Comparison of single and multiple doses of prophylactic antibiotics in experimental streptococcal endocarditis

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ABSTRACT Single-doses or short-term administration of β-lactam antibiotics alone or combined with aminoglycoside antibiotics have failed to consistently prevent experimental streptococcal endocarditis induced by high inocula of bacteria poorly susceptible to killing by these antibiotics. The optimal duration of administration of antibiotics for successful prophylaxis under these circumstances has not been established. We therefore tested, in rats with catheter-induced sterile aortic vegetations, the duration of administration of antibiotic necessary to prevent endocarditis induced by bacterial inocula 100 to 10,000 times the 90% infective dose of two tolerant viridans-group streptococci and two Streptococcus faecalis strains. Multiple-dose regimens of amoxicillin alone or of amoxicillin combined with gentamicin were studied. Against the two viridans group streptococci, successful prophylaxis was achieved with multiple doses of amoxicillin alone given over 24 to 48 hr and by the combination of amoxicillin and gentamicin given for 6 to 24 hr. Against the two S. faecalis strains, multiple-dose regimens with amoxicillin alone failed, but the combination of amoxicillin and gentamicin was successful when administered for 48 to 72 hr. Thus, after challenge with high bacterial inocula, repeated doses of a β-lactam antibiotic alone were sufficient to prevent viridans streptococcal endocarditis, but multiple doses of a bactericidal combination (β-lactam plus aminoglycoside), as necessary for the treatment of established endocarditis, were a prerequisite for successful prophylaxis of S. faecalis endocarditis.


STUDIES ON THE PREVENTION of streptococcal endocarditis both in rabbits and in rats have demonstrated that prophylaxis can be successfully achieved with a single dose of cell wall–active antibiotics or of bacteriostatic antibiotics such as clindamycin and erythromycin.1–5 However, this protection was limited to the minimum bacterial inoculum infecting 90% of untreated animals (ID90) when the test streptococcal strain was not killed by the antibiotic.4,6 In contrast, when the test streptococcus was rapidly killed, protection was afforded even after challenge with inocula exceeding by far the ID90.4 Since the majority of viridans streptococci and virtually all strains of Streptococcus faecalis isolated from patients with endocarditis7–9 are poorly susceptible to bacterial killing by cell wall–active antibiotics, single-dose prophylaxis of experimental endocarditis due to these strains has shown limited efficacy.4,6

Recent experiments investigating the mode of action of prophylactic antibiotics in the absence of bacterial killing in rats have suggested that the prolonged inhibition of bacterial growth might be an important mechanism of protection, allowing the resting organisms that have seeded the vegetations to be cleared before growth starts. In these experiments, repeated administration of antibiotics, providing prolonged serum inhibitory activity, permitted the circumvention of the limited efficacy of single-dose prophylaxis that is observed with inocula greater than the ID90.10

Most recent recommendations on the prevention of bacterial endocarditis in humans have advocated single-dose or short-term (one additional dose after 6 hr) prophylaxis.11–12 However, most failures have been observed after single-dose or short-term prophylaxis.13 This might simply be due to the fact that these regimens are those in common use, but is possibly also due to the magnitude of the bacterial inoculum size.14 The purpose of the present study was to investigate whether
multiple doses of amoxicillin alone or of the combination of amoxicillin plus gentamicin would successfully prevent streptococcal endocarditis after challenge with high bacterial inocula, a condition in which single doses of amoxicillin alone or the combination of amoxicillin plus gentamicin have failed.4,6

Materials and Methods

Microorganisms. Four streptococcal strains, two viridans streptococci (Streptococcus sanguis and Streptococcus intermedius) and two S. faecalis strains (S. faecalis 309 and S. faecalis 1209) were used in the present experiments. The strains have been previously used in experimental endocarditis in rats5,6 and in rabbits.2,5

Determination of minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs), and rates of killing. The MICs of amoxicillin and of gentamicin for the four test strains were determined by a standard broth dilution technique with an inoculum of 10^5 organisms from an overnight culture.15 The MBCs were determined by subculturing, on penicillin-containing (Difco Laboratories, Detroit) blood agar plates, 10-fold and 100-fold dilutions of a 0.1 ml sample from each dilution of antibiotic showing no turbidity after 18 hr of incubation. After incubation for 48 hr, the number of colonies from each subculture on blood agar plates was counted and the MBC was determined as the lowest dilution of antibiotic that showed 99.9% killing.

