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# Pharmacodynamic Effects of Subinhibitory Concentrations of β-Lactam Antibiotics In Vitro

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**The pharmacodynamic effects of subinhibitory concentrations of different β-lactam antibiotics were investigated. A postantibiotic effect (PAE) was induced for different bacterial species by exposure to 10× MIC of several β-lactam antibiotics for 2 h in vitro. The antibiotic-bacterial combinations used in this study were imipenem-*Pseudomonas aeruginosa*, benzylpenicillin-*Streptococcus pneumoniae* and -*Streptococcus pyogenes*, cefcanel-*S. pyogenes*, ampicillin-*Escherichia coli*, and piperacillin-*E. coli*. After the induction of the PAE, the exposed cultures as well as the unexposed controls were washed and diluted. Thereafter, the cultures in the postantibiotic phase (PA phase) and the cultures not previously treated with antibiotics were exposed to 0.1, 0.2, and 0.3× MIC of the relevant drug and the growth curves were compared. When bacteria in the PA phase were exposed to sub-MICs, a substantial prolongation of the time before regrowth was demonstrated, especially in antibiotic-bacterial combinations for which a PAE was found. In contrast, sub-MICs on cultures not previously exposed to suprainhibitory antibiotic concentrations yielded only a slight reduction in growth rate compared with the controls. Thus, it seems important to distinguish the direct effects of sub-MICs on bacteria not previously exposed to suprainhibitory concentrations from the effects of sub-MICs on bacteria in the PA phase.**

The effects on bacteria of subinhibitory antibiotic concentrations (sub-MICs) were noted early in the antibiotic era. In 1944, Eagle and Musselman reported a temporary inhibition of the growth of spirochetes after exposure to sub-MICs of penicillin in vitro (10). Clinical experience at that time also revealed satisfactory results when patients with pneumococcal pneumonia were treated with low doses of penicillin which could hardly have yielded concentrations in serum above the MIC (38). Furthermore, Eagle and coworkers showed, both in vitro and in a rabbit model, that gram-positive bacteria exposed to a suprainhibitory concentration of penicillin did not return to a growth phase immediately after the concentration in serum had fallen under the MIC (9, 11). This effect was later named the postantibiotic effect (PAE) and is one of many explanations for the success of intermittent dosage with antibiotics (7). Another factor of importance for the success of discontinuous antibiotic dosing is the function of a normal host defense system. For example, it does not seem to be necessary to keep the level of β-lactam antibiotics in serum above the MIC to clear an infection in immunocompetent animals, whereas several studies using neutropenic animals have shown the importance of maintaining levels of β-lactam antibiotics in serum above the MIC in order to avoid regrowth of gram-negative bacteria (3, 14, 33). One reason for the difference between immunocompetent and neutropenic animals is that bacteria exposed to sub-MICs in immunocompetent animals are more susceptible to phagocytic cell functions (13, 19, 24, 26, 41). Sub-MICs can also exert a direct effect on the virulence of the bacteria by modifying the bacterial cell surface and the excretion of exoenzymes (22, 31, 36, 40).

However, in the treatment of infections caused by gram-positive bacteria, the effects of sub-MICs may explain the success of intermittent dosage schedules even in the absence of immune factors. We have shown earlier that when β-hemolytic streptococci in the postantibiotic phase (PA phase) induced by 10× MIC of benzylpenicillin for 2 h in vitro were exposed to sub-MICs, a long delay was demonstrated before regrowth. This was in contrast to the cultures only exposed to the sub-MICs, where no major differences between the growth curves of these cultures and the unexposed controls were seen (27). The effects of sub-MICs on bacteria previously exposed to antibiotics seem thus to be different from the direct effects of sub-MICs. The aim of the present study was to further investigate the effect of sub-MICs of different β-lactam antibiotics on bacteria in the PA phase.

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## MATERIALS AND METHODS

**Cultures.** The strains used in the study were as follows: *Pseudomonas aeruginosa* ATCC 27853; *Escherichia coli* ATCC 25923; *Streptococcus pneumoniae* ATCC 6306; *Streptococcus pyogenes*, group A M12, NCTC P1800; and clinical isolates of *P. aeruginosa* (1139) and *S. pyogenes* group A (U118 and U120). The gram-negative strains were grown in Mueller-Hinton broth (supplemented with 50 mg of $Ca^{2+}$ per liter and 25 mg of $Mg^{2+}$ per liter), and the gram-positive strains were grown in Todd-Hewitt broth. The streptococci and pneumococci were cultured for 6 h at 37°C in 5% $CO_2$ in air, resulting in approximately $5 \times 10^8$ CFU/ml, and the other strains were cultured for 6 h at 37°C, resulting in approximately $10^9$ CFU/ml.

