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Successful Prophylaxis Against Experimental Streptococcal Endocarditis with Bacteriostatic Antibiotics

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Because bacteriostatic concentrations of vancomycin are effective in prophylaxis against endocarditis due to *Streptococcus sanguis* in rats, the efficacy of three other bacteriostatic antibiotics was investigated against three different streptococcal species that cause subacute endocarditis in humans: *Streptococcus intermedius*, *S. sanguis*, and *Streptococcus mitior*. Rats were challenged by intravenous injection of 2×10^5 colony-forming units of streptococci 24 hr after intracardiac insertion of a transaortic catheter and 30 min after intravenous injection of various doses of clindamycin, erythromycin, and doxycycline. Significant protection was achieved with all three antibiotics, but only clindamycin was fully effective against all three species at doses that simulated serum levels achievable in humans after oral administration. Endocarditis was prevented by antibiotic concentrations in serum far below minimal bactericidal concentrations for these streptococci. Furthermore, serum at the time of bacterial challenge was not bactericidal. Therefore, single doses of nonbactericidal antibiotics prevented endocarditis in rats by mechanisms other than bacterial killing.

Recommendations for antibiotic prophylaxis against bacterial endocarditis require the administration of multiple high doses of bactericidal antibiotics and parenteral administration for high-risk patients [1]. These recommendations are based largely on studies in rabbits and have presented a number of practical problems. Prolonged or parenteral administration of prophylactic antibiotics is far more expensive and less acceptable to both patients and their dentists than a single oral dose. Poor compliance may jeopardize the effectiveness of regimens involving multiple or parenteral doses [2]. There are also important limitations in the experimental data on which the recommendations for prophylaxis during dental procedures are based. The experimental studies involved only rabbits and a single strain of *Streptococcus sanguis* [3, 4]. Moreover, failure of certain antibiotic regimens in the rabbit model could be due to the number of microorganisms used to induce infection, which greatly exceeded that found in humans after dental manipulation [2]. These problems as well as possible failures with the presently recommended regimens in humans [5] have prompted us to continue experimental studies of the prevention of bacterial endocarditis.

Recent experiments with vancomycin in rats [6] and rabbits [7] introduced the possibility of mechanisms other than bacterial killing operating in the prevention of endocarditis. Therefore, the present study was undertaken to test three nonbactericidal antibiotics (erythromycin, clindamycin, and doxycycline), against three species of viridans streptococci that are responsible for infective endocarditis.

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Materials and Methods

Microorganisms. Two previously described species of α-hemolytic streptococci were used, *Streptococcus intermedius* (described elsewhere as *S. sanguis* [6]) and *S. sanguis* biotype II (provided by Dr. D. T. Durack, Duke University Medical Center, Durham, N.C. [3, 4]), as well as a strain of *Streptococcus mitior* that was also isolated from a patient with bacterial endocarditis. MICs and MBCs of clindamycin (Upjohn Co., Kalamazoo, Mich.), erythromycin (Eli Lilly and Co., In-

dianapolis), and doxycycline (Pfizer, Zurich) were determined by broth dilution tests, using Mueller-Hinton broth (Difco Laboratories, Detroit) and 5 × 10^5 microorganisms as the inoculum. Special care was taken to avoid the carry-over of antibiotics [8]. The MBC was defined as the lowest concentration of antibiotic that produced 99.9% killing after 24 hr of incubation.

Antibiotic serum levels and serum bactericidal activity. Serum levels of antibiotics were determined in five rats at each time point by the agar diffusion technique [9]. The serum bactericidal activity of antibiotics against the three streptococci was determined by standard methods [10] 30 min after iv injection of 50 mg of clindamycin or erythromycin/kg of body weight or 10 mg of doxycycline/kg. The serum bactericidal activity was defined as the highest serum dilution that provided 99.9% killing after 24 hr of incubation.

Production and prophylaxis of endocarditis. Sterile vegetations were produced in female Wistar rats (weight, 180–200 g) by a modification of a previously described method [6]. In brief, a polyethylene catheter (model no. PP 10; 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 0.5 ml of various concentrations of antibiotics or 0.85% NaCl. Thirty minutes later 0.5 ml of saline containing 10^5 cfu of bacteria, the minimal inoculum needed to produce endocarditis in >80% of control rats, was injected iv. Rats were killed 48–72 hr 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 hr of incubation at 37 C. This method permitted the detection of 5 × 10^2 cfu/g of vegetation.

Statistical analysis. The χ^2 test with Yates's correction and Student's *t*-test (unpaired) were used for statistical comparisons.

Results

Antibiotic sensitivity, antibiotic serum levels, and serum bactericidal activity. All three streptococcal species had very low MICs for the three antibiotics tested (figure 1), but MBCs were largely above achievable serum levels (figure 2). At the time of bacterial challenge, serum bactericidal activity against all three streptococcal species was undetectable with any of the three antibiotics.

