Postantibiotic effects and postantibiotic sub-MIC effects of benzylpenicillin on viridans streptococci isolated from patients with infective endocarditis


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We investigated the postantibiotic effects (PAEs) and the postantibiotic sub-MIC effects of benzylpenicillin on three strains of viridans streptococci isolated from infective endocarditis patients. The PAEs of benzylpenicillin on penicillin tolerant Streptococcus sanguis TW-70 (0-4-3-9 h), penicillin tolerant S. sanguis TW-80 (0-3-6-3 h) and nontolerant Streptococcus oralis TW-186 (0-5-3-1 h) were dependent on exposure time. The PAEs were not concentration dependent for S. sanguis TW-70 and S. sanguis TW-80 above the MIC, and for S. oralis TW-186 above 16 x MIC. The antimicrobial effects of benzylpenicillin at sub-MIC concentrations were examined in bacteria pretreated with benzylpenicillin (8 x MIC) for 2 h and compared with untreated bacteria. At the sub-MICs tested, the regrowth of pretreated S. oralis TW-186 cells was more prolonged than that of untreated cells and bactericidal action was seen only in pretreated cells. These effects (so-called 'postantibiotic sub-MIC effects') were not observed in penicillin tolerant S. sanguis TW-70. The presence of the postantibiotic sub-MIC effect may be an important factor in determining the dosing regimen for infective endocarditis.

Introduction

The persistent suppression of bacterial growth after short exposure to antimicrobials is known as a postantibiotic effect (PAE) (Craig & Gudmundsson, 1991). It is an important factor in determining antimicrobial dosing regimens since a prolonged PAE justifies extending dosage intervals beyond the time when antimicrobial concentrations at the site of infection fall below the MIC (Craig & Gudmundsson, 1991).

Recent studies have shown that the antimicrobial effects at sub-MICs on various bacteria were enhanced in the PAE phase (Odenholt, Holm & Cars, 1989; Odenholt-Tornqvist, Holm & Cars, 1990; Oshida et al., 1990; Odenholt-Tornqvist, Lowdin & Cars, 1991, 1992; Kikuchi, Totsuka & Shimizu, 1992b; Kikuchi et al., 1993). These so-called 'postantibiotic sub-MIC effects' might contribute to the longer duration of the PAEs in-vivo than in-vitro.

Viridans streptococci are the organisms most frequently involved in infective endocarditis (Tunkel & Mandell, 1992; van der Meer et al., 1992), and have frequently

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exhibited penicillin tolerance (Slater & Greenwood, 1983; Meylan, Flancioli & Glauser, 1986; Holbrook et al., 1988; Powley, Meeson & Greenwood, 1989; James, Young & White, 1991; van der Meer et al., 1991; Kikuchi et al., 1992a). The optimal dosing regimen of an antimicrobial for treatment of infective endocarditis is determined by bactericidal activity, and pharmacokinetic pharmacodynamic parameters such as a PAE (Cremieux & Carbon, 1992).

We investigated the in-vitro PAEs and the postantibiotic sub-MIC effects of benzylpenicillin on penicillin tolerant and nontolerant viridans streptococci isolated from infective endocarditis patients.

Materials and methods

Organisms and culture methods

*Streptococcus sanguis* TW-70 (= GIFU 8328 = ATCC 10556; type strain of *S. sanguis*) originally isolated from an infective endocarditis patient (Washburn, White & Niven, 1951) was purchased from the Department of Microbiology, School of Medicine, Gifu University (Gifu, Japan). *S. sanguis* TW-80 and *Streptococcus oralis* TW-186 were isolated from the blood of infective endocarditis patients. The bacteria were cultured on Columbia agar (Oxoid, Basingstoke, UK) with 5% horse blood (Nihon Bio-Test, Tokyo, Japan) (BA) at 37°C anaerobically (AnaeroPack, Mitsubishi Gas Chemical, Tokyo, Japan) and were then grown overnight in Todd-Hewitt broth (THB; Oxoid).

Antibiotics and susceptibility tests

Benzylpenicillin (lot no. PCKWS-9-14) was purchased from Meiji Seika Kaisha (Yokohama, Japan). MICs and MBCs were determined by a microbroth dilution method (Holbrook et al., 1988). Tolerance was defined as an MBC/MIC ratio ≥32 (Slater & Greenwood, 1983). Eagle's effect was defined according to Eagle & Musselman (1948).