**Antibiotics.** The antibiotics were obtained as reference powders with known potency from the following pharmaceutical companies: imipenem from Merck, Sharp & Dohme, Sweden AB, Bromma, Sweden; benzylpenicillin, ampicillin, and cefcanel from Astra Research Centre, Södertälje, Sweden; and piperacillin from Lederle Cyanamid International, N.J. Dilutions were made with distilled water on the same day as the experiments were performed.

**Determination of MICs.** MICs were determined in fluid media by using a twofold serial dilution with an inoculum of

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$10^5$ CFU of the test strain per ml and were read after 24 h. The MIC was defined as the lowest concentration of the antibiotic allowing no visible growth.

**Induction of the PA phase and determination of the PAE.** The following antibiotic-bacterial combinations were used in the experiments: imipenem-*P. aeruginosa* ATCC 27853 (four experiments) and -*P. aeruginosa* 1139 (two experiments); benzylpenicillin-*S. pyogenes* U118 (two experiments) and -*S. pneumoniae* ATCC 6306 (two experiments); cefcanel-*S. pyogenes* NCTC P1800 (two experiments) and U120 (two experiments); ampicillin-*E. coli* ATCC 25923 (two experiments); and piperacillin-*E. coli* ATCC 25923 (two experiments) (Table 1). After incubation, the test strains were diluted by a factor of $10^{-1}$ in each respective medium, to provide a bacterial density of approximately $5 \times 10^7$ to $1 \times 10^8$ CFU/ml. The strains, in the exponential growth phase, were then exposed to 10× MIC of the different antibiotics for 2 h at 37°C, except for cefcanel–β-hemolytic streptococci, for which 5× MIC was used in consideration of the non-protein-bound concentration in serum attainable in humans. The exposed strains were washed three times for 5 min each time at $1,400 \times g$ to eliminate the antibiotic and were then resuspended in fresh media. Controls, not exposed to the antibiotics, were treated similarly. Depending on the rate of killing during the 2 h of induction, the exposed cultures were either diluted by a factor of $10^{-2}$ in fresh media (cefcanel-*S. pyogenes* NCTC P1800 and U120, benzylpenicillin-*S. pyogenes* U118, and piperacillin-*E. coli*) or were left undiluted (imipenem-*P. aeruginosa* ATCC 27853 and 1139, ampicillin-*E. coli*, and benzylpenicillin-*S. pneumoniae*) in order to obtain approximately $10^5$ CFU/ml. The controls were all diluted by a factor of $10^{-3}$ in fresh media in order to reach a similar bacterial count. Each of the cultures with bacteria in the PA phase and the controls were thereafter divided into four different tubes. To determine the PAE, one tube of each culture was reincubated at 37°C for another 10 h. Samples were withdrawn at 0, 2 (before and after dilution), 3, 4, 5, 6, 8, and 11 h and if necessary diluted with phosphate-buffered saline. Three different dilutions of the samples were seeded on blood agar plates and counted for determination of the number of CFU. Only plates with 10 to 1,000 colonies were counted, and the mean of counts from the three dilutions was used. The PAE was defined according to the following formula (7): $PAE = T - C$, where $T$ is the time required for the viable counts of the antibiotic-exposed cultures to increase by 1 $\log_{10}$ above the counts observed immediately after washing and $C$ is the corresponding time for the unexposed cultures.

**Determination of the effects of sub-MICs.** The remaining three tubes of the control cultures and the cultures in the PA phase were exposed to 0.1, 0.2, and 0.3× MIC of the same antibiotic used for the induction of the PAE and reincubated at 37°C for another 10 h. Samples were withdrawn and the numbers of viable bacteria were determined as described above.

The effect of sub-MICs (sub-MIC effect [SME]) on bacteria not preexposed to antibiotics was defined as follows: $SME = T_S - C$, where $T_S$ is the time taken for the cultures exposed only to sub-MICs to increase by 1 $\log_{10}$ above the counts observed immediately after washing and $C$ is the corresponding time for the unexposed cultures.

The effect of sub-MICs on bacteria in the PA phase (PA SME) was defined as follows: $PA\ SME = T_{PA} - C$, where $T_{PA}$ is the time taken for the cultures previously exposed to antibiotics and then exposed to different sub-MICs to in-

TABLE 1. Effects of subinhibitory antibiotic concentrations on bacteria previously (PA SME) or not previously (SME) exposed to antibiotics

