Laboratory Investigation

Endocarditis

Antibiotic prophylaxis of experimental endocarditis after dental extractions

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Abstract

In rats with catheter-induced sterile aortic valve vegetations we studied the efficacy of single-dose amoxicillin and single-dose erythromycin prophylaxis for the prevention of bacterial endocarditis after extractions of periodontally diseased teeth. Endocarditis after extractions occurred in 89% of control animals and was due to group G streptococci, to Staphylococcus aureus, or to both organisms. A single-dose of amoxicillin or erythromycin successfully prevented endocarditis due to these bacterial species. The analysis of the bacteremia (by culturing blood drawn 1 min after extraction on penicillinase-containing blood agar plates) indicated that amoxicillin did not influence the incidence or the magnitude of circulating group G streptococci and S. aureus, while erythromycin apparently suppressed them. However, when care was taken to eliminate blood erythromycin by a lysis-centrifugation process, the incidence and magnitude of bacteremia after erythromycin prophylaxis was similar to that in control rats. We conclude that single doses of amoxicillin and erythromycin successfully prevent experimental endocarditis after dental extractions. Since this prophylaxis was operative by mechanisms other than the prevention of the circulation of bacteria before seeding the valvular vegetations, it suggests that recommendations for prevention of bacterial endocarditis should not be aimed only at providing adequate antibiotic blood levels to suppress the bacteremia produced by the invasive procedure.


Several mechanisms have been proposed to describe the manner in which antibiotics prevent bacterial endocarditis. One hypothesis postulates that antibiotics may confer protection against bacterial endocarditis by reducing the incidence and the magnitude of bacteremia after certain procedures, thus decreasing the chances that circulating bacteria will colonize the damaged valvular endothelium in subjects at risk of developing endocarditis. Recommendations for prophylaxis against bacterial endocarditis in humans have thus advocated the administration of antibiotics before procedures likely to result in bacteremia so as to produce peak serum levels of antibiotics at the time microorganisms are likely to gain access to the blood stream. While several studies in humans have investigated the incidence of bacteremia after dental extractions, investigations on the influence of antibiotic prophylaxis on the bacterial recovery rate after such procedures have given apparently conflicting results. Indeed, a wide variation of the incidence of postextractions bacteremia after penicillin prophylaxis has been reported, ranging from 0% to 53%, but these variations were in fact due to the different preparations, routes of administration, and doses of penicillin given for prophylaxis, as well as to the different culture techniques used.

Thus, there could be some interest in the investigation of these controversial issues in experimental preparations under well-controlled conditions.

In the present study, we have used a preparation of bacterial endocarditis that results from the extraction of periodontally diseased teeth in rats with catheter-induced sterile aortic vegetations. Since endocarditis in this preparation follows the bacteremia originating from the oral flora of the animal and not from the intravenous injection of bacteria, it mimics more closely the human condition than previous preparations.
of endocarditis\textsuperscript{12} and permits the investigation of the efficacy of antibiotic prophylaxis against endocarditis as well as its effect on the bacteremia produced by dental extractions. We studied the effect of single doses of amoxicillin or erythromycin on the development of bacteremia and on the prevention of bacterial endocarditis after extraction of periodontally diseased teeth.

**Materials and methods**

**Induction of periodontal disease in rats.** Periodontal disease was produced in rats by a method previously described.\textsuperscript{11} In brief, gingival irritation was induced in female Wistar rats (180 to 200 g) by placement of a black silk ligature (size 000) at the cervical margins of the first left and right maxillary molars and the animals were maintained on a powdered, high-sucrose diet (Kaye’s diet 2000, Teklad, Madison, WI) ad libitum for 18 weeks. This results in periodontal disease in at least 70\% of rats. After 18 weeks and immediately before catherization of the animals, the presence of periodontal disease was evaluated by one of us (D. O.), according to the following three clinical criteria: presence of a ligature, presence of plaque in the area next to the ligature, and presence of erythema. Only rats fulfilling the three clinical criteria of periodontal disease were used for these experiments.