Determination of the PAE

The PAE was determined by the method described by Craig & Gudmundsson (1991). The test strains grown overnight were diluted 10⁻² in fresh THB and incubated for 2 h at 37°C. These cultures were diluted 10⁻² and incubated for a further 2 h. The cultures were then exposed to benzylpenicillin at various concentrations (0·25–256 × MIC) for various times (0·5–8·0 h). To eliminate the drug, the exposed strains were washed three times by centrifugation at 3500 g for 10 min and resuspended in the same volume of THB. Controls were washed and resuspended similarly. The strains exposed were diluted 10⁻¹ or 10⁻², and the controls were diluted 10⁻³ or 10⁻⁴ in THB to achieve approximately the same inoculum. Both cultures were reincubated and samples withdrawn at respective times. Samples, if necessary diluted in sterile saline, were seeded on to BA plates in duplicate. After 48 h incubation anaerobically, viable cells were counted. The PAE was calculated by the following formula:

\[
\text{PAE} = T - C;
\]

where \( T \) is the time required for the viable count of the test culture to increase by 1 log₁₀ after drug removal and \( C \) is the time required for the viable cell count of the
untreated control culture to increase by $1 \log_{10}$ after completion of the same procedure used on the test culture for drug removal. Each experiment was repeated three to six times.

**Determination of the sub-MIC effect**

Pretreatment with benzylpenicillin at $8 \times \text{MIC}$ for 2 h (PAE induction) and washing procedures were as described above. The cultures were resuspended after washing and diluted $10^{-2}$ in THB. These cultures were exposed to benzylpenicillin at $1/16 \times \text{MIC} - 1 \times \text{MIC})$. Unpretreated control cultures were similarly exposed to the same concentrations of benzylpenicillin. These cultures were reincubated (time 0) and samples withdrawn at timed intervals. Viable cell counts were determined as described above. The postantibiotic sub-MIC effect was evaluated by the following two parameters.

1. Postantibiotic sub-MIC enhancement (PASE), which was represented in Figure 1, was defined by the following formula (Kikuchi et al., 1993):

$$\text{PASE} = S' - S,$$

where $S$ represents the difference between the growth time (time for the viable cell counts to increase $1 \log_{10}$) at each sub-MIC of benzylpenicillin without pretreatment (without PAE induction) and the growth time of the untreated control culture, and $S'$ is the difference between the growth time at each sub-MIC of benzylpenicillin with pretreatment (with PAE induction) and the growth time of the control culture with only pretreatment (only PAE induction).

2. A change in viable cell counts at 4 h was defined as the difference between the viable cell counts at 4 h and those at time 0.

Each experiment was repeated three times.

![Figure 1](https://example.com/figure1.png)

*Figure 1. Schematic representation of the postantibiotic effect (PAE) and the postantibiotic sub-MIC enhancement (PASE) of the postantibiotic sub-MIC effect. □, Pretreatment with benzylpenicillin ($8 \times \text{MIC}$); ○, control without pretreatment; ■, sub-MIC benzylpenicillin without pretreatment; ■, control with pretreatment (PAE); □, sub-MIC benzylpenicillin with pretreatment.*
Table. Susceptibilities to benzylpenicillin of test strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/L)</th>
<th>MBC (mg/L)</th>
<th>Eagle effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. sanguis</td>
<td>TW-70</td>
<td>0.12</td>
<td>&gt;128</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>TW-80</td>
<td>0.12</td>
<td>&gt;128</td>
</tr>
<tr>
<td>S. oralis</td>
<td>TW-186</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Statistical analysis
Statistically significant differences were analysed by the Mann–Whitney U test (StatView II; Abacus Concepts Inc. Berkley, CA).

Results

Antimicrobial susceptibilities to benzylpenicillin
The MIC and MBC values and the Eagle effect for three strains are shown in the Table. Each S. sanguis was tolerant, and the S. oralis strain was nontolerant to benzylpenicillin. The Eagle effect was found only in S. sanguis TW-70.