Prophylaxis of endocarditis with clindamycin. Despite the lack of bacterial killing in vitro, clindamycin at doses of 50 and 20 mg/kg achieved very efficient prophylaxis against the three streptococci tested (figure 1). The 20 mg/kg dose produced peak serum levels below the 4.5 μg/ml achieved in humans after an oral dose of 450 mg

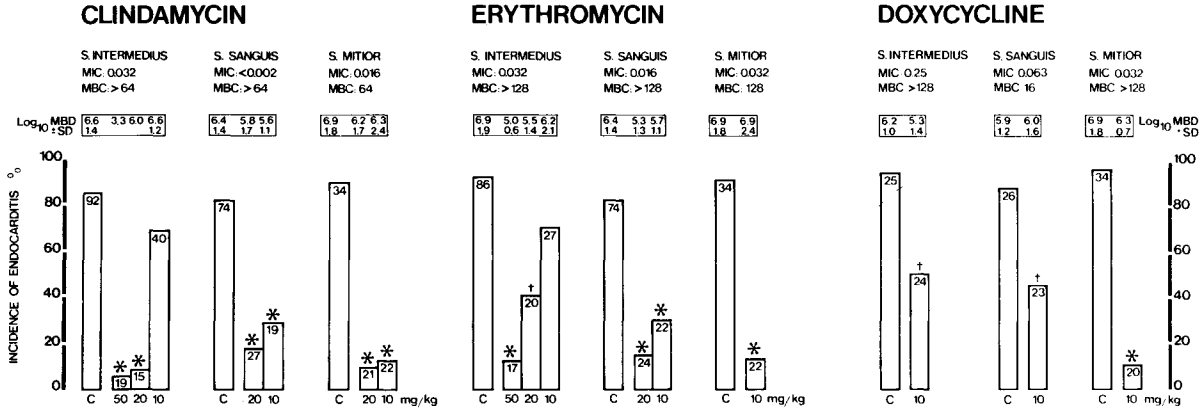

Figure 1. Incidence of endocarditis due to *Streptococcus intermedius*, *Streptococcus sanguis*, and *Streptococcus mitior* after prophylaxis with various doses of clindamycin, erythromycin, and doxycycline administered iv 30 min before bacterial challenge. Numbers within the bars indicate the total number of rats in the groups; numbers beneath the bars indicate the doses (mg/kg of body weight) of the prophylactic antibiotics. Results for control rats (C) that were injected iv with 0.85% NaCl are shown. Above the bars the log of the mean (± SD) bacterial density (MBD) recovered from infected vegetation in each group are shown in boxes. The MICs and MBCs for the antibiotics are also indicated. Statistical comparisons are as follows: (*) indicates $P < 0.01$ in comparison with controls; (†) indicates $P < 0.05$ in comparison with controls.

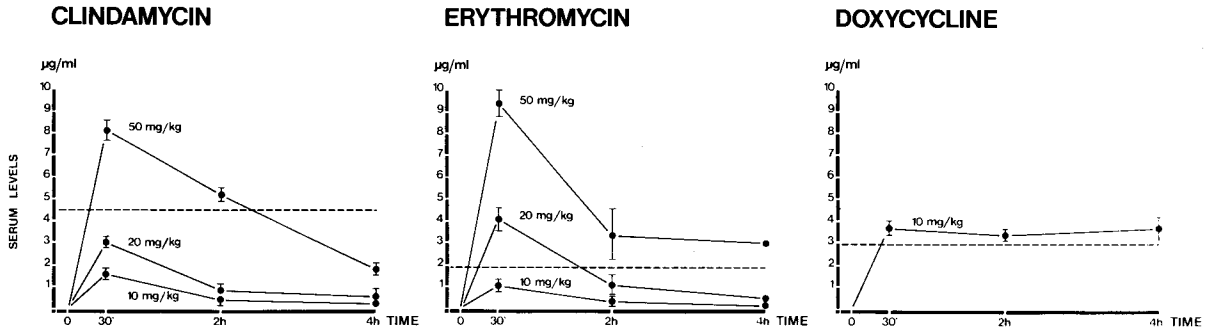

Figure 2. Serum levels of clindamycin, erythromycin, and doxycycline 0.5, 2, and 4 hr after single iv injections in rats. Dotted lines represent peak levels achieved in humans after oral doses of 450 mg of clindamycin, 500 mg of erythromycin, and 200 mg of doxycycline [11].

[11] (figure 2). The 10 mg/kg dose was not uniformly effective.

Prophylaxis of endocarditis with erythromycin. Although the MICs and MBCs of erythromycin for the three streptococcal species were similar to those of clindamycin, erythromycin was less efficient than clindamycin in preventing endocarditis. With the dose of 10 mg/kg, which produced peak serum levels similar to the 0.5–2.0 μg/ml achieved in humans after an oral dose of 500 mg [11] (figure 2), only endocarditis due to *S. sanguis* and *S. mitior* was significantly prevented. The incidence of infection with *S. sanguis* was ~30%.