With the use of concentrations of 25 μg amoxicillin per milliliter (Beecham Research Laboratories, Brentford, England), of 8 μg gentamicin per milliliter (Schering Corporation, Kenilworth, NJ), or of both, the rates of killing of the four strains were determined in tryptic soya broth (TSB, Difco Laboratories) with a 10^6 inoculum from an overnight culture. These concentrations of amoxicillin and gentamicin were chosen because they were similar to peak serum levels obtained in rats 30 min after the injection of 40 mg/kg iv amoxicillin and of 4 mg/kg im gentamicin. In man, these serum levels are achieved 2 hr after an oral dose of 3 g of amoxicillin16 and 30 min after an intravenous dose of 1.5 mg/kg of gentamicin.17 At various times after the inoculation of the bacteria into the antibiotic containing broth, 10^-1, 10^-3, and 10^-5 dilutions of a 0.1 ml sample were subcultured on penicillin-containing blood agar plates and incubated for 48 hr for colony counts.

Serum levels of antibiotics and determination of the serum bactericidal activity. Serum levels of antibiotics were determined 30 min and 1, 2, and 4 hr after the injection of 40 mg/kg iv amoxicillin or 4 mg/kg im gentamicin into groups of five rats by a standard agar diffusion technique. Bacillus subtilis was used as the test organism and normal rat serum was used as the diluent.18 The serum bactericidal activity 30 min, 1, 2 and 4 hr after the administration of amoxicillin (or of the combination of amoxicillin plus gentamicin) to rats was determined for each of the four strains by standard methods with use of an inoculum of 10^6 colony-forming units (cfu) from an overnight culture.19

The serum bactericidal activity was defined as the highest serum dilution providing 99.9% killing of the initial inoculum after 18 hr of incubation.

Production of endocarditis and natural history of infection. Sterile vegetations were produced in female Wistar rats (180 to 200 g) by a modification of a previously described method.20 In brief, a polyethylene catheter (PP 10, Portex, Hythe, Kent, England) was inserted through the right carotid artery across the aortic valve and secured with a silk ligature. Twenty-four hours after catheterization, rats were injected via the tail vein with 0.5 ml of saline containing 10^8 cfu from an overnight culture of the test organisms. The number of bacteria injected intravenously was adjusted by counting the organisms in an hemocytometer, confirmed by colony counts and expressed in colony-forming units per milliliter.

The ID_90 was 10^6 cfu/ml for S. sanguis, 10^5 cfu/ml for S. intermedius, and 10^4 cfu/ml for both S. faecalis strains. Thus, the 10^5 cfu/ml inoculum size used in the prophylaxis experiments was 100 times the ID_90 for S. sanguis, 1000 times the ID_90 for S. intermedius, and 10,000 times the ID_90 for both S. faecalis strains.

Rats were killed 72 hr after single-dose prophylaxis and 5 days after the last dose of drug when multiple doses of antibiotic(s) were given. The aortic vegetations were excised, weighed, homogenized in 1 ml of saline, serially diluted, and plated on penicillinase-containing blood agar plates. The colonies were counted after 24 and 48 hr of incubation at 37°C. This method permitted the detection of 10^2 cfu/g of vegetation.

Prophylaxis of endocarditis with amoxicillin and with amoxicillin plus gentamicin. In each experiment with one of the four test strains, amoxicillin was given at a dose of 40 mg/kg iv and gentamicin at a dose of 4 mg/kg im. The control groups were given intravenous saline. Depending on the strain tested, the following prophylactic regimens, including either amoxicillin alone or combined amoxicillin plus gentamicin, were tested: (1) single-dose antibiotic prophylaxis 30 min before bacterial challenge, (2) antibiotic prophylaxis 30 min before bacterial challenge, followed 6 hr later by one additional dose of antibiotic (alone or combined), (3) antibiotic prophylaxis 30 min before bacterial challenge, followed by four additional doses at 6 hr intervals (providing antibiotic serum levels for 28 to 30 hr), by six additional doses (providing serum levels for 40 to 42 hr), by eight additional doses (providing serum levels for 52 to 54 hr), or by 12 additional doses (providing antibiotic serum levels for 76 to 78 hr).

Statistical evaluation. For each strain, the incidence of endocarditis in the prophylaxis groups was compared with the incidence in the control group by the chi-square test with the Yates correction.