Determination of the PAEs
The PAEs of benzylpenicillin were not concentration dependent (fixed exposure time 2 h) for S. sanguis TW-70 (0.8–1.2 h) and S. sanguis TW-80 (2.0–2.3 h) above the MIC. The PAE of benzylpenicillin (fixed exposure time 2 h) on S. oralis TW-186 was prolonged in a concentration dependent manner up to 16 × MIC and reached a maximum response (0.5–2.7 h). The PAEs were increased on S. sanguis TW-70 (0.4–3.9 h above 1 h of exposure time), on S. sanguis TW-80 (0.3–6.3 h above 0.5 h of exposure time), and on S. oralis TW-186 (1.0–3.1 h above 1 h of exposure time) in a exposure time dependent manner (exposure concentration fixed at 4 × MIC) up to 8 h and did not reach maximum responses at the longest exposure time in this study.

Determination of the sub-MIC effects
Figure 2 shows the sub-MIC effects of benzylpenicillin on S. sanguis TW-70 with or without pretreatment. The postantibiotic sub-MIC effects were not found in this strain following exposure to benzylpenicillin. Figure 3 shows the sub-MIC effects of benzylpenicillin on S. oralis TW-186 with or without pretreatment. The regrowth at each sub-MIC was more prolonged with than without pretreatment. Furthermore, the bactericidal effect at sub-MIC, which did not occur without pretreatment, was found with pretreatment. These postantibiotic sub-MIC effects of benzylpenicillin were described as the PASEs (Figure 4) and the changes in viable cell counts at 4 h (Figure 5). The positive PASEs on S. oralis TW-186 were concentration dependent up to 1/2 × MIC, but PASEs on S. sanguis TW-70 were not found.

Discussion
There have been a few reports of PAEs on viridans streptococci. Dornbusch, Henning & Linden (1989) demonstrated that two new penems, FCE22101 and FCE24362, had
PAEs on penicillin tolerant viridans streptococci isolated from infective endocarditis patients. We agreed with these results. However, Holbrook et al. (1989) did not find any benzylpenicillin PAEs on five strains of penicillin tolerant S. sanguis.

The mechanism of β-lactam PAEs is poorly understood. One possible explanation is that the PAE is the time required for the organism to resynthesise PBPs (Craig & Gudmundsson, 1991) or the period during which the cell regenerates active PBPs after the β-lactam has dissociated from the covalently bound β-lactam-PBP complexes (Tomasz, 1979). Another theory is that the PAE represents the time taken for restoration of the fine balance between autolytic enzymes and newly activated PBPs (Winstanley & Hastings, 1989). Two strains of S. sanguis used in our experiments showed penicillin tolerance, which might be explained in terms of defective autolytic enzymes (Handwerger & Tomasz, 1985) or some alteration in autolysis regulation (Liu & Tomasz, 1987; Kikuchi et al., 1992a). Therefore, the difference in concentration dependence of the PAEs between S. sanguis and S. oralis may represent differences in restoration of the balance between autolytic enzymes and PBPs.

The significance of the PAE for treatment of infective endocarditis has been shown in some experimental models. Gengo et al. (1984) demonstrated that the effectiveness

![Figure 2](https://academic.oup.com/jac/article-abstract/34/5/687/776000/28)

**Figure 2.** The effects of sub-MICs of benzylpenicillin on S. sanguis TW-70 with or without pretreatment of benzylpenicillin. Each data point represents the mean from three different experiments. •, Control; ○, PAE; □, 1 MIC; □, 2 MIC; ▲, 1 MIC; △, 2 MIC.
of methicillin in experimental *S. aureus* endocarditis was closely related to the total time during which serum concentrations exceeded the MBC of methicillin, the duration of the PAE and the time to enter the log growth phase. Ingerman et al. (1986) observed that ciprofloxacin, with an efficient bactericidal activity and a long PAE, could permit longer dosing intervals in experimental endocarditis due to *Pseudomonas aeruginosa* than ceftazidime and BMY-28142, which did not exhibit PAEs. PAEs have been demonstrated *in vitro*, for imipenem on *P. aeruginosa* and penicillin plus gentamicin on *Enterococcus faecalis*, but these bacteria, antimicrobial combinations did not show PAEs in an experimental infective endocarditis model (Hessen, Pistakis & Levinson, 1988, 1989).

