Bacterial Adhesion in the Pathogenesis of Infective Endocarditis

EFFECT OF SUBINHIBITORY ANTIBIOTIC CONCENTRATIONS ON STREPTOCOCCAL ADHESION IN VITRO AND THE DEVELOPMENT OF ENDOCARDITIS IN RABBITS

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ABSTRACT Bacterial adhesion to the constituents of nonbacterial thrombotic endocarditis (NBTE) is important in the pathogenesis of endocarditis. Subinhibitory concentrations (subMIC) of some antibiotics decrease bacterial adhesion to epithelial cells in vitro. We utilized an in vitro assay system to study the effect of subMIC of various antibiotics on streptococcal adhesion to a fibrin-platelet matrix (simulating NBTE). The results were (*a*) bacterial adhesion of *Streptococcus sanguis* and *Streptococcus faecalis* to NBTE was significantly reduced by vancomycin, penicillin, tetracycline, chloramphenicol and streptomycin ($P < 0.01$ vs. controls) but not rifampin or trimethoprim-sulfametrole; (*b*) the effect was dose-dependent and increased with duration of exposure to antibiotic; (*c*) reduction in bacterial adhesion did not correlate with altered retention by hydrophobic-interaction chromatography.

This reduction in adhesion correlated with a diminished capacity of subMIC exposed *Streptococcus sanguis* (¼ vancomycin minimum inhibitory concentration (MIC) × 4 h) to produce endocarditis in vivo. After intravenous inoculation of 10^6 colony-forming units of preincubated organisms into rabbits with traumatized aortic valves, 6 of 22 developed endocarditis vs. 17 of 22 controls ($P = 0.03$).

These results may be relevant to prophylaxis of endocarditis since exposure of bacteria to subMIC of various antibiotics may reduce bacterial adherence both, to mucosal surfaces, and to damaged cardiac valves.

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INTRODUCTION

Subinhibitory concentrations (subMIC)[1] of many antibiotics have profound effects on the exposed bacteria including a decreased growth rate, morphologic alterations, enhanced leukocytic ingestion or killing, and reduced bacterial adhesion to epithelial cells (1). SubMIC of various antibiotics decreased the adhesion of *Escherichia coli* to intestinal or genitourinary epithelial cells in vitro (2, 3); these observations may be relevant to the prophylaxis and/or therapy of enteric diseases or urinary tract infections, but no in vivo correlative experiments have been performed.

We have shown previously that adhesion of oral streptococci to the constituents of nonbacterial thrombotic endocarditis (NBTE—fibrin plus platelets) is important in the pathogenesis of infective endo-

[1] *Abbreviations used in this paper*: BHIB, brain-heart infusion broth; CFU, colony-forming units; MIC, minimum inhibitory concentration; subMIC, subinhibitory concentration; NBTE, nonbacterial thrombotic endocarditis.

carditis caused by these organisms (4). Several experimental manipulations decreased streptococcal adhesion to fibrin-platelet matrices in vitro, including removal of extracellular dextran by enzymatic digestion and preincubation of the organisms with anti-whole-cell antibody (4, 5). Antibody also protected rabbits from the development of *Streptococcus sanguis* endocarditis in vivo (5). Similar phenomena were recently observed in experimental endocarditis due to *Candida albicans* (6). The effect of subMIC on the adhesion of oral streptococci is unknown.

The purposes of these studies were (*a*) to determine the influence of subMIC of various antibiotics on bacterial adhesion to the constituents of NBTE in vitro, and (*b*) to determine if a reduced bacterial adhesion after exposure to subMIC of antibiotics correlates with a reduced propensity to produce endocarditis in vivo.

TABLE I

Effect of Subinhibitory Concentrations (¼ MIC) of Antibiotics on Adhesion of Streptococci to Fibrin-Platelet Matrices In Vitro

Drug	Adhesion ratio × 10⁴*	
	S. sanguis	*S. faecalis*
None (controls)	643±74	676±35
Penicillin	192±60	344±40
Tetracycline	195±46	261±51
Trimethoprim-sulfametrole	1291±81	859±52
Rifampin	543±42	299±40
Streptomycin	293±31	239±35
Chloramphenicol	221±45	227±42
Vancomycin	113±38	188±65

* Data represent mean±SD.