| Antibiotic-bacterial combination | Mean (range) h of effect | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PAE | 0.1× MIC | | 0.2× MIC | | 0.3× MIC | | 0.5× MIC | |
| | | SME | PA SME | SME | PA SME | SME | PA SME | SME | PA SME |
| Imipenem-*P. aeruginosa* ATCC 27853 | −0.8 (−1.1 to 0.1) | 0.6 (0.1 to 1.5) | 1.8 (0.6 to 3.7) | 2.4 (1.1 to 5.5) | 5.1[a] (3.0 to >9) | 4.0[a] (2.6 to >9) | >9[a] (6.2 to >9) | ND[b] | ND |
| Imipenem-*P. aeruginosa* 1139 | 1.0 (0.6 to 1.4) | 0.1 (0 to 0.2) | 3.2 (2.5 to 3.9) | 3.8 (3.4 to 4.2) | 7.0 (5.1 to 8.8) | >8.8[a] (6.7 to >8.8) | >21.8[a] (7 to >21.8) | ND | ND |
| Benzylpenicillin-*S. pyogenes* U118 | 2.3 (2.2 to 2.3) | 0 (−0.2 to 0.1) | 2.7 (2.4 to 3.0) | −0.4 (−0.5 to 0.1) | 2.9 (2.8 to 3.0) | 0.3 (−0.5 to 1.0) | 4.8 (3.3 to 6.2) | ND | ND |
| Benzylpenicillin-*S. pneumoniae* ATCC 6306 | 2.0 (1.6 to 2.2) | 0 (−0.6 to 0.5) | 3.4 (2.8 to 5.0) | 0.4 (4.1 to 7.5) | 4.5 (3.3 to 5.4) | 1.2 (0.4 to 2.0) | 5.8 (4.1 to 7.5) | ND | ND |
| Cefcanel-*S. pyogenes* NCTC P1800 | 2.3 (1.7 to 2.8) | ND | ND | ND | ND | 0.5 (0 to 1) | 3.8 (2.9 to 4.6) | 1.1 (0 to 2.2) | >22.6 (>20 to >22.8) |
| Cefcanel-*S. pyogenes* U120 | 3.3 (1.4 to 5.2) | ND | ND | ND | ND | 0.2 (0 to 0.3) | 6.6 (5.7 to >10.6) | 0.3 (0 to 0.5) | >23.1 (9.2 to >22.6) |
| Ampicillin-*E. coli* ATCC 25923 | −0.1 (−0.3 to 0) | −0.2 (−0.2 to −0.3) | 0.4 (0.1 to 0.8) | 0.1 (−0.3 to 0.5) | 1.5 (1.4 to 1.6) | 0.4 (0.3 to 0.4) | 3.0 (3.0 to 3.0) | ND | ND |
| Piperacillin-*E. coli* ATCC 25923 | −0.5 (−0.4 to −0.6) | 0 (0 to 0) | −0.2 (−0.3 to −0.1) | 0.4 (0.3 to 0.5) | 0 (−0.1 to 0.1) | 0.5 (0.4 to 0.6) | 0.8 (0.7 to 0.8) | ND | ND |

[a] Values calculated from the mean values of CFU because the time for the cultures to grow 1 $\log_{10}$ CFU extended the period of observation.
[b] ND, not determined.