The sub-MIC effects of the antimicrobials bacteria in terms of morphology, growth rate, susceptibility to phagocytic killing and virulence are well known (Lorian & Gemmell, 1991). Since the half-lives of most antimicrobials in humans are longer than those in rodents, the duration of sub-MIC effects is longer in humans. Cremieux & Carbon (1992) reported that the core concentration of benzylpenicillin within a vegetation was 1/3 to 1/8 of the peripheral concentration of the vegetation in experimental rabbit infective endocarditis. These findings indicate the significance of sub-MICs for treatment of infective endocarditis. Antimicrobials that have postantibiotic sub-MIC effects in addition to sub-MIC effects on the bacteria should be more effective in treating endocarditis than those without postantibiotic sub-MIC effects. For agents with
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Figure 4. The postantibiotic sub-MIC effects of benzylpenicillin calculated by the postantibiotic sub-MIC enhancements (PASEs) on S. sanguis TW-70 (■) and S. oralis TW-186 (□). Each value represents the mean ± s.d. from three different experiments.

postantibiotic sub-MIC effects, more widely spaced intermittent dosing intervals would be possible than for those with only PAEs. In the present study, we wish to emphasise the enhanced bactericidal activity associated with postantibiotic sub-MIC effects. Eradication of S. oralis TW-186 by benzylpenicillin in infective endocarditis caused by this strain is likely, even if the drug concentration in the vegetation is below the MIC. In contrast, postantibiotic sub-MIC effects on S. sanguis TW-70 were absent. The reason for this discrepancy is not clear.

The mechanism of the postantibiotic sub-MIC effect is unknown, but the following hypothesis has been proposed for β-lactams. When bacteria are exposed to a supra-inhibitory concentration of penicillin, the drug binds covalently to the PBPs. After removal of the excess drug, most of the PBPs remain inactivated, and a low drug concentration is sufficient to bind the newly produced PBPs (Odenholt et al., 1989; Odenholt-Tornquist et al., 1991). We proposed the following explanation for the absence of a postantibiotic sub-MIC effect of benzylpenicillin on S. sanguis TW-70. When the bacteria are exposed to a high, supra-inhibitory concentration of benzylpenicillin, pronounced inhibition of RNA and protein synthesis is induced, in comparison with effects caused by concentrations near the MIC (Mychajlonka, McDowell & Shockman, 1980). The influence of different drug concentrations at the time of inhibition of RNA and protein synthesis (also affecting autolysis), and of inhibition of peptidoglycan metabolism could explain the Eagle effect (Fontana et al., 1990). The interaction of the Eagle effect and tolerance on viridans streptococci is well known (Holbrook et al., 1988; Kikuchi et al., 1992a). Since S. sanguis TW-70 showed tolerance with the Eagle effect to benzylpenicillin, pronounced tolerance was induced in bacteria treated by benzylpenicillin at 8 × MIC. It was therefore difficult for benzylpenicillin to kill this strain at concentrations approaching the MIC. Since the PAEs of benzylpenicillin on S. sanguis TW-70 were shorter than those on S. oralis TW-186, reactivation
The postantibiotic sub-MIC effects of benzylpenicillin calculated by the changes in viable cell counts at 4 h on \( S. \) sanguis TW-70 (b) and \( S. \) oralis TW-186 (a). Each value represents the mean \( \pm \) S.D. from three different experiments. *, \( P < 0.05 \); **, \( P < 0.01 \). ◻, No pretreatment of benzylpenicillin; ◆, pretreatment of benzylpenicillin.

of the PBPs might be rapid after drug removal (Tuomanen, 1986) and sub-MIC benzylpenicillin could not suppress regrowth.

Penicillin tolerant streptococci are particularly difficult to eradicate in animal models of endocarditis (Brennan & Durack, 1983; Hess, Dankert & Durack, 1983; Lowy et al., 1983; Meeson et al., 1990). Benzylpenicillin monotherapy of penicillin tolerant streptococci cannot be expected to eradicate bacteria in vegetations in the absence of postantibiotic sub-MIC effects.

Further studies are proposed to clarify the in-vivo and clinical implications of PAEs and postantibiotic sub-MIC effects for treatment of infective endocarditis.

References


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