METHODS

In vitro studies

Preparation of bacteria, preparation of artificial fibrin-platelet matrices, and *determination of in vitro adhesion.* These were performed as previously described (4). Adhesion of bacteria to artificial platelet-fibrin matrices was expressed as the ratio of adherent bacteria divided by the number in the initial inoculum. For purposes of comparison the ratio was multiplied by 10^4.

Influence of antibiotic. The following antibiotics were added to the organisms at ¼ MIC for 6 h (in brain-heart infusion broth [BHIB] plus 5% sucrose) and adhesion determined: penicillin, trimethoprim-sulfametrole, rifampin, streptomycin, chloramphenicol, tetracycline, and vancomycin.

Influence of antibiotic concentration. S. sanguis or *S. faecalis* were exposed to penicillin, streptomycin, or vancomycin for 4 h at 37°C at the following concentrations (given as fraction of the MIC): 1/64, 1/32, 1/16, ⅛, and ¼.

Influence of time of exposure to subMIC. The organisms (*S. sanguis* or *S. faecalis*) were exposed to ¼ MIC of vancomycin or penicillin for varying time intervals (0.5, 1, 2, 3, 4, or 6 h) before determination of bacterial adhesion to the fibrin-platelet matrices.

Influence of antibiotic exposure on bacterial surface hydrophobicity. Streptococci were exposed to each antibiotic (see above) at ¼ MIC in BHIB plus 5% sucrose at 37°C for 4 h and retention assessed in sepharose-phenyl columns as described previously (7).

Production of endocarditis. Endocarditis was produced by 1 h of polyethylene catheter-induced aortic valve trauma as described previously (4, 5). A single inoculum ($\simeq 10^6$ colony-forming units [CFU]) was injected intravenously and the catheter was then removed. The presence or absence of endocarditis was confirmed 72 h after catheterization as described previously (4, 5).

Experimental design. The control group ($n = 22$) was injected with *S. sanguis* grown in BHIB plus 5% sucrose for 4 h which was centrifuged, washed, and resuspended in saline to ensure a final inoculum of $\simeq 10^6$ CFU in 1 ml. The other group ($n = 22$) received *S. sanguis* treated identically except the organism was exposed to ¼ MIC vancomycin for the 4 h treatment period.

RESULTS

MIC. The MIC (microtiter broth-dilution method) for the various antibiotics examined against *S. sanguis* and *S. faecalis* were (in μg/ml): 0.03 and 2 for penicillin, 1 and 4 for vancomycin, 16 and 8 for streptomycin, 1 and 128 for chloramphenicol, 0.06 and 4 for rifampin, 0.5 and 4 for trimethoprim-sulfametrole and 0.25 and 32 for tetracycline, respectively.

Effect of various antibiotics. The influence of antibiotic exposure (all at ¼ MIC × 6 h) of the various agents on bacterial adhesion of *S. sanguis* and *S. faecalis* to fibrin-platelet matrices in vitro is shown in Table I. Although trimethoprim-sulfametrole increased adhesion all the other drugs decreased adhesion in these experiments. The mean adhesion ratio as a percentage of controls (for *S. sanquis* was as follows: penicillin 30%, streptomycin 45.6%, chloramphenicol 34.4%, tetracycline 30%, rifampin 83%, and vancomycin 17.6%. The most effective agents were penicillin, tetracycline, and vancomycin ($P < 0.01$ vs. controls). An identical pattern was observed with *S. faecalis* (Table I).

Effect of antibiotic concentrations. The decreased adhesion of *S. sanguis* or *S. faecalis* observed after exposure to penicillin or vancomycin was dose-dependent (Fig. 1). For example, with *S. sanguis* and vancomycin, the mean adhesion ratio as a percentage of control were as follows: 1/64 MIC—82.3%, 1/16 MIC—44.6%, ¼ MIC—17.6% (the latter two values, $P < 0.01$ vs. controls) (Fig. 1). Similar results were observed with *S. faecalis.*

Effect of time of exposure. The reduction of adhesion of *S. sanguis* to fibrin-platelet matrices was dependent on the length of time the organism was incubated with vancomycin at ¼ MIC (Fig. 2). A significant decrease in adhesion was apparent after 1 h (69.3% of control, $P = 0.02$) and a maximum effect