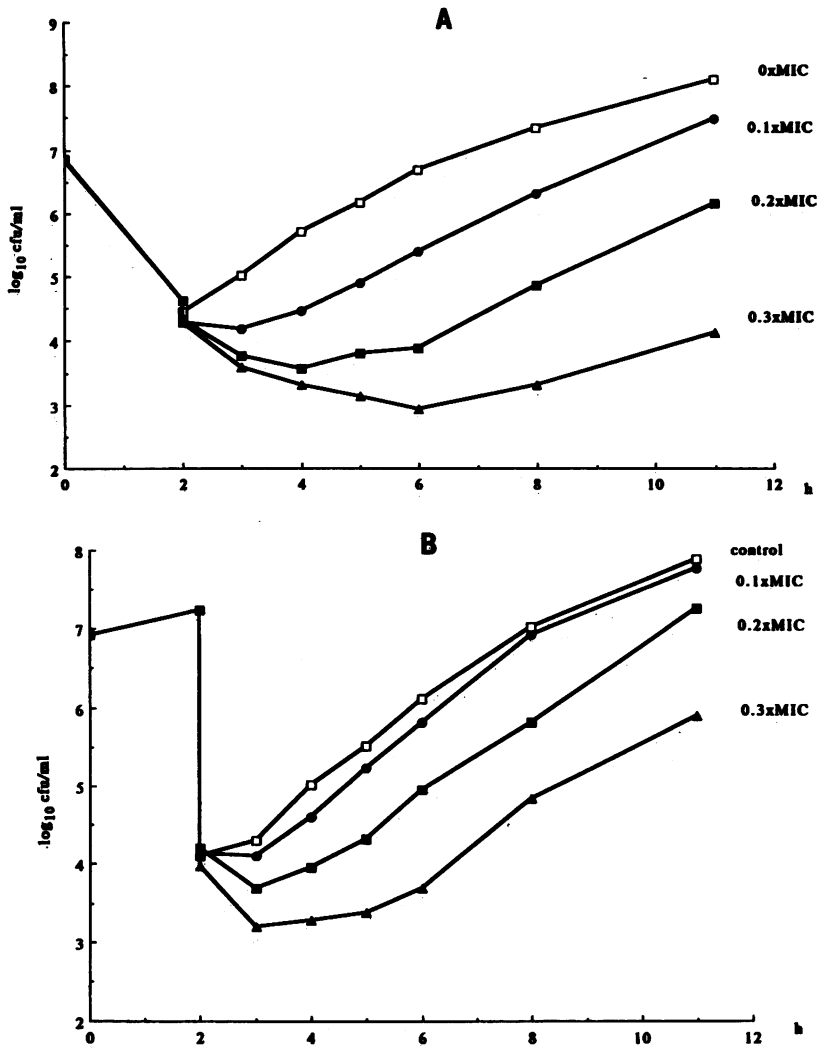


FIG. 1. (A) Effect of different subinhibitory concentrations of imipenem on *P. aeruginosa* ATCC 27853 in the PA phase (PA SME). The bacteria were exposed to 10× MIC of imipenem for 2 h, washed, and then exposed to 0, 0.1, 0.2 or 0.3× MIC. (B) Effect of different subinhibitory concentrations of imipenem on *P. aeruginosa* previously not exposed to imipenem (SME). Mean values based on CFU from four experiments are shown.

crease by 1 $\log_{10}$ above the counts observed immediately after washing and *C* is the time defined above.

## RESULTS

**Minimum antibiotic concentrations.** The MICs were as follows: imipenem-*P. aeruginosa* ATCC 27853, 8 mg/liter; imipenem-*P. aeruginosa* 1139, 2 mg/liter; benzylpenicillin-*S. pyogenes* U118, 0.016 mg/liter; benzylpenicillin-*S. pneumoniae*, 0.016 mg/liter; cefcanel-*S. pyogenes* U120, 0.05 mg/liter; cefcanel-*S. pyogenes* NCTC P1800, 0.05 mg/liter; ampicillin-*E. coli* ATCC 25923, 8 mg/liter; and piperacillin-*E. coli* ATCC 25923, 4 mg/liter. The MICs for the ATCC strains are in close range to those found by other authors.

**PAEs, PA SMEs, and SMEs.** The results of all experiments are listed in Table 1. Examples from some of the experiments are given in Fig. 1 and 2. The effect of subinhibitory imipenem concentrations on *P. aeruginosa* ATCC 27853 in the PA phase is shown in Fig. 1A. The PA SME following treatment with 0.3× MIC of imipenem lasted more than 11 h. Figure 1B demonstrates the effect of a sub-MIC of imipenem on the same bacterial strain not previously exposed to a supra-MIC. Exposure to 0.3× MIC yielded an SME of 4 h. The effects of sub-MICs of piperacillin on *E. coli* are shown in Fig. 2. In this combination, which does not exhibit a PAE, the effect of the sub-MICs was seen to be much less, both on the bacteria in PA phase and on the cultures not preexposed to the drug. The PA SME following treatment with 0.3× MIC was 0.8 h, and the SME was 0.5 h.

## DISCUSSION

One of the prime objectives of antimicrobial therapy is to provide an optimal amount of active drug at the site of the infection. It was long believed that one of the reasons for the success of intermittent-dosage regimens was a delayed antibiotic concentration time course in tissue fluid compared with that in serum, suggesting that even when antibiotic concentrations were not measurable in serum, adequate levels were still present in tissue fluid (17). However, it is now generally believed that in the majority of clinical situa-

