Effects of sub-bactericidal concentrations of antibiotics in experimental models of endocarditis

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Subinhibitory and sub-bactericidal concentrations of antibiotics significantly influence host-bacterial interactions. This paper reviews the pathogenesis of endocarditis and examines how subinhibitory concentrations of antibiotics may influence the course of the disease. At present significant effects have been documented only for the stage of bacterial adherence to the damaged valve.

A discussion of subinhibitory antibiotic concentrations in endocarditis may appear to fly in the face of progress given our solid knowledge of the necessity for bactericidal antibiotic therapy for this infection. Nonetheless, such is our task, and rather than attempt to make a case for the role they may play in clinical practice, we will review our understanding of the pathogenesis of endocarditis and examine at what stages subinhibitory antibiotic concentrations might influence the disease.

Although the concept of subinhibitory concentrations (sub-MIC) of antibiotics seems straightforward, the effects on bacteria are varied and for the most part not characterized at the biochemical level. In-vitro aspects that have been investigated for some antibiotics and organisms are primarily morphological or functional (e.g. growth rate, susceptibility to serum killing and phagocytosis, and adherence to surfaces) (Washington, 1979). Limited studies in animals have in some cases demonstrated at least partial protection against infection (Zak & Kradolfer 1979). These investigations have shown that generalizations are hard to make and one must consider each drug-organism-infection combination individually.

It is also apparent from detailed studies of pathogenesis that infections develop through a sequence of stages, with different virulence factors acting at each stage. In endocarditis, three major stages in the pathogenesis of the disease have been identified, although knowledge of the molecular mechanisms involved is limited. These are (1) bacteraemia, (2) adherence of micro-organisms to the valve, and (3) vegetation development.

Bacteraemia occurs in a variety of well described circumstances (Everett & Hirschmann 1977), putting a patient at risk for developing endocarditis. More rapid clearance of micro-organisms from the blood stream would lessen the risk of valve colonization. However, in neither of two studies has exposure of micro-organisms to inhibitory but sub-bactericidal antibiotic concentrations altered their clearance following intravenous injections (Bernard, Francioli & Glauser, 1981; Lowy et al., 293
The organisms studied in each case were strains of *Streptococcus sanguis*, tolerant to penicillin and vancomycin to which they were exposed.

Gram-negative bacteria infrequently cause endocarditis, one reason for this being susceptibility to complement lysis in serum (Durack & Beeson, 1977). Enhanced serum susceptibility in the presence of sub-MIC of antibiotics would be expected to decrease the infectivity of relatively resistant strains.

Adherence of circulating micro-organisms to the damaged valve is the second stage in the development of endocarditis. Autopsy and experimental animal model studies implicate the lesion of non-bacterial thrombotic endocarditis (NBTE) as the lesion on cardiac valves which make them susceptible to developing endocarditis (Freedman, 1982). We have examined the adherence of *Str. fecaelis* and *Str. sanguis* strains to this substrate in vitro in the presence of sub-MIC of several antibiotics (Scheld et al., 1981). Vancomycin, penicillin, tetracycline, chloramphenicol, and streptomycin all significantly reduced adherence to simulated NBTE, while rifampicin and trimethoprim-sulfamethoxazole did not. The observed reductions in adherence were dose and time dependent. The in-vivo significance of these observations was confirmed by demonstrating that sub-MIC of vancomycin (the most potent of the antibiotics in the in-vitro studies) reduced the capacity of *Str. sanguis* to cause endocarditis in the rabbit model. Only 6 of 22 rabbits infected with organisms preincubated with \( \frac{1}{2} \) the MIC of vancomycin for 4 h developed endocarditis while 17/22 infected with unexposed organisms became infected.

Similar observations were made by Bernard et al. (1981), who also demonstrated reduced infectivity of a tolerant *Str. sanguis* pretreated with inhibitory but sub-bactericidal concentrations of vancomycin. Supportive in vitro studies by Ramirez-Rondez, Fuxench & Reyes (1983) used excised traumatized cardiac valves from dogs, showing that adherence of strains of *Staphylococcus aureus*, *Str. sanguis*, and an enterococcus were reduced with sub-MIC of penicillin, ampicillin, vancomycin, and erythromycin, but not gentamicin. Thus far, these studies are in agreement, although only a limited number of strains have been tested, particularly in an animal model.