Results

MICs and MBCs. The MICs and MBCs of amoxicillin and gentamicin for the four test strains are listed in table 1. All strains had high MBC values, a phenomenon that is common among streptococci when careful measures to avoid the antibiotic carryover are taken.7,8

Antibiotic serum levels in rats. The amoxicillin serum levels (mean ± SD levels of five rats at each time interval) after the intravenous injection of 40 mg/kg amoxicillin were 23.7 ± 3 μg/ml at 30 min, 8.8 ± 1 μg/ml at 1 hr, 3.5 ± 0.4 μg/ml at 2 hr, 0.6 ± 0.3 μg/ml at 4 hr. The gentamicin serum levels (mean ± SD levels of five rats at each time interval) after the intravenous injection of 4 mg/kg gentamicin were 32.0 ± 1.7 μg/ml at 30 min, 3.5 ± 1.2 μg/ml at 1 hr, 0.6 ± 0.2 μg/ml at 4 hr.

TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC Amoxicillin</th>
<th>MBC Amoxicillin</th>
<th>MIC Gentamicin</th>
<th>MBC Gentamicin</th>
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<td>8</td>
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</tr>
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<td>128</td>
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<td>2</td>
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<td>128</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>S. faecalis 1209</td>
<td>1</td>
<td>128</td>
<td>4</td>
<td>16</td>
</tr>
</tbody>
</table>

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\(\mu g/ml\) at 4 hr, and undetectable at 6 hr. The mean (± SD) gentamicin serum levels (\(\mu g/ml\); in five rats at each time interval) after the intramuscular injection of 4 mg/kg gentamicin were 8 ± 2.1 at 30 min, 4.6 ± 1.8 at 1 hr, 1.9 ± 0.9 at 2 hr, and undetectable at 4 hr.

Serum bactericidal activity (SBA). After the intravenous injection of 40 mg/kg of amoxicillin to rats, no SBA (in five animals) against any of the four streptococcal strains could be detected 30 min or later after injection. After the administration of combined amoxicillin plus gentamicin (4 mg/kg), the SBAs against both viridans streptococci were 1:4 at 30 min, 1:2 at 2 hr, and undetectable at 4 hr. Against both \(S.\) \textit{faecalis} strains, the SBAs of combined amoxicillin plus gentamicin were 1:8 at 30 min, 1:4 at 2 hr, and undetectable at 4 hr.

Rates of killing of the four test strains. Figure 1 shows the rates of killing of the four test strains by 25 \(\mu g/ml\) amoxicillin with and without the addition of 8 \(\mu g/ml\) gentamicin. With concentrations of 8 \(\mu g/ml\) of gentamicin alone, all four test strains showed survival of 100% or more of the initial inoculum after 24 hr of incubation (not shown). \(S.\) \textit{sanguis} and \(S.\) \textit{intermedius} were killed by peak concentrations of amoxicillin within 24 hr and 48 hr of incubation, respectively. Neither \(S.\) \textit{faecalis} strains exhibited a significant decrease of colony counts within 48 hr of exposure to peak amoxicillin concentrations. When exposed to the combination of amoxicillin plus gentamicin at peak concentrations, killing of more than 99.9% of the original inoculum of all four test strains was achieved within 6 hr, thus demonstrating synergism on exposure to this combination.

Antibiotic prophylaxis of \(S.\) \textit{sanguis} and \(S.\) \textit{intermedius} endocarditis. The results obtained with the different prophylactic regimens against each of the two viridans streptococci are shown in figure 2.

Regimens with amoxicillin alone. Single-dose amoxicillin was ineffective for prophylaxis against endocarditis induced by \(10^6\) cfu of either viridans streptococci. Four subsequent doses of amoxicillin completely prevented \(S.\) \textit{sanguis} endocarditis, but failed to protect against

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Rates of killing in vitro of four streptococcal strains (1 = \(S.\) \textit{sanguis}; 2 = \(S.\) \textit{intermedius}; 3 = \(S.\) \textit{faecalis} 309; 4 = \(S.\) \textit{faecalis} 1209) incubated in 25 \(\mu g/ml\) of amoxicillin alone (open triangles) or in the combination of 25 \(\mu g/ml\) of amoxicillin plus 8 \(\mu g/ml\) of gentamicin (open squares). The rates of killing of the four test strains by 8 \(\mu g/ml\) of gentamicin alone are not shown, but all four test strains showed 100% survival of the inoculum at 24 hr. The closed circles represent control strains in tryptic soya broth.}
\end{figure}
endocarditis induced by S. intermedius. Indeed, eight subsequent doses of amoxicillin had to be administered after challenge with the latter strain to successfully prevent endocarditis.