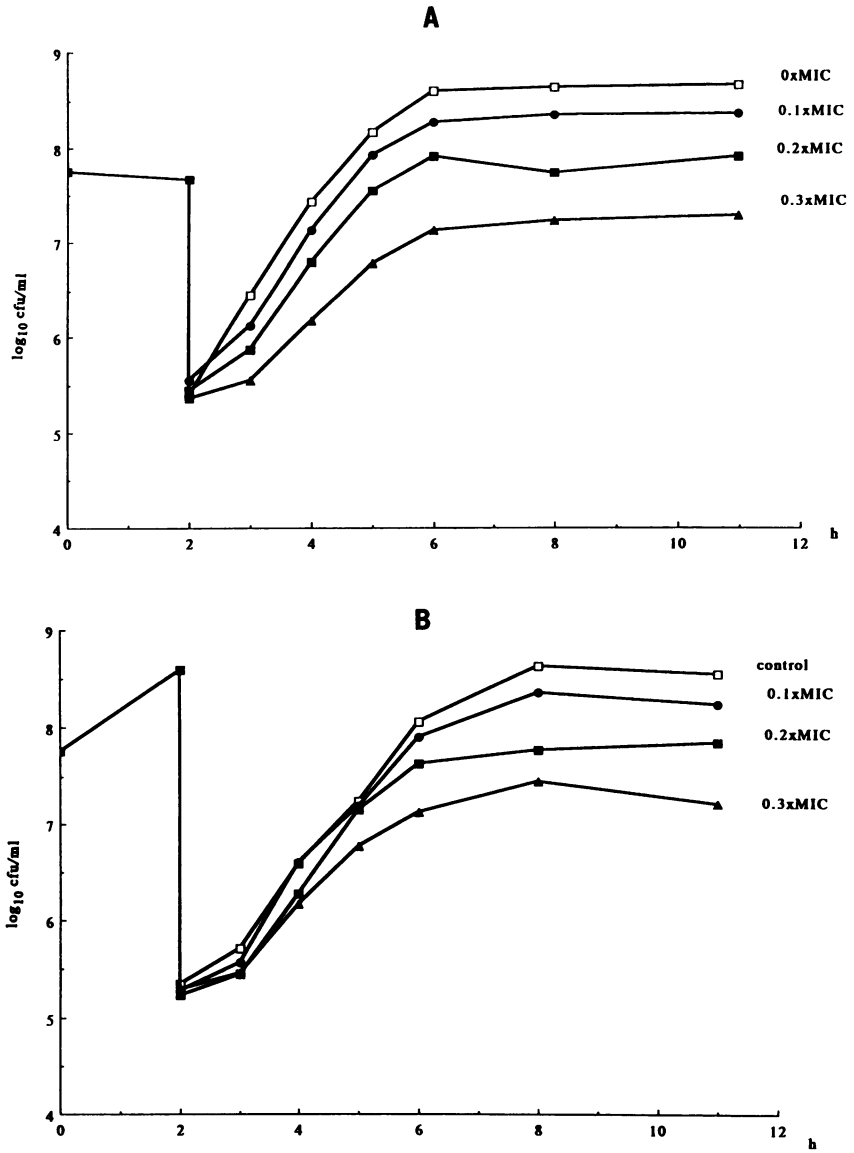


FIG. 2. (A) Effect of subinhibitory concentrations of piperacillin on *E. coli* previously exposed to 10× MIC of piperacillin. The bacteria were exposed to 10× MIC of piperacillin for 2 h, washed, and then exposed to 0, 0.1, 0.2 or 0.3× MIC. (B) Effect of subinhibitory concentrations of piperacillin on *E. coli* previously not exposed to piperacillin. Mean values based on CFU from two experiments are shown.

tions, the free levels of the antibiotic in serum can be used to predict the free levels in the tissue fluid (6, 34).

The concept of a therapeutic level of antibiotics originated from early studies on the sulfonamides and was thereafter readily applied to the penicillins (38). It was suggested that in the in vivo situation, the concentration of the penicillins should be maintained above this critical level by the use of continuous administration. However, in the late 1940s, doubts were raised about the necessity for continuous administration of penicillin (1, 9), and later Eagle et al. showed that intermittent therapy was as effective in normal mice as continuous therapy (8). The therapeutic level was later expressed in terms of concentrations that inhibited the bacteria over 24 h in vitro.

This MIC has thereafter been the instrument of expressing the activity of antibiotics in vitro. However, discrepancies between the MIC tests of an antibiotic performed in vitro and the outcome of treatment of a given infection are common, and the value of a conventional MIC determination has often been criticized, since this titration uses static concentrations which will not reflect the fluctuating concentrations between supra- and sub-MICs seen in vivo. In addition, the MIC determinations simply measure the ability of the antibiotic to prevent the appearance of visible growth after 24 h and disregard factors such as protein binding, immune defense, and compound stability (4, 15, 25).

The direct pharmacodynamic effects of sub-MICs can be measured in different ways. Bacteria exposed to sub-MICs may change in cell wall structure, change in ribosome density, or form filaments (18, 20–22). In addition to morphological changes, sub-MICs may also have a direct inhibitory effect on the bacterial growth in vitro. Lorian introduced the definition of MAC, minimal antibiotic concentration, to describe the lowest concentration of an antibiotic that caused a 1 $\log_{10}$ decrease in the number of microorganisms or resulted in a structural change in the

bacteria observed by light or electron microscopy (19). Svanborg-Edén et al. (36) demonstrated in 1978 that *E. coli* exposed to sub-MICs adhered less readily to human urinary tract epithelial cells, and in the beginning of the 1980s Redjeb et al. (32) showed that 16 of 20 patients with lower urinary tract infections treated with sub-MICs of ampicillin had cleared their urine of bacteria in 2 days, compared with the controls, who all still had bacterial levels of $>10^5$ CFU/ml in the urine. It is also known that sub-MICs of antibiotics can affect the expression of exoenzymes in bacteria (16, 23).

Different animal studies have also shown that sub-MICs can have a therapeutic effect in animals with an intact host defense system. In a rabbit model with implanted tissue cages, we have shown that 0.3× MIC of benzylpenicillin inhibited streptococcal multiplication in the cages for several hours (28). Similar results were demonstrated by Zak and Kradolfer in a mouse model, in which no regrowth of staphylococci or *E. coli* could be seen in the peritoneal fluid for up to 18 h after an intramuscular injection of ampicillin which gave one-third of the MIC in the fluid (42). Roosendaal et al. have shown in a rat pneumonia model that sustained sub-MICs of ceftazidime have a therapeutic effect in immunocompetent rats infected with *Klebsiella pneumoniae* but not in neutropenic rats (33).