**Production of sterile aortic vegetations in rats.** Eighteen weeks after placement of the ligatures, sterile aortic vegetations were produced by insertion of a polyethylene catheter (PP 10, Portex, Hythe, Kent, England) across the aortic valve through the right carotid artery.\textsuperscript{13} This procedure induces in at least 95\% of rats the development of sterile vegetations that might be colonized by circulating bacteria with subsequent development of endocarditis.

**Gingival cultures.** Twenty-four hours after catheterization and immediately before dental extractions, gingival cultures were obtained by the atraumatic collection of plaque from both periodontally diseased teeth. The plaque material was suspended in 1 ml of tryptic soya broth (TSB, Difco Laboratories, Detroit), and serially diluted in normal saline before it was plated onto penicillinase- (Difco Laboratories) containing blood agar plates and incubated under aerobic conditions. The dilution procedure permitted the detection of $10^4$ colony-forming units (cfu) of aerobic organisms.

**Extractions of periodontally diseased teeth and detection of postextraction bacteremia.** Immediately after the gingival cultures were obtained, the two periodontally diseased teeth were extracted by one of us (D. O.). The two extractions were accomplished in a 4 min period. One minute after the extractions, 1 ml of blood was drawn from a jugular vein and immediately plated for culture onto penicillinase-containing blood agar plates.

**Prophylaxis of endocarditis with amoxicillin or erythromycin before dental extractions.** Thirty minutes before dental extractions, groups of rats were randomly assigned to three prophylactic regimens: control rats received 0.5 ml of intravenous normal saline, one group of rats received 40 mg/kg intravenous amoxicillin (Beecham Research Laboratories, Brentford, England), and one group received 20 mg/kg iv erythromycin glucosinate (Eli Lilly and Co., Indianapolis). These doses of the two antibiotics have been previously shown to produce peak serum levels in rats that are similar to those in humans after the recommended prophylactic oral doses.\textsuperscript{14, 15}

**Sacrifice of the animals.** The animals were killed 72 hr after dental extractions. One milliliter of blood was drawn and immediately plated for colony counts onto penicillinase-containing blood agar plates. The aortic vegetations were aseptically excised, weighed, homogenized in 1 ml of normal saline, serially diluted, and plated onto penicillinase-containing blood agar plates. This method permitted the detection of $10^2$ cfu/g of vegetation. In addition, undiluted samples of vegetation homogenates were incubated under anaerobic conditions using the Gas-Pack Catalyst system (BBL, Cockeysville, MD). The spleens were aseptically removed, homogenized in 2 ml of normal saline, and directly plated onto penicillinase-containing blood agar plates. All plates were counted after 48 hr of incubation. The autopsies of animals that died before the 72 hr period of observation after dental extractions was completed were performed within 8 hr of death. Only the aortic vegetations from these animals were processed for culture.

**Identification of the organisms and susceptibility tests in vitro.** Bacteria recovered from the gingivae, from the blood, from the aortic vegetations, and from the spleen homogenates were identified and specified with the use of the API system (Analytab Products, Plainview, NY). All cultures were obtained under aerobic conditions because preliminary studies had shown that no anaerobic organisms were detected in anaerobic cultures of the vegetation homogenates, thus demonstrating that only aerobic bacteria were able to induce endocarditis in this preparation. The in vitro susceptibility of the organisms recovered from the vegetations of rats that had been given antibiotic prophylaxis was determined by standard disk procedures.\textsuperscript{16} The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of amoxicillin and erythromycin for the group G streptococcal strain used in some experiments were determined by use of a standard macrobroth-dilution technique with an inoculum of $10^6$ organisms from an overnight culture.\textsuperscript{17} The MBC was the lowest dilution of antibiotic that showed 99.9\% killing.

