Journal of Antimicrobial Chemotherapy (1985) **15**, *Suppl. A*, 297–302

Successful prophylaxis of experimental streptococcal endocarditis with single doses of sublethal concentrations of penicillin

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Penicillin prophylaxis against experimental endocarditis due to a strain of *Streptococcus intermedius* isolated from a patient with endocarditis was studied in rats. The minimum bactericidal concentration of penicillin for this strain was more than 64 mg/l and was higher than the peak penicillin serum level obtained in rats 30 min after the iv injection of 60 mg/kg, and in man after an oral dose of 2 g of phenoxymethyl penicillin. Moreover timed kill curves performed in the presence of 64 mg/l of penicillin showed no decrease in the number of colony-forming units during the first 6 h of incubation and only a 95% decrease after 24 h. In addition, no bactericidal activity could be detected in the serum 30 min after penicillin injection, that is at the time of bacterial challenge. Using the minimum bacterial inoculum needed to produce endocarditis in 90% of control animals (ID_{90}), penicillin successfully prevented endocarditis due to this strain. We conclude that penicillin may prevent streptococcal endocarditis by other mechanisms than bacterial killing.

Introduction

Penicillin is one of the most commonly recommended antibiotics for prophylaxis of bacterial endocarditis in susceptible patients undergoing oral procedures. Studies in the rabbit model of endocarditis have suggested that high and repeated doses of bactericidal antibiotics, including penicillin, were necessary to achieve successful protection (Durack & Petersdorf, 1973; Pelletier, Durack & Petersdorf, 1975) Furthermore, antibiotics like clindamycin or tetracycline were ineffective, suggesting that bacterial killing was the main mechanism by which antibiotics protected against endocarditis. However the number of micro-organisms used to induce infection in these experiments was very high and probably greatly exceeded that found in man after dental manipulation.

Recent experiments performed with lower inocula have shown that single doses of vancomycin, amoxicillin and even "bacteriostatic" antibiotics such as clindamycin or erythromycin were effective in preventing endocarditis (Bernard, Francioli & Glauser, 1981; Glauser & Francioli, 1982; Glauser *et al.*, 1983a). These studies raised the possibility of mechanisms other than bacterial killing operating to prevent endocarditis, since protection was achieved in the absence of bactericidal activity of the antibiotics used for prophylaxis.

Mechanisms of action other than bacterial killing are not only interesting but highly desirable if one considers that up to 40 to 50% of *Streptococcus viridans* isolated either

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from the mouth flora (Dankert & Hess, 1982) or from patients with endocarditis (Glauser *et al.*, 1983*b*) were shown to be tolerant to penicillin or amoxicillin.

The purpose of the present study was to test the efficacy of penicillin, the antibiotic most commonly used for endocarditis prophylaxis, against a strain of *Str. viridans* highly resistant to its bactericidal activity.

Material and methods

Micro-organism

A previously described strain of *Str. intermedius* isolated from a patient with bacterial endocarditis was used (Glauser & Francioli, 1982). Minimal inhibitory concentration (MIC) of penicillin were determined by broth dilution tests, using Mueller–Hinton broth (Difco Laboratories, Detroit, U.S.A.), and 5×10^5 cfu as the inocula. The minimal bactericidal concentration (MBC) were determined by subculturing on penicillinase-containing blood agar plates 0·1 ml of an undiluted sample. After incubation for 48 h the number of colonies on each plate was counted, and the MBC was determined as the lowest dilution of antibiotic that showed 99·9% killing.

Killing curve with 64 mg/l of penicillin were performed in trypticase soya broth (Difco Laboratories, Detroit, U.S.A.) using an inoculum of 10^6 cfu/ml of an overnight culture of *Str. intermedius*. This concentration was chosen because it was well above achievable serum levels obtainable in humans after a 2 g oral dose of phenoxymethyl penicillin.

Penicillin serum levels and serum bactericidal activity

Serum levels of sodium benzyl penicillin were determined in five rats by an agar diffusion technique (Sabath & Anhalt, 1980) at 30 min, 1 and 2 h after iv injection of 60 mg/kg. This dosage was chosen because it produces peak serum levels in rats similar to those achieved in man after an oral dose of 2 g of phenoxymethyl penicillin. The serum bactericidal activity of penicillin against *Str. intermedius* was determined by standard methods (Anhalt, Sabath & Barry, 1980) 30 min after iv injection of 60 mg of penicillin/kg of body weight. The serum bactericidal activity was defined as the highest serum dilution that gave 99·9% killing after 24 h of incubation.

Production and prophylaxis of endocarditis

Sterile vegetations were produced in femal Wistar rats (weight 180–200 g) by a modification of a previously described method. In brief, a polyethylene catheter (model no. PP10; Portex, Hythe, Kent, England) was passed through the aortic valve via the right carotid artery and secured with a silk ligature. Twenty-four hours after catheterization, rats were injected in the tail vein with saline or 60 mg/kg of sodium penicillin. Thirty minutes later 0·5 ml of saline containing 10^5 cfu of bacteria, the minimal inoculum needed to produce endocarditis in more than 90% of control rats, was injected intravenously (Glauser *et al.*, 1983*a*). Rats were killed 72 h after bacterial challenge, and aortic vegetations were excised, weighed, homogenized in 1 ml of 0·85% NaCl, serially diluted, and plated. Colony counts were done after 48 h of incubation at 37°C.