The direct effects of sub-MICs described above must, however, be distinguished from the effects of sub-MICs on bacteria previously exposed to supra-MICs. Tuomanen showed that after exposure of a pneumococcal strain to 50× MIC of benzylpenicillin, reexposure to subinhibitory concentrations prolonged the time before regrowth compared with the time for controls (39). In an earlier study (27), we demonstrated that when β-hemolytic streptococci in the PA phase were again exposed to 0.2 and 0.3× MIC after washing, no regrowth was observed for more than 24 h. In contrast, bacteria which had only been exposed to sub-MICs showed growth curves that were almost identical to those of the controls (27). Later, Oshida et al., using the same experimental design, showed that staphylococci in the PA phase were also more susceptible to sub-MICs of β-lactam antibiotics compared with the controls (30). In the present study we have extended our earlier findings and could demonstrate that also in other antibiotic-bacterial combinations, the effects of sub-MICs on bacteria in the PA phase (PA SME) are much more pronounced than the direct effect of sub-MICs (SME) and than the PAE (Table 1). We also showed that, with one exception, the PA SME was substantially longer for antibiotic combinations exhibiting a PAE. When imipenem and *P. aeruginosa* ATCC 27853 were tested, a PAE was not demonstrated but the PA SME still lasted for over 11 h. However, it has earlier been shown that this strain of *P. aeruginosa* exhibits a PAE in vitro when lower inocula are used (5, 29).

The mechanisms behind the effects of subinhibitory concentrations of β-lactam antibiotics on bacteria in the PA phase are not clear. However, it seems reasonable to hypothesize that the following events could explain this phenomenon. When bacteria are exposed to a suprainhibitory concentration of an antibiotic, the drug binds covalently to the active sites of the penicillin-binding proteins (PBPs). Synthesis of PBPs is known to continue during penicillin treatment. When excess drug is removed and a repeated challenge with subinhibitory concentrations is given, most of the PBPs are still inactivated, and only a low drug concentration is needed to inhibit the newly produced PBPs. This results in a prolonged inhibition of cell multiplication until a critical number of free PBPs is once more available (27, 39). Thus, it seems that the PAE can be substantially prolonged in vitro when the bacteria are reexposed to sub-MICs of β-lactam antibiotics.

There are few reports of the effects of sub-MICs in vivo where the animals had previously been exposed to suprainhibitory concentrations of the antibiotic. The early results of Eagle and Musselman (11) concerning the slow recovery period of bacteria after antibiotic exposure are now regarded as being caused by sub-MICs, since the gamma phase of penicillin elimination was not taken into consideration in that rabbit model (12). Sande et al. reported a postantibiotic effect of 6 to 12 h for ampicillin against *S. pneumoniae* in a rabbit meningitis model (35). However, later, when β-lactamase was injected intracisternally in the rabbits, no PAE was revealed and the earlier results were considered to be the effect of sub-MICs (37). Gerber et al. reported a prolonged inhibition of *P. aeruginosa* after the concentration of ticarcillin had fallen under the MIC in a thigh infection model in immunocompetent mice (14). We have also demonstrated a 12-h inhibition of streptococcal growth in rabbits with implanted tissue cages after the concentration in the cages had decreased to below the MIC for the strain (28).

Pharmacodynamic parameters such as the rate of bacterial killing and the time before regrowth of surviving bacteria are factors which may influence the optimal dosage interval. The PAE is one of many factors that have been suggested as a possible reason for the success of intermittent antibiotic dosage regimens (7). However, the duration of the concentration above the MIC plus the duration of the PAE for β-lactam antibiotics will not always cover the entire dosage interval. Furthermore, in humans treated with intermittent-dosage schedules of antibiotics, suprainhibitory concentrations will always be followed by subinhibitory levels. Our experiments show that in certain antibiotic-bacterial combinations where a PAE is found, there is a long delay before regrowth can be achieved with subinhibitory concentrations, even in the absence of immune factors, and it seems that the pharmacodynamic effects of sub-MICs in these antibiotic-bacterial combinations may be of more importance for the inhibition of growth between doses than is the PAE. However, we do not think it is necessary to distinguish the PAE from the effect of sub-MICs, since it is the combined effect of supra- and sub-MICs that will prevent the bacterial regrowth between the doses. To obtain an optimal effect in the treatment of a bacterial infection, it may be important to use different dosage schedules depending on the type of microorganism, the type of antibiotic used, and the status of the host defense system.