Prophylaxis of endocarditis with doxycycline. Although half of that in controls, the incidence of endocarditis due to *S. intermedius* and *S. sanguis* in doxycycline-treated rats was still >40% with the dose of 10 mg/kg. This dose produced peak serum levels similar to the 2.8 μg/ml achieved in humans after an oral dose of 200 mg [11] (figure 2).

Quantitative cultures of infected vegetations. For all three streptococcal species, the number of bacteria/g of infected vegetations was similar in control rats to that in rats in which prophylaxis failed (figure 1).

Discussion

Previous studies in rabbits that had been used to establish current recommendations for the prophylaxis against bacterial endocarditis [1] suggested that bacterial killing is the mechanism responsible for successful protection. This conclusion was based on two observations. (*1*) There seemed to be a correlation between the efficacy of antibiotics or combinations of synergistic antibiotics in killing bacteria in vitro and the protection obtained in vivo [3]. (*2*) Bacteriostatic antibiotics such as tetracycline failed to protect from infection [12]. These results served as a basis for the current American Heart Association recommendations of high doses and prolonged administration of bactericidal antibiotics for prophylaxis of endocarditis in humans [1]. However, these experiments in rabbits involved extremely large bacterial inocula. Recent experiments from two different laboratories using inocula that are 2 to 3 log smaller and a single dose of vancomycin as the prophylactic antibiotic have shown that experimental endocarditis can be prevented in the absence of bacterial killing [6, 7]. Even this smaller inoculum provides a stringent test of antibiotic prophylaxis because the resulting bacteremia is far greater than that observed in humans after oral procedures [2] and produces 80%–100% endocarditis in animals. The rats [6], like high-risk human patients, have a permanent intracardiac foreign body. Therefore, it was of interest to use this model to reexamine the efficacy of single doses of orally absorbable, nonbactericidal antibiotics.

Previous attempts to protect rabbits with clindamycin at doses of 10 mg/kg against high inocula (10^8 cfu) of *S. sanguis* failed [4]. In the present study, we found that this same dose produced similar peak concentrations in rats and often failed to protect against smaller inocula (10^5 cfu) of three streptococcal species. In contrast, excellent protection was afforded by doubling the dose (20 mg/kg). This protection was achieved by clindamycin levels below those which follow a single 450-mg oral dose in humans. Thus, peak serum levels conveniently achievable in patients pro-

tected rats from three streptococcal species which colonize the mouth and cause endocarditis in humans. Furthermore, rats were protected at clindamycin levels below the MBCs for these streptococci and in the absence of serum bactericidal activity.

Erythromycin protected rats from endocarditis due to *S. sanguis* and *S. mitior* at doses resulting in serum levels achievable in humans after an oral dose of 500 mg. Against both *S. intermedius* in the present experiments and *S. sanguis* in rabbits [4], erythromycin was protective only at doses that resulted in serum levels far above those obtained in humans after oral administration of 500 mg of the drug [11]. Like tetracycline hydrochloride in rabbits [12], doxycycline failed to provide sufficient protection in rats against infection with two of the three species tested. Thus, both erythromycin and doxycycline protected rats against only some streptococcal species at doses usually given orally to patients. As with clindamycin, protection was achieved in the absence of serum bactericidal activity.

Since sublethal concentrations of vancomycin in previous studies [6, 7] and three bacteriostatic antibiotics in the present experiments provide protection against endocarditis, mechanisms other than killing must be involved. We investigated in two ways the possibility that bacteriostatic antibiotics might have merely delayed the growth of bacteria in vegetations. (*1*) We compared the numbers of streptococci in vegetations from infected controls and from animals infected in spite of prophylaxis. If antibiotics suppressed rather than prevented infection, rats in which prophylaxis failed should have fewer organisms in their vegetations than untreated control animals. In fact, we found the same high numbers in both groups of rats (figure 1). (*2*) We examined animals one week rather than three days after challenge (data not shown) and found no increase in the percentage of animals in which prophylaxis failed. We conclude that bacteriostatic antibiotics prevented, not just suppressed, infection—that is, prophylaxis was an all-or-none phenomenon.

Another possible mechanism is suggested from previous experiments with vancomycin. In both rats and rabbits, vancomycin appears to protect by inhibiting adherence of streptococci to sterile vegetations [6, 7]. This interpretation is supported by demonstration in vitro that nonbactericidal concentrations of vancomycin and penicillin prevent adherence of streptococci to platelet-fibrin clots [7, 13]. MICs of clindamycin have similar negative effects on the adherence of *Escherichia coli* [14] and alter surface structures of streptococci that mediate adherence [15, 16]. These observations raise the possibility that one mechanism by which clindamycin or other bacteriostatic antibiotics prevented endocarditis is interference with adherence.

Regardless of the mechanism responsible for protection, the present experiments suggest that the use of stringent antibiotic regimens aimed at killing bacteria might not be required for adequate prevention of bacterial endocarditis in humans.

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