Only one study has attempted to elucidate the mechanism of reduced adherence. Lowy et al. (1983), pretreated a tolerant *Str. sanguis* with penicillin and demonstrated reduced adherence to simulated NBTE in vitro as well as to traumatized cardiac valves of rabbits following intravenous injections of the bacteria. They went on to show that lipoteichoic acid (LTA) was an important factor in organism adherence to NBTE and that exposure of the organism to penicillin induced LTA release from the cell wall. This mechanism may also be responsible for the reduced adherence demonstrated by us and others with vancomycin, which like penicillin, is a cell wall active agent. Adherence of bacteria to NBTE is known to be mediated by several additional mechanisms, including dextran on the cell surface of streptococci, and potentially fibronectin binding protein of *Staph. aureus* and some streptococci. Whether sub-MIC of antibiotics alters surface dextran is not known, but sub-MIC of clindamycin, erythromycin and chloramphenicol reduce fibronectin binding to *Staph. aureus*. Interestingly penicillin increased fibronectin binding (Proctor et al., 1983).

How the vegetation develops after valve colonization has been less well investigated. In past studies, we observed that potent anticoagulation prevented gross vegetation formation (although infection persisted), thus demonstrating that coagulation activation was a fundamental step in vegetation development (Hook & Sande 1974). Recent studies by us have shown that bacteria adherent to the valve surface stimulate
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the expression of tissue factor (tissue tromboplastin) thereby activating coagulation via the extrinsic pathway, leading to fibrin deposition and vegetation growth (Drake, Rodgers & Sande, 1984). How bacteria on the valve stimulated the local host cells (endothelial cells and valve stromal cells) to express tissue factor is under investigation. Potentially, released cytotoxin or cell wall constituents such as lipoteichoic acid could mediate this effect, and thus be influenced by inhibitory or sub-MIC of antibiotics. The influence of sub-MIC of penicillin on LTA release has been discussed; sub-MIC of clindamycin have been documented to decrease haemolysin production in Escherichia coli and Str. pyogenes (Boe, Dellinger & Minshew, 1983).

We believe that there is a dynamic phase in the progression of endocarditis where, following adherence, micro-organisms must "struggle" to resist mechanical dislodge-ment and reproduce to develop a critical mass of bacterial colonies that produce sufficient coagulation activation to provide a protective fibrin covering before the infection becomes irreversibly established on the valve. It is likely that most patients who have developed endocarditis had only minimally-sized lesions susceptible to colonization, and had infecting bacteraemias of low magnitude. This is a very different situation than that created in most experimental model studies where in addition to extensive valve trauma and high infecting inocula, catheters are left in place, causing continued valve damage and local coagulation activation independent of infection. We have examined the course of experimental Staph. aureus endocarditis induced with minimal valve trauma (60 min), low infecting inocula ($10^4$), and catheter removal immediately following inoculation (Drake & Sande, unpublished observations). Rather than manifesting a rapid progressive course, with continuous bacteraemia from the time of infection, the disease was indolent for one to three days, during which animals were afebrile and blood cultures of some intermittently positive in low titre. This was followed at variable times by an accelerated phase with fever and continuous high level bacteraemia prior to death. Vegetations of animals in this phase contained many organisms while those of animals in the indolent phase had significantly fewer. Not all animals developed endocarditis, and it is likely that some self cured. We have termed this early phase, in which the disease may either resolve or accelerate, the 'waffling phenomenon', and believe the course of the disease in this model reflects what usually occurs in man. If such a situation truly exists, then the presence of inhibitory or sub-MIC of antibiotics might favour the course of resolution.

This has not been tested in the Staph. aureus model described, but we have examined the effectiveness of cefotaxime in the early and later treatment of enterococcal endocarditis (Sullam et al., unpublished observations). Although cefotaxime is inactive against enterococci in broth, MICs for the test strain determined in serum or whole blood were 16–32 mg/l. Initiation of treatment 1 h after inoculation failed to eradicate the infection, although bacterial titres in the vegetations remained below those of controls during the four days of treatment. When treatment was started 12 h after inoculation and catheters left in place, no influence on bacterial growth in vegetations was observed. In both experiments, bacteraemia was suppressed and survival improved relative to controls. These observations of clinical improvement without eradication of bacteria from vegetations parallels the reported response of patients treated with bacteriostatic drugs. We were unable therefore to abort the infection immediately after valve colonization using cefotaxime. Whether other drugs will do this remains to be seen.

In conclusion, inhibitory or sub-MIC of antibiotics have been shown to decrease
adherence of bacteria to NBTE and to reduce infectivity in the animal model, thus establishing the relevance of in-vitro observations. In the few instances examined, exposure of organisms to these antibiotic concentrations did not alter bacterial clearance. In a single study, inhibitory antibiotic administered shortly after valve colonization reduced the clinical manifestations of endocarditis but did not abort the infection. As more knowledge is gained about the influence of sub-MIC of antibiotics on bacterial surface structure and extracellular products, use of antibiotics in this manner may extend our understanding of bacterial virulence and disease pathogenesis.

References