Regimens with the combination of amoxicillin plus gentamicin. The combination given as a single dose failed to consistently protect against endocarditis due to either of the viridans streptococci. Against challenge with S. sanguis, however, a single dose of amoxicillin + gentamicin was slightly more effective than it was against S. intermedius. Indeed, one subsequent dose of amoxicillin + gentamicin completely prevented S. sanguis endocarditis, while four subsequent doses of the combination were required to significantly protect the animals from S. intermedius infection.

Antibiotic prophylaxis of S. faecalis 309 and S. faecalis 1209 endocarditis. The results obtained with the different prophylactic regimens against each of the 2 S. faecalis strains are shown in figure 3.

Regimens with amoxicillin alone. Single-dose amoxicillin failed to protect against endocarditis due to either S. faecalis strains. Eight subsequent doses of the drug had to be given to significantly protect against S. faecalis 309 (p = .003 when compared with controls), but a failure rate of 23% was still observed. Against S. faecalis 1209, eight doses of amoxicillin alone totally failed to prevent endocarditis.

Regimens with the combination of amoxicillin plus gentamicin. Single-dose amoxicillin + gentamicin failed to prevent endocarditis due to either S. faecalis strains. Four subsequent doses of amoxicillin + gentamicin significantly protected against S. faecalis 309 endocarditis (p = 0.001 when compared with controls), but the failure rate of 36% was high, while six subsequent doses of amoxicillin + gentamicin completely protected the animals. After challenge with S. faecalis...
1209, even eight subsequent doses of the combination resulted in a high failure rate (36%); endocarditis induced by this strain could only be successfully prevented by 12 subsequent doses of amoxicillin + gentamicin.

Discussion

Previous studies in rats have shown that successful prophylaxis of streptococcal endocarditis depends both on the susceptibility of the test strain to killing by the antibiotic and on the bacterial inoculum size used for challenge. When bacteria are rapidly killed by the antibiotic, single-dose prophylaxis is successful irrespective of the number of organisms used to induce endocarditis, and therefore provides a wide margin of efficacy. In contrast, against the so-called tolerant strains (a group that includes most viridans streptococcal strains isolated from the mouth flora and from patients with endocarditis), cell wall–active antibiotics as well as bacteriostatic antibiotics such as clindamycin and erythromycin have only prevented endocarditis induced by the ID$_{90}$, but have not prevented endocarditis induced by higher bacterial numbers. Recent experiments in rats have suggested that the prolonged inhibition of bacterial growth, as provided by repeated administration of antibiotics after a prophylactic dose, might circumvent the limited efficacy of single-dose antibiotic prophylaxis.

The present results confirm our previous observations that single-dose prophylaxis with amoxicillin alone, or with the combination of amoxicillin plus gentamicin, does not reliably prevent streptococcal endocarditis induced by high bacterial numbers. In addition, the results clarify the conditions necessary for inoculum-independent successful antibiotic prophylaxis of both viridans streptococcal and enterococcal (S. faecalis) endocarditis.

With regard to viridans streptococcal endocarditis, the results show that amoxicillin alone administered every 6 hr for 24 to 48 hr after bacterial challenge prevented endocarditis induced by inoculum sizes from 100 to 1000 times the ID$_{90}$ of the test strains. The combination of amoxicillin plus gentamicin significantly reduced the need for additional drug administration.

With regard to the prophylaxis of enterococcal endocarditis, in contrast to viridans streptococcal endocarditis, the prolonged administration of amoxicillin alone for 48 hr after challenge failed to reliably prevent the infection, since this regimen was unsuccessful against one of the two S. faecalis strains tested. These differences in the efficacy of antibiotic prophylaxis against viridans and enterococcal endocarditis might be partly related to the higher bacterial inocula relative to the ID$_{90}$ of the S. faecalis strains when compared with the inocula of two viridans streptococci. However, similar observations regarding the prophylaxis of S. faecalis endocarditis were made by Durack et al. and Guze et al., who used inocula of 10$^6$ to 10$^7$ S. faecalis cfu/ml that caused a 100% infection rate in controls. As in the present study, these authors not only found that single-dose ampicillin prophylaxis failed to prevent S. faecalis endocarditis, but also that two to five subsequent doses of ampicillin alone as well as two to three subsequent doses of the combination of ampicillin plus gentamicin did not consistently prevent endocarditis. More prolonged prophylactic regimens were not tested in these experiments. In the present experiments, when amoxicillin plus gentamicin was administered for the prevention of enterococcal endocarditis, successful prophylaxis was achieved if the combination was given for 36 or 72 hr. Thus, as was observed after challenge with the two viridans streptococcal strains, against enterococcal endocarditis the combined β-lactam plus aminoglycoside regimens were superior to the regimens including a β-lactam alone.