The interaction between sub-MICs of antibiotics and the immune system (especially phagocytosis) is probably also of great importance for therapeutic success when antibiotics are administered at long intervals (24, 41). With β-lactam antibiotics, an initial dose sufficient to yield suprainhibitory concentrations, followed by doses that will maintain subinhibitory levels, seems to be a potentially successful method of clearing an infection with gram-positive cocci, even in the absence of immune factors.

## REFERENCES

1. **Altemeier, W. A.** 1948. Penicillin therapy with prolonged interval dosage schedules. Ann. Surg. **128**:708–713.
2. **Bakker-Woudenberg, I. A. J. M., and R. Roosendaal.** 1988. Impact of dosage regimens on the efficacy of antibiotics in the immunocompromised host. J. Antimicrob. Chemother. **21**:145–147.
3. **Bakker-Woudenberg, I. A. J. M., J. C. van den Berg, P. Fontinje, and M. F. Michel.** 1984. Efficacy of continuous versus

intermittent administration of penicillin G in *Streptococcus pneumoniae* pneumonia in normal and in immunodeficient rats. Eur. J. Clin. Microbiol. **3**:131–135.
4. **Barza, M.** 1978. A critique of animal models in antibiotic research. Scand. J. Infect. Dis. Suppl. **14**:109–117.
5. **Bustamente, C. I., G. L. Drusano, B. A. Tatem, and H. C. Standiford.** 1984. Postantibiotic effect of imipenem on *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **26**:678–682.
6. **Cars, O.** 1991. The pharmacokinetics of antibiotics in tissues and tissue fluids: a review. Scand. J. Infect. Dis. Suppl. **74**:23–33.
7. **Craig, W. A., and S. Gudmundsson.** 1986. The postantibiotic effect, p. 515–536. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
8. **Eagle, H., R. Fleischman, and M. Levy.** 1953. Continuous vs. discontinuous therapy with penicillin. The effect of the interval between injections on therapeutic efficacy. N. Engl. J. Med. **248**:481–488.
9. **Eagle, H., R. Fleischman, and A. D. Musselman.** 1950. The bactericidal action of penicillin in vivo: the participation of the host and the slow recovery of the surviving organisms. Ann. Intern. Med. **33**:544–571.
10. **Eagle, H., and A. D. Musselman.** 1944. The spirocheticidal action of penicillin in vitro and its temperature coefficient. J. Exp. Med. **80**:493–505.
11. **Eagle, H., and A. D. Musselman.** 1949. The slow recovery of bacteria from the toxic effects of penicillin. J. Bacteriol. **58**:475–490.
12. **Ebert, S. C., J. Leggett, B. Vogelman, and W. A. Craig.** 1988. Evidence for a slow elimination phase for penicillin G. J. Infect. Dis. **158**:200–202.
13. **Gemmel, C. G., P. K. Petersen, D. Schemling, Y. Kim, J. Matthews, L. Wannamaker, and P. G. Quie.** 1981. Potentiation of opsonization and phagocytosis of *Streptococcus pyogenes* following growth in the presence of clindamycin. J. Clin. Invest. **67**:1249–1256.
14. **Gerber, A. U., H. P. Brugger, C. Feller, T. Stritzko, and B. Stadler.** 1986. Antibiotic therapy of infections due to *Pseudomonas aeruginosa* in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. J. Infect. Dis. **153**:90–97.
15. **Greenwood, D.** 1981. In vitro veritas? Antimicrobial susceptibility tests and their clinical relevance. J. Infect. Dis. **144**:380–385.
16. **Grimwood, K., M. To, H. R. Rabin, and D. E. Woods.** 1989. Inhibition of *Pseudomonas aeruginosa* exoenzyme expression by subinhibitory antibiotic concentrations. Antimicrob. Agents Chemother. **33**:41–47.
17. **Kunin, C. M.** 1981. Dosage schedules of antimicrobial agents: a historical review. Rev. Infect. Dis. **3**:4–11.
18. **Lorian, V.** 1975. Some effects of subinhibitory concentrations of antibiotics on bacteria. Bull. N.Y. Acad. Med. **51**:1046–1055.
19. **Lorian, V.** 1986. Effects of low antibiotic concentrations on bacteria, p. 596–668. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
20. **Lorian, V., and B. Atkinson.** 1975. Abnormal forms of bacteria produced by antibiotics. Am. J. Clin. Pathol. **64**:678–688.
21. **Lorian, V., and B. Popoola.** 1972. The effect of nitrofurudantin on the morphology of gram-negative bacilli. J. Infect. Dis. **125**:187–189.
22. **Lorian, V., and A. Waluschka.** 1972. Blood cultures showing aberrant forms of bacteria. Am. J. Clin. Pathol. **57**:406–409.