**Magnitude of group G streptococcal bacteremia in rats challenged with intravenous bacteria with and without amoxicillin prophylaxis.** Since postextraction blood samples were drawn only at one time point (1 min) after dental extractions, we investigated in further experiments whether amoxicillin prophylaxis might speed up the bacterial clearance from the blood of rats. For these experiments we used an amoxicillin-sensitive group G streptococcal strain (MIC 0.064, MBC 0.25 $\mu$g of amoxicillin/ml) that had caused endocarditis after dental extractions in control rats. The organism was grown overnight in TSB, and diluted in saline to a final concentration of $10^7$ cfu/ml. Thirty minutes before the intravenous injection of a 0.5 ml bacterial suspension, groups of normal female Wistar rats (180 to 200 g) received either 40 mg/kg of intravenous amoxicillin or saline. Two, 5, 10, and 15 min after the bacterial challenge, 1 ml of blood was drawn from a jugular vein and immediately plated on penicillinase-containing blood agar plates. The time interval of 15 min for the last blood sampling was chosen because in previous experiments in rats, circulating streptococci were barely detectable 15 min after intravenous bacterial challenge.\textsuperscript{18} Plates were incubated at 37$\ C$ for 48 hr before colony counts were performed.

**Studies of the effect of erythromycin carryover on the magnitude of group G streptococcal bacteremia.** In rats given erythromycin prophylaxis before dental extractions, no procedure was used to inactivate erythromycin present in the postextraction blood samples before plating onto blood agar plates. We thus tested in normal rats whether erythromycin prophylaxis might modify the bacterial recovery rate after intravenous bacterial challenge. The same group G streptococcal strain used in the described above experiments (MIC of erythromycin 0.064 $\mu$g/ml, MCB 0.5 $\mu$g/ml) was used for challenge and grown overnight in TSB before dilution in normal saline to a final concentration of $10^7$ cfu/ml. Thirty minutes before intravenous injection of a 0.5 ml bacterial suspension, normal female Wistar
rats (180 to 200 g) were given either 20 mg/kg of intravenous erythromycin or saline. Five minutes after bacterial challenge, 1 ml of blood was drawn from a jugular vein of each animal. This time interval was chosen because it was similar to that for blood sampling after teeth extractions. One half of all blood samples (drawn either from control rats or from rats given erythromycin prophylaxis) were immediately plated onto blood agar plates. The other half were processed for minimizing the carryover of erythromycin by a modification of a method previously described.\(^\text{19}\) In brief, red cells were lysed by the addition to the 1 ml blood sample of 3 ml of ice-cold distilled water and by agitating the suspension in the vortex mixer for 30 sec. Tonicity was restored by the addition of 1 ml of 0.6M KCl, and the suspension was centrifuged at 4°C for 10 min at 2500 rpm. The supernatant was discarded and the procedure was repeated once. The sediment containing the bacteria was then washed in 5 ml of TSB before suspension in 1 ml of TSB and plating onto blood agar.

**Statistical evaluation.** The incidence of bacterial endocarditis, the mortality rates, and the recovery rates of group G streptococcal and *Staphylococcus aureus* strains from postextraction blood cultures in each prophylaxis group were compared with controls by the Fisher’s exact test. The levels of circulating group G streptococci and *S. aureus* after dental extractions were compared in the various groups of rats by the nonparametric Kruskal-Wallis analysis of variance.

**Results**

**Development of bacterial endocarditis after dental extractions.** Bacterial endocarditis developed in 17 of 19 (89%) control rats after dental extractions. Twelve of the 19 control rats (63%) died within the 72 hr observation period and all had endocarditis.

Nine of 17 animals with endocarditis had monomicrobial infections that were caused either by group G streptococci (seven cases) or by *S. aureus* (two cases). Eight rats had bimicrobial endocarditis, and all of these had group G streptococci as one of the two infecting bacterial species recovered from the vegetations. All of these rats died before the time at which they were scheduled to be killed. In addition to group G streptococci, six of the eight vegetations grew *S. aureus*, and in two, the vegetations grew *Streptococcus mitis*. Thus, group G streptococci caused 15 of 17 (91%) cases of endocarditis and *S. aureus* caused eight of 17 (44%) cases of endocarditis in control rats.

The microorganisms causing endocarditis were invariably found in the gingival cultures before dental extractions.