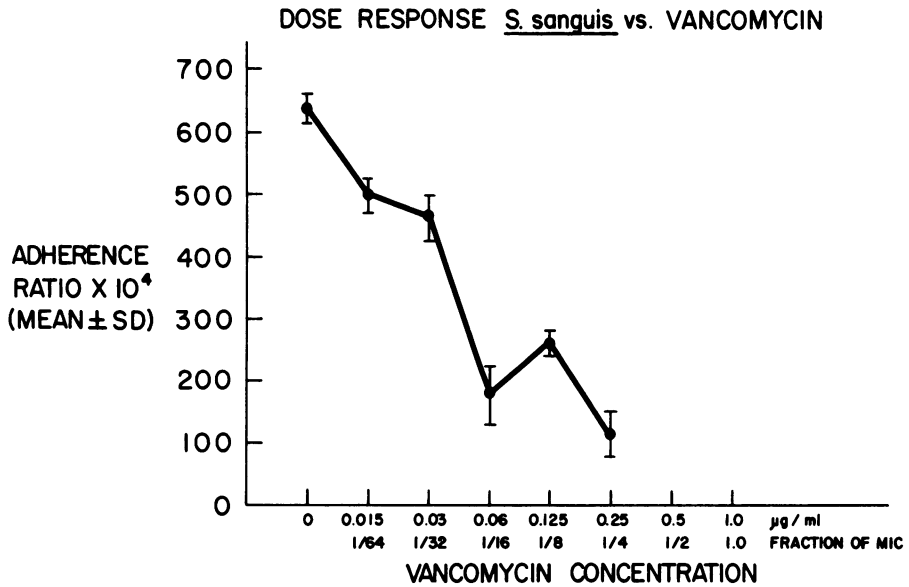

FIGURE 1 Adherence of *S. sanguis* to fibrin-platelet matrices in vitro following incubation in BHIB for 4 h with vancomycin at varying concentrations below MIC.

was observed after 4–6 h of incubation (17–32% of control, $P < 0.01$). Similar results were noted with *S. faecalis*.

Effect of subMIC on bacterial cell surface hydrophobicity. Exposure of *S. sanguis* or *S. faecalis* to a variety of antibiotics (¼ MIC × 4 h) did not significantly alter surface hydrophobicity since the percent retention by the columns was virtually complete, and ranged from 86–100% of the controls for both organisms.

Effect of exposure to subMIC vancomycin on development of streptococcal endocarditis in vivo. The reduced adhesion of *S. sanguis* after exposure to ¼ MIC vancomycin to fibrin-platelet matrices observed in vitro directly correlated with a reduced ability of vancomycin-exposed organisms to produce endocarditis in vivo. After injection of control *S. sanguis* (inoculum = $\log_{10}$ 6.18 CFU), 17 of 22 rabbits developed endocarditis vs. only 6 of 22 with endocarditis after injection of vancomycin-exposed *S. sanguis* (inoculum $\log_{10}$ 6.31 CFU) ($P = 0.03$ by Fischer exact test).

DISCUSSION

The purpose of these studies was to examine the hypothesis that preincubation of streptococci with subMIC of antibiotics would reduce their ability to stick to NBTE and thus render them less likely to produce endocarditis. Fibrin and platelets were used as the test surface in vitro because they form the nidus for colonization (NBTE) on damaged cardiac valves for circulating organisms (4). The results of both in vitro and in vivo investigations are consistent with this hypothesis.

Several classes of antibiotics were tested to determine if the effect was common to representative drugs that inhibit bacterial growth. Exposure of *S. sanguis* or *S. faecalis* to subMIC of penicillin, vancomycin, streptomycin, tetracycline, and chloramphenicol significantly reduced their adhesion to platelet-fibrin surfaces in vitro. The effect was most pronounced with penicillin and vancomycin. The reduction in adhesion was dose-related (greater with increasing drug concentrations) and time-dependent (maximal at 4–6 h of incubation). Incubation of *S. sanguis* with subMIC of penicillin has also recently been reported to reduce adhesion to damaged heart valves in vitro with a dose-