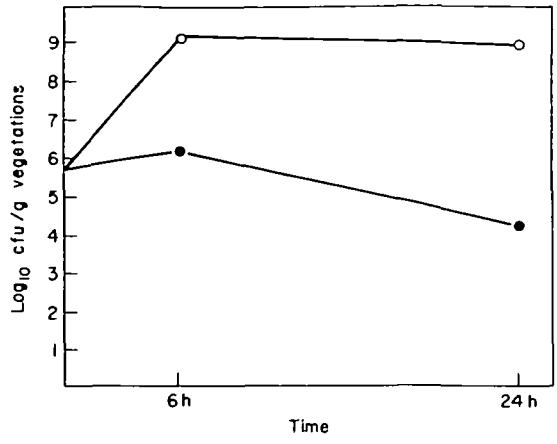

Figure 1. Rate of *in-vitro* killing of *Str intermedius* by 64 mg of penicillin/l a concentration 4 times higher than the serum level in rats 30 min after the iv injection of 60 mg/kg of penicillin ○, Control; ●, benzyl penicillin 64 mg/l

Statistical analysis

The χ^2 test with Yates's correction was used for statistical comparisons.

Results

Susceptibility of Str. intermedius to penicillin

Minimal inhibitory and bactericidal concentrations for the *Str. intermedius* of penicillin were 0·032 and >64 mg/l respectively. Survival of *Str. intermedius* in 64 mg/l of penicillin showed no decrease in cfu during the first 6 h of incubation and only a 90–99% killing after 24 and 48 h of incubation (Figure 1). Therefore, this *Str. intermedius* was tolerant to the bactericidal action of penicillin.

Penicillin serum levels and serum bactericidal activity (Figure 2)

Penicillin serum levels (±S.D.) in 5 rats 30 min, 1 h and 2 h after injection of 60 mg/kg iv were 16±1, 2±0·1 and approximately 0·01 mg/l, respectively. There was no detectable serum bactericidal activity 30 min after injection of penicillin, that is at the time of injection of bacteria in the prophylaxis experiments.

Prophylaxis of Str. intermedius *endocarditis by intravenously administered penicillin* (Figure 3)

The incidence of *Str. intermedius* endocarditis was reduced from 95% (22/23 rats) in controls to 20% (4/20) in rats given penicillin 30 min before bacterial challenge ($P<10^{-4}$).

Discussion

Earlier studies in rabbits have shown that high and prolonged serum levels of bactericidal antibiotics, including penicillin, were necessary for adequate prophylaxis

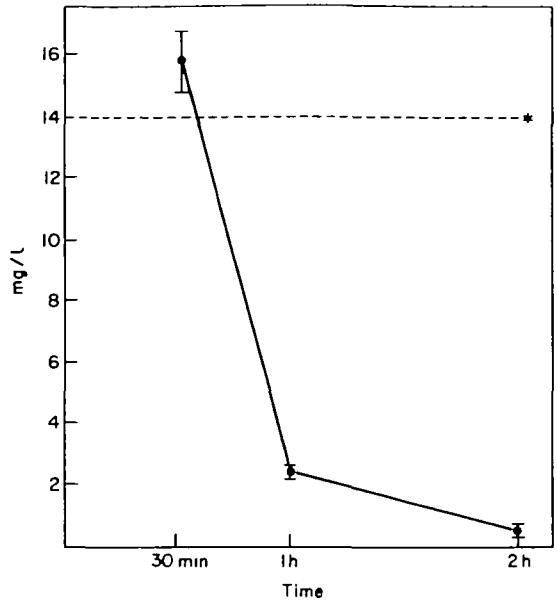

Figure 2. Serum levels (±S D) of sodium penicillin 30 min, 1 h and 2 h after a single iv injection of 60 mg/kg in rats The dotted line represents peak level achieved in humans after an oral dose of 2 g of phenoxymethyl penicillin

against *Str. viridans* endocarditis (Durack & Petersdorf, 1973).

Recently, strains of viridans streptococci isolated from the oral flora of children with cardiac disease (Dankert & Hess, 1982) and from their blood after dental extractions (Holloway, Dankert & Hess, 1980) were often shown to be tolerant to penicillin. In addition, we found that 17 out of 25 strains of viridans streptococci isolated from patients with endocarditis were tolerant to amoxicillin, 13 of them having MBCs

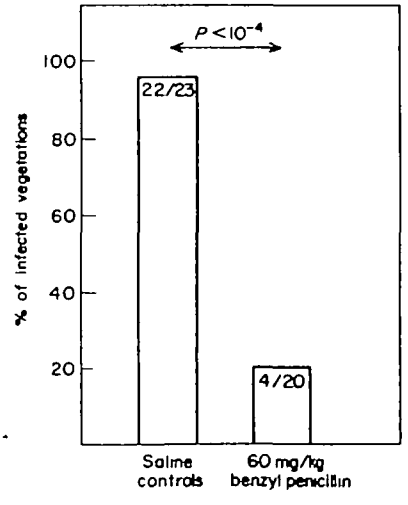

Figure 3. Incidence of endocarditis due to *Str. intermedius* after prophylaxis with 60 mg/kg of sodium penicillin administered iv 30 min before bacterial challenge. Numbers within the bars indicate the number of rats in each group

greater than 32 mg/l, a concentration which is higher than the peak serum levels achieved after a 3 g oral dose (Glauser *et al.*, 1983*b*).