The exact mechanisms by which the multiple-dose antibiotic regimens were successful in preventing endocarditis after challenge with high bacterial inocula are unknown. Previous observations have indicated that the number of bacteria adhering to the vegetations after challenge is related to the magnitude of the inoculum size used for challenge. When high inocula were used, sustained bacteriostatic blood levels were required to successfully prevent endocarditis due to tolerant organisms, probably by allowing all adherent organisms to be cleared from the vegetations. It is conceivable that a similar mechanism operated in the present experiments (against viridans streptococci) after prophylaxis with amoxicillin alone. On the other hand, the fact that multiple doses of the combination of amoxicillin plus gentamicin were clearly more effective than amoxicillin alone suggests that this combination operated, at least partially, through a killing mechanism. Moreover, it was striking that for the prevention of S. faecalis endocarditis, only the combined amoxicillin plus gentamicin regimen was consistently successful. Such combinations are required to produce a bactericidal effect on enterococci, which are notoriously insensitive to bacterial killing by most cell wall–active antibiotics.

Only a few studies have compared the efficacy of single versus multiple doses of antibiotics in relation to
the bacterial inoculum sizes used for challenge. In early experiments in rabbits, Durack et al.\textsuperscript{1} used a bacterial inoculum size of $10^6$ cfu of a \textit{S. sanguis} strain that resulted in a 100% infection rate in controls. This inoculum probably represented from 10 to 100 times the ID$_{90}$ for that organism.\textsuperscript{3} In these experiments, only multiple doses of penicillin G and of penicillin V, as well as a large single dose of procaine penicillin or of the combination of penicillin G and benzathin penicillin (providing serum inhibitory levels for at least 24 hr after challenge) were effective in the prevention of \textit{S. sanguis} endocarditis. With regard to \textit{S. faecalis} infection, as previously mentioned, from one to five doses of ampicillin or two to three doses of ampicillin plus gentamicin failed to reliably prevent enterococcal endocarditis.\textsuperscript{22, 23} Thus, previous experiments in rabbits, as well as our present studies in rats, suggest that multiple dose regimens are necessary for the prevention of experimental streptococcal endocarditis induced by high bacterial numbers.

The clinical relevance of data derived from the animal preparation of endocarditis to patients has been questioned, mainly on the grounds that the bacterial numbers used to induce experimental endocarditis exceeded by far those detected in the blood of patients undergoing dental or urogenital procedures. It should be pointed out, however, that the number of bacteria circulating after intravenous bacterial injections into animals is several logs less than the total number of bacteria injected.\textsuperscript{1} For instance, after the intravenous injection of $10^4$ \textit{S. faecalis} 1209 (an inoculum size that corresponds to the ID$_{90}$ for this strain), a mean of $10^5$ bacteria/ml of blood was found in the heart blood of five rats at 2 min after injection, and \textit{S. faecalis} colonies were barely detectable 15 min after injection.* More importantly, our recent studies on the production of endocarditis in rats after the recent of periodontally diseased teeth have failed to demonstrate a relationship between the total number of a given streptococcal species circulating immediately after tooth extraction and the likelihood of these streptococci to subsequently produce endocarditis.\textsuperscript{24}

The number of bacteria circulating and their stickiness is not known in those very few patients who are going to develop endocarditis. More importantly, there are groups of patients who are at particularly high risk of developing endocarditis after bacteremic episodes. Since our present understanding of the mode of action of prophylactic antibiotics indicates that prolonged antibiotic levels are required to provide the best margin of safety for the prevention of endocarditis, the Swiss recommendations for the prophylaxis of endocarditis,\textsuperscript{25} unlike the British recommendations\textsuperscript{11} and the most recent recommendations of the American Heart Association,\textsuperscript{12} suggest that multiple-dose regimens administered over 48 hr should be used for the prophylaxis of endocarditis in high-risk subjects. Cost-benefit estimates will need to be made of the potential impact of such prophylactic multiple-dose regimens.

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