**Effect of amoxicillin and erythromycin prophylaxis on the development of bacterial endocarditis after dental extractions.** Bacteriologically positive vegetations were observed in two of 19 (10%) rats after amoxicillin prophylaxis (p < 10\(^{-5}\) compared with control). However, the presence of true endocarditis in these two animals was considered doubtful, since only a relatively small number of amoxicillin-resistant *Enterobacter cloacae* colonies (10\(^4\) cfu/g of vegetation) was found at autopsy of one rat that died before the end of the 72 hr observation period, possibly resulting from the premortem colonization of the aortic vegetations. No *E. cloacae* endocarditis was found in control rats, nor was this organism recovered from the blood cultures after dental extractions. The other doubtful endocarditis occurred in a rat that harbored low titers of amoxicillin resistant *S. aureus* on the vegetation at death (10\(^5\) cfu/g), while (in contrast to control rats with frank endocarditis) the blood and the spleen cultures were sterile. In this case, contamination of the vegetations during the sacrifice procedure might have occurred. Overall, only two of 19 (10%) rats died after amoxicillin prophylaxis (p < 10\(^{-3}\) compared with control), including the one with possible *E. cloacae* endocarditis.

Among rats given erythromycin prophylaxis, one of 13 (7%) animals developed endocarditis (p < 10\(^{-5}\) compared with control). This animal had bimicrobial endocarditis due to both *Morganella morganii* (10\(^5\) cfu/g of vegetation) and to an erythromycin-sensitive *S. mitis* strain (10\(^7\) cfu/g of vegetation). Both strains were also cultured from the blood and from the spleen homogenate. Overall, one of 13 (7%) rats died after erythromycin prophylaxis (p < 10\(^{-2}\) compared with control) and had sterile vegetations at autopsy.

**Quantitative bacteremia after dental extractions in rats with and without antibiotic prophylaxis.** Four groups of microorganisms, group G streptococci, *S. aureus*, gram-negative bacilli, and streptococci other than group G, were regularly cultured 1 min after dental extractions from the blood of control rats and rats in each prophylaxis group. Since endocarditis in control rats was invariably due to group G streptococci and to *S. aureus* strains, we report here only the quantitative results of postextraction bacteremia due to these two bacterial species.

**Postextraction bacteremia due to group G streptococci (figure 1).** Group G streptococci were detected in the blood cultures of 13 of 19 (73%) control animals. In five rats that developed group G streptococcal endocarditis, these microorganisms were undetectable at the time the blood samples were drawn. After amoxicillin prophylaxis, group G streptococci were found in 10 of 19 rats (52%) (p = NS compared with controls) and in four of 13 animals (31%, p = .04, compared with controls) after erythromycin prophylaxis. When detectable, the magnitude of bacteremia (in cfu/ml) was not significantly different in the three groups (p = .05 by Kruskal-Wallis analysis).

**Postextraction bacteremia due to *S. aureus* (figure 2).** *S. aureus* strains were detected in the blood of 10 of 19 (54%) control rats. In three control rats that developed
of 13 (31%) after erythromycin prophylaxis (p = NS compared with control). When detectable, the magnitude of bacteremia (in cfu/ml) did not differ in the three groups (p = .7 by Kruskal-Wallis analysis).

Thus, despite successful prophylaxis of group G streptococcal and S. aureus endocarditis, amoxicillin did not significantly influence the incidence or the magnitude of postextraction bacteremia due to these two bacterial species, as reflected by blood cultures 1 min after extraction, while erythromycin prophylaxis apparently decreased the incidence of bacteremia due to the group G streptococci.

Magnitude of group G streptococci bacteremia in rats challenged with intravenous bacteria with and without amoxicillin prophylaxis. The results of these experiments are shown in figure 3. At each time after intravenous challenge, when blood was taken for cultures, there was no substantial difference in the number of group G streptococcal cfu recovered from the blood of control rats and from the blood of rats given amoxicillin prophylaxis.