To assess whether the phenomenon of *in-vitro* tolerance holds any significance *in vivo*, Hess, Dankert & Durack (1983) studied the ability of penicillin to prevent experimental endocarditis caused by tolerant and non-tolerant strains of *Str. sanguis*. They found that a single dose of penicillin was successful when the strain of streptococcus was highly sensitive to the bactericidal action of the antibiotic, but was much less effective when the strains were tolerant and not killed *in vitro* by concentrations of penicillin similar to those achieved *in vivo*. Moreover the efficacy of penicillin against tolerant strains were greatly increased by the addition of streptomycin which provided a better bactericidal activity. These results were interpreted as showing that killing was indeed a critical factor for efficacy of prophylaxis and, because of the occurrence of tolerance among viridans streptococci, this raises concern about the efficacy of the single dose amoxicillin regimen recently recommended by the Working Party of the British Society for Antimicrobial Chemotherapy (1982).

In contrast to the experiments mentioned above (Durack and Petersdorf, 1973; Hess, Dankert and Durack 1983), we and others recently found that single doses of vancomycin (Bernard *et al.*, 1981; Scheld *et al.*, 1981), amoxicillin (Glauser *et al.*, 1983*a*) and even bacteriostatic antibiotics (Glauser & Francioli, 1982) could afford excellent protection against *Str. viridans* endocarditis in the absence of bacterial killing. With the present study, penicillin can be added to the list of antibiotic operating in the absence of bacterial killing. Indeed, the *Str. intermedius* strain had a MBC for penicillin much higher than the peak serum level of the antibiotic and therefore was not expected to be killed by the drug *in vivo*. This was also attested by the killing curves and the absence of any detectable bactericidal activity in the serum at the time of injection of the bacteria.

The discrepancy between the present results and those of Durack & Petersdorf (1973) and Hess *et al.* (1983) regarding the requirement for penicillin to be bactericidal in order to prevent endocarditis might be explained by the differences in the size of the inocula used for bacterial challenge. Indeed, we have shown in rats that streptococcal endocarditis can be efficiently prevented by single doses of non-bactericidal concentrations of amoxicillin, provided that, like in the present experiment, the inoculum size is not higher than the minimum inoculum able to induce endocarditis in 90% of control animals (ID_{90}). With higher inocula the efficacy of prophylaxis of amoxicillin was abolished unless the strain of streptococcus used was highly sensitive to the bactericidal action of the drug (Glauser *et al.*, 1983*a*). Therefore, a bactericidal activity appears to be required for efficient prophylaxis only when infection is induced by inocula 10 or 100 times higher than the ID_{90}. Durack & Petersdorf (1973) used an inoculum of 10^8 cfu and presented evidence that this inoculum was much higher than the ID_{90} (Pelletier, Durack & Petersdorf, 1975). Hess, Dankert & Durack (1983) used an inoculum of 10^7 cfu, a size which is likely to be also above the ID_{90}. In contrast, the present experiments using penicillin as prophylactic antibiotic were carried out with an inoculum known to be close to the ID_{90} (Glauser *et al.*, 1983*a*). These differences in inoculum size probably explain why, in the absence of killing, penicillin prevented endocarditis in the present experiment, while it did not in other studies. Even though the inoculum we used was smaller, it provides a stringent test of antibiotic prophylaxis because the resulting bacteraemia is far greater than that observed in humans after

oral procedure (Petersdorf, 1978) and produces 80–100% endocarditis in animals.

The mechanisms by which antibiotics can prevent endocarditis in the absence of killing are not completely elucidated. The cell-wall active antibiotics such as penicillin, amoxicillin or vancomycin have been shown *in vitro* to interfere with streptococcal adhesiveness to platelets fibrin matrices mimiking cardiac vegetations. Moreover there are *in-vivo* experimental suggestions that inhibition of adherence might be one of the mechanisms of action of antibiotics involved in endocarditis prophylaxis. (Scheld *et al.*, 1981; Lowy *et al.*, 1983; Glauser *et al.*, 1983*a*).

Whatever the mechanism of protection, the present experiment demonstrates that short-lived serum levels of penicillin similar to those achieved in man after an oral dose of 2 gm of phenoxymethyl penicillin can efficiently prevent the development of endocarditis, provided the bacterial inoculum size used for challenge is limited to the ID_{90}.

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