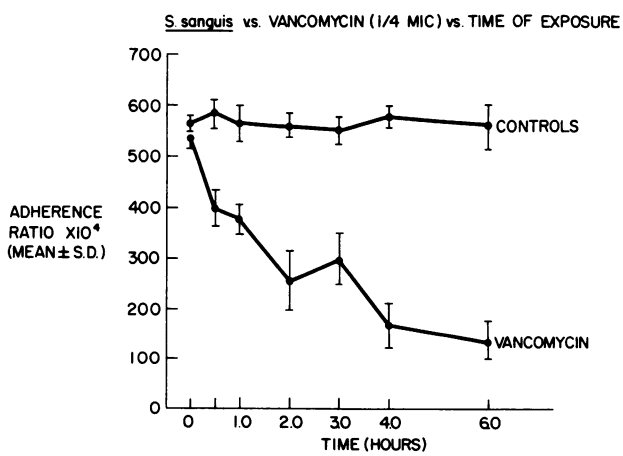

FIGURE 2 Adherence of *S. sanguis* to fibrin-platelet matrices in vitro after incubation in BHIB with vancomycin at ¼ MIC for variable time periods compared with control organisms incubated in BHIB without vancomycin.

response and effective incubation time similar to those reported here (Ramirez-Ronda et al.).[2]

The exact mechanism by which penicillin reduces bacterial adhesive properties is not known but correlates with the release of lipoteichoic acids from the cell envelope. Incubation of *S. sanguis* or *S. pyogenes* with sublethal doses of penicillin stimulates release of lipoteichoic acids into the culture supernate, and correlates with a reduced adhesion to buccal mucosal cells (8). The mechanism of vancomycin inhibition of bacterial adhesion is not known.

Preincubation with tetracycline, chloramphenicol, and streptomycin, all agents that primarily affect ribosomal function and protein synthesis, also reduced adhesion of the organisms to fibrin-platelets in vitro in our experiments. In previously reported studies, adhesion of *E. coli* to human oral epithelial cells was also impaired by preincubation with the aminoglycosides, chloramphenicol, and tetracycline (8). Specific alterations of the surface properties (a decrease in mannose-specific ligand binding activity and degree of piliation) were noted. Thus antibiotics with various modes of action are capable of disrupting the intricate surface mechanisms that confer optimal adhesive capacity of bacterial cells to human tissues.

Tylewska et al. (9) suggested that a change in surface charge or hydrophobicity by subMIC of penicillin and rifampin might reduce adhesion of *Streptococcus pyogenes* to epithelial cells. However, no significant changes in hydrophobic properties as determined by hydrophobic interaction chromatography of *S. sanguis* with subMIC of vancomycin were found in our study.

The potential in vivo significance of these observations was tested in a model of *S. sanguis* endocarditis. Prior studies have suggested that the ability of bacteria and fungi to adhere to fibrin and platelets in vitro is predictive of their ability to produce endocarditis in this model (4, 6). In the present experiment 10^6 CFU of *S. sanguis*, preincubated for 4 h with ¼ MIC of vancomycin (a process that reduced adhesion in vitro) and injected into animals with traumatized aortic valves, produced endocarditis in only 27% of the animals compared with 77% of animals injected with the same number of control organisms. While this study demonstrates a correlation between in vitro adhesion and production of endocarditis, other mechanisms, as yet unexplored, could also be operative. Bernard et al.[3] recently reported similar findings in a rat model of endocarditis after incubation of a vancomycin-tolerant strain of *S. sanguis* with inhibitory concentrations of vancomycin.

These studies suggest that the exposure of streptococci to low concentrations of certain bacteriostatic and bactericidal antibiotics may reduce their ability to produce endocarditis. The prevention of this disease may thus not always require high levels of bactericidal antibiotics as previously suggested (10). The interaction of organisms with low concentrations of antibiotics on mucosal surfaces or within the bloodstream may exert a valuable prophylactic role.

[2] Ramirez-Ronda, C. H., and R. Gutierrez. 1980. 20th Interscience Conference of Antimicrobial Agents and Chemotherapy Abstr. No. 642.

[3] Bernard, J. P., P. Francioli, and M. P. Glauser. 1981. Prophylaxis of endocarditis due to vancomycin-tolerant *Streptococcus sanguis*: a study on the mechanism of protection by vancomycin. *In* Program, 12th International Congress of Chemotherapy, Florence, Italy. Abstr. No. 1304.

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