 (0.4–0.7)	1.3 (1.2–1.5)	0.0	0.1 (0.0–0.1)	0.2 (0.1–0.2)
	134/94	1	0.4 (0.2–0.6)	0.4 (0.3–0.4)	0.6 (0.4–0.7)	0.1 (0.0–0.1)	0.0	0.1 (0.0–0.1)
Lag phase[b]	1159/93	4	2.0 (1.7–2.0)	5.8 (5.3–6.2)	6.2 (6.0–6.5)	0.0	0.2 (0.1–0.3)	0.2 (0.1–0.2)
	1000/93	4	1.7 (1.5–2.0)	4.5 (4.1–5.1)	5.5 (5.0–5.8)	0.1 (0.0–0.1)	0.0	0.1 (0.1)
	134/94	4	0.6 (0.4–0.8)	1.5 (1.0–1.9)	2.4 (2.1–2.8)	0.1 (0.0–0.1)	0.1 (0.0–0.1)	0.1 (0.1)

[a] Experiments were done in triplicate.
[b] Experiments were done with QID in triplicate and VAN in duplicate.

QID and VAN were determined with these strains. MICs were evaluated by a microdilution method in Iso-Sensitest broth according to the guidelines of the National Committee for Clinical Laboratory Standards (7). The MICs, in milligrams per liter, for *S. aureus* 306/94 were as follows: QID, 0.25; VAN, 1.0; MOX, 0.125; GEN, 1.0; ROX, 1.0; CTX, 2.0; CRO, 2.0. The MICs of QID and VAN were 0.25 and 1.0, respectively, for *S. aureus* 1159/93 and 0.5 and 1.0, respectively, both for *S. aureus* 1000/93 and for *S. aureus* 134/94. For studies on nongrowing bacterial cultures, the 16-h cultures were used. The time of antibiotic exposure was 1 and 4 h. For studies on exponentially growing cultures, the 16-h cultures, which contained 10^9 CFU/ml, were diluted (1:100) in fresh prewarmed Iso-Sensitest broth and incubated at 37°C with shaking for 1 h. After 1 h, these cultures contained an inoculum of 10^7 CFU/ml of an exponentially growing culture. The time of antibiotic exposure was 1 h. The 4-h exposure time was omitted because QID and MOX showed a rapid bactericidal effect; thus, the bacterial count was too low for PAE determination by viable plate count after dilution. At the end of the exposure time, cultures were diluted 1:500 or 1:2,000 in fresh Iso-Sensitest broth prewarmed to 37°C. Dilution, rather than washing, was used to remove the antibiotics, since washing bacterial cells can itself slow down the growth rate (5). One hundred milliliters of each diluted culture was then reincubated at 37°C with shaking. A control culture to which no antibiotic had been added was handled in the same manner. Another control was used to verify that the antibiotics no longer had either a bactericidal or a bacteriostatic effect against the bacteria after dilution (concentration, 0.008 to 0.002 times the MIC).

Viable counts were determined immediately after dilution (0 h) and then every hour until a visible turbidity was reached. Viability counts were performed by making 10-fold dilutions of the sample with a 0.9% NaCl solution (Merck, Darmstadt, Germany) and then plating an aliquot of 0.05 ml on china blue lactose agar (Oxoid) plates. Plates were read after 20 to 48 h at 37°C. The PAE was defined as described by Craig and Gudmundsson (5). All experiments were performed in duplicate or in triplicate (Tables 1 and 2).

The PAE on the exponentially growing cultures of *S. aureus* 306/94 ranged from 2.5 to 5.0 h for QID, from 1.3 to 2.5 h for GEN, from 1.2 to 1.7 h for ROX, from 0.8 to 1.9 h for MOX, and from 0.5 to 0.9 h for VAN, CTX, and CRO (Table 1). Because of the multiresistance of the three MRSA strains, only a PAE for QID and VAN has been determined with these strains. The PAE of QID ranged from 1.3 to 4.8 h, and for VAN it was between 0.8 and 1.1 h (Table 2). In conclusion, QID produced long PAEs against all of the strains tested. None of the other antibiotics tested produced a comparably long PAE. Only GEN showed a PAE of more than 2 h against *S. aureus* 306/94 (Table 1). In contrast to other reports (4, 8), we found equal PAEs for the constitutive, erythromycin-resistant MRSA strains and for the inducible strain.

For lag-phase cultures of *S. aureus*, a significant PAE was only found for QID (Table 2). After an exposure time of 1 h, values for QID ranged from 0.4 to 1.5 h for all strains tested. For ROX, the PAE ranged from 0.1 to 0.4 h, and for the other antibiotics it was between 0.0 and 0.2 h (two experiments with each antibiotic). During an exposure time of 4 h, the PAEs of QID increased highly. With the exception of the constitutive *S. aureus* strain (0.6 to 2.4 h), the PAEs were between 1.7 and 6.9 h. No increases in the PAEs of the other antibiotics were seen after an increase of the exposure time. These results show that QID produces equally long PAEs on lag-phase bacterial cultures of *S. aureus* and that under these conditions the PAE of the constitutively resistant strain is reduced in the same way as the PAE on exponentially growing cultures.

The high accumulation of QID in gram-positive bacteria may be the reason for the long PAE of QID on static bacteria. The internal concentration of QID is 58-fold higher than the external concentration and is maintained by the specific binding of QID to bacterial ribosomes (2). A very stable ternary quinupristin-ribosome-dalfopristin (1:1:1 stoichiometry) complex is formed (1). The period of bacterial growth suppression may be a result of this stable complex, which leads to an inhibition of protein synthesis. The dissociation of the ternary complex and the diffusion out of the bacteria may allow protein synthesis followed by bacterial growth.

The results of this study demonstrate the variability of PAE measurements obtained by different methods. Because the conditions in vivo differ from the in vitro situation, one has to be careful with recommendations for dosing schedules.

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