Studies of group G streptococci bacteremia in rats with and without erythromycin prophylaxis before intravenous bacterial challenge, and of the influence of the carryover effect of erythromycin (figure 4). When the blood of rats given erythromycin prophylaxis was drawn 5 min after intravenous challenge with 10⁵ group G streptococci and directly plated onto agar plates, sterile blood cultures were observed in five of 10 rats, while in the remaining five animals the yield was 10 or less cfu/ml of blood. In
contrast, after elimination of erythromycin from the blood by hemolysis, centrifugation, and washing before plating, the blood cultures from all 10 rats grew a number of group G streptococci similar to that in blood from control rats. This demonstrated that, rather than preventing bacteremia, the carryover of erythromycin from the cultivated blood to the agar plates had negatively influenced the recovery of bacteria.

Discussion

In our experiments, a high incidence of severe bacterial endocarditis could be produced after extraction of periodontally diseased teeth in untreated (control) rats with catheter-induced sterile aortic vegetations. This confirms our previous findings in a preparation that mimics human endocarditis more closely than the preparation produced by intravenous bacterial challenge. In our current preparation, dental extraction produced endocarditis that was caused by organisms that were found in dental plaque before extractions and were recovered from the blood cultures after extractions, thus demonstrating a direct relationship between dental extraction, bacteremia, and the subsequent development of bacterial endocarditis.

Both single-dose amoxicillin and single-dose erythromycin provided successful prevention of severe monomicrobial and bimicrobial endocardial infections and prevented death from these infections. Both regimens have been shown to successfully prevent viridans streptococcal endocarditis in rats after intravenous bacterial challenge. In the present study, the only failure of prophylaxis occurred in a rat that received erythromycin and had bimicrobial endocarditis due to M. morganii and S. mitis. While 

\[ \text{FIGURE 4. Bacterial counts 5 min after intravenous challenge with } 10^5 \text{ cfu of group G streptococci in rats given intravenous saline (controls) or erythromycin prophylaxis (20 mg/kg iv). For two groups of rats (controls, erythromycin), 1 ml blood was directly plated onto agar plates. For the remaining two groups (controls + lysis/centrifugation, erythromycin + lysis/centrifugation), 1 ml of blood was hemolyzed and centrifugated and the bacterial sediment was washed before plating. Mean log}_{10} \text{ cfu} \pm \text{SD per milliliter of blood is indicated for each group of animals.} \]

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over effect of erythromycin in serial blood cultures from rats challenged intravenously with group G streptococci after erythromycin prophylaxis, taking great care to wash out the erythromycin in the blood samples before plating for culture. Since no difference was found in the magnitude of bacteremia after erythromycin prophylaxis when compared with that in control rats after such precautions, one can reasonably infer that single-dose erythromycin, like single-dose amoxicillin, successfully prevented bacterial endocarditis by means other than the modification of the incidence or the magnitude of postextraction bacteremia.

In early experiments in rabbits, and Durack and Petersdorf speculated that it was unlikely that prophylactic antibiotics could be operative by completing their antibacterial action within the time bacteria are circulating after intravenous bacterial challenge. Evidence for this hypothesis was provided by the fact that a high dose of procaine penicillin G was equally effective for endocarditis prophylaxis in rabbits when given before and after intravenous bacterial challenge. We have observed a similar efficacy of antibiotics given after bacterial challenge in preventing endocarditis due to viridans streptococci and enterococci in rats. Our present experiments in rats with endocarditis developing after dental extractions provide direct experimental evidence that successful antibiotic prophylaxis was operative, against the organisms that entered the blood stream, by mechanisms other than killing of bacteria before they seed the damaged valvular endothelium. Previous observations have suggested that mechanisms such as the prevention of bacterial adherence, or as more recently observed, the suppression of bacterial growth after adherence to the damaged valves, might be operative in the prophylaxis of endocarditis.

In conclusion, after dental extractions, endocarditis was produced regularly by organisms found in the dental plaque and circulating in the blood. Single doses of amoxicillin or erythromycin given before dental extractions successfully prevented endocarditis without modifying the postextraction bacteremia, thus demonstrating that successful prophylaxis was operative by means other than the mere killing of bacteria.

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