23. **Lorian, V., A. Waluschka, and B. Popoola.** 1973. Pneumococcal β-hemolysin produced under the effect of antibiotics. Appl. Microbiol. **25**:290–294.
24. **McDonald, P. J., B. L. Wetherall, and H. Pruul.** 1981. Postantibiotic leucocyte enhancement: increased susceptibility of bacteria pretreated with antibiotics to activity leucocytes. Rev. Infect. Dis. **3**:38–44.
25. **Merrikin, D., and G. N. Rolinson.** 1979. Antibiotic levels in experimentally infected mice in relation to therapeutic effect and antibacterial activity in vitro. J. Antimicrob. Chemother. **5**:423–429.
26. **Milantovic, D.** 1983. Antibiotics and phagocytosis. Eur. J. Clin. Microbiol. **2**:414–425.
27. **Odenholt, I., S. E. Holm, and O. Cars.** 1989. Effects of benzylpenicillin on group A β-hemolytic streptococci during the postantibiotic phase in vitro. J. Antimicrob. Chemother. **24**:147–156.
28. **Odenholt, I., S. E. Holm, and O. Cars.** 1990. Effects of supra- and sub-MIC benzylpenicillin concentrations on group A β-hemolytic streptococci during the postantibiotic phase in vivo. J. Antimicrob. Chemother. **26**:193–201.
29. **Odenholt, I., B. Isaksson, L. Nilsson, and O. Cars.** 1989. Postantibiotic and bactericidal effect of imipenem against *Pseudomonas aeruginosa*. Eur. J. Clin. Microbiol. Infect. Dis. **8**:136–141.
29a. **Odenholt, I., E. Löwdin, and O. Cars.** 1990. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 148.
30. **Oshida, T., T. Onta, N. Nakanishi, T. Matsushita, and T. Yamaguchi.** 1990. Activity of sub-minimal inhibitory concentrations of aspoxicillin in prolonging the postantibiotic effect against *Staphylococcus aureus*. J. Antimicrob. Chemother. **26**: 29–38.
31. **Raponi, G., N. Keller, B. P. Overbeek, M. Rosenberg-Arsak, K. P. M. van Kessel, and J. Verhoef.** 1990. Enhanced phagocytosis of encapsulated *Escherichia coli* strains after exposure to sub-MICs of antibiotics is correlated to changes of the bacterial cell surface. Antimicrob. Agents Chemother. **34**:332–336.
32. **Redjeb, S. B., A. Slim, A. Horchani, S. Zmerilli, A. Boujnah, and V. Lorian.** 1982. Effects of ten milligrams of ampicillin per day on urinary tract infections. Antimicrob. Agents Chemother. **22**:1084–1086.
33. **Roosendaal, R., I. A. J. M. Bakker-Woundenberg, M. van den Berghe-van Raffe, and M. F. Michel.** 1986. Continuous versus intermittent administration of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia in normal and leukopenic rats. Antimicrob. Agents Chemother. **30**:403–408.
34. **Ryan, M. D., O. Cars, and B. Hoffstedt.** 1986. The use of antibiotic serum levels to predict concentrations in tissues. Scand. J. Infect. Dis. **18**:381–388.
35. **Sande, M. A., O. M. Korzeniowski, G. M. Allegro, R. O. Brennan, O. Zak, and W. M. Scheld.** 1981. Intermittent or continuous therapy of experimental meningitis due to *Streptococcus pneumoniae* in rabbits: preliminary observations on the postantibiotic effect in vivo. Rev. Infect. Dis. **3**:98–109.
36. **Svanborg-Edén, C., T. Sandberg, and K. Alestig.** 1978. Decrease in adhesion of *E. coli* to human urinary tract epithelial cells in vitro by subinhibitory concentrations of ampicillin. Infection **6**(Suppl. 1):121–124.
37. **Täuber, M. G., O. Zak, W. M. Scheld, B. Hengstler, and M. A. Sande.** 1984. The postantibiotic effect in the treatment of experimental meningitis caused by *Streptococcus pneumoniae* in rabbits. J. Infect. Dis. **149**:575–583.
38. **Tilett, W. S., M. J. Cambier, and J. E. McCormick.** 1944. The treatment of lobar pneumonia and pneumococcal empyema with penicillin. Bull. N.Y. Acad. Med. **20**:140–143.
39. **Tuomanen, E.** 1986. Newly made enzymes determine ongoing cell wall synthesis inhibitors. J. Bacteriol. **176**:535–543.
40. **Tylewska, S., C. Hjertén, and T. Wadström.** 1981. Effect of subinhibitory concentrations of antibiotics on the adhesion of *Streptococcus pyogenes* to pharyngeal epithelial cells. Antimicrob. Agents Chemother. **30**:563–566.
41. **Van der Auwera, P.** 1991. Interactions between antibiotics and phagocytosis in bacterial killing. Scand. J. Infect. Dis. Suppl. **74**:42–48.
42. **Zak, O., and F. Kradolfer.** 1979. Effects of subminimal inhibitory concentrations in experimental infections. Rev. Infect. Dis. **5**:862–879.