Experimental Rationale for the Use of Combinations of Cell Wall Active Agents plus Aminoglycosides in the Therapy of Enterococcal Endocarditis

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Among the many dramatic examples of the efficacy of penicillin was its initial remarkable success in treating endocarditis due to viridans streptococci -a disease which carried with it one hundred percent (100%) mortality in the pre-antibiotic era. Early studies showed that this disease could be cured with a total dose of as little as 5,000,000 units of penicillin as long as the drug were given in divided doses over a sufficient time period (three weeks or more).

These dramatic results with viridans streptococcal endocarditis, however, were not reproduced for enterococcal endocarditis and, although there were no early controlled trials, failure and relapse rates of greater than fifty percent (>50%) were recorded in patients treated with penicillin alone, even in relatively high doses. Following Thomas Hunter's initial observation that a combination of penicillin and streptomycin resulted in cure of a patient with enterococcal endocarditis, others tried this therapy with success. It was subsequently shown in the laboratory that penicillin was essentially bacteriostatic in its activity against enterococci whereas the combination of penicillin plus streptomycin resulted in rapid bactericidal activity. Our early studies demonstrated that synergism with streptomycin could be obtained when it was combined with other cell wall active agents including cycloserine, bacitracin, cephalosporins and vancomycin. Moreover, combinations of streptomycin with agents which did not inhibit cell wall synthesis (including drugs such as colistin, erythromycin, chloramphenicol and other inhibitors of ribosomal protein synthesis) produced no synergism in combination with streptomycin.

Encouraged by the studies of Plotz and Davis who showed that it was possible to measure the uptake of radio labeled streptomycin in *E. coli*, we studied the effect of various antimicrobials on streptomycin uptake in enterococci. These studies showed that cell wall active agents produced a marked increase in the uptake of radio labeled streptomycin which correlated with the enhanced bactericidal activity of the combination. These studies provided a rational basis for combination therapy, namely that cell wall active agents (which by themselves exhibit only bacteriostatic activity against enterococci) overcome a natural permeability barrier to streptomycin, allowing increased uptake of the streptomycin which leads to its bactericidal

activity. Subsequent studies with ribosomes confirmed that it was the binding of streptomycin to ribosomes which perturbed protein synthesis and accounted for the killing activity of streptomycin.

In our early studies we were also struck by the fact that there were some strains of enterococci which were not killed synergistically by penicillin-streptomycin combinations. These agents exhibited high-level (MIC > 2000 µg/ml) resistance to streptomycin and were not killed despite the fact that the combination resulted in increased intracellular uptake of streptomycin. The basis for this became clear when we studied the ribosomes of these organisms and found that mutations of the 30S subunit led to streptomycin resistance. These studies formed the basis for a simple screening test (the determination of high-level aminoglycoside resistance) to determine whether or not combinations of cell wall active agents and aminoglycosides would produce synergism against enterococci. Subsequent studies in our laboratory showed that strains with ribosomal resistance to streptomycin were still subject to killing by gentamicin in combination with cell wall active agents and this ultimately led to the widespread use of combinations of beta-lactams (or vancomycin) plus gentamicin for therapy of enterococcal endocarditis.

It should be noted that a number of the earlier studies of penicillin therapy of enterococcal endocarditis are likely compromised by the fact that penicillin-susceptible *Streptococcus bovis* (which are killed effectively by penicillin) were often lumped together with true enterococci as "group D streptococci". Undoubtedly some of the early successes reported for penicillin or ampicillin monotherapy of enterococcal endocarditis were due to including these organisms as "enterococci" which they are not. It should also be noted that while enterococci are intrinsically more resistant than other streptococci to the inhibitory effect of beta-lactam antimicrobials (as reflected in elevated minimal inhibitory concentrations for penicillin and ampicillin against enterococci compared with true streptococci), they are not intrinsically "tolerant" to cell wall active antibiotics. We were able to demonstrate this conclusively by obtaining enterococci from an antibiotic virgin population in the British Solomon Islands in the late 1960s. The minimal inhibitory concentration of penicillin for these organisms is similar to the MIC of penicillin for *Enterococcus faecalis* strains currently isolated in the United States. However, the Solomon Islands strains were rapidly lysed by penicillin. Despite this, exposure to as few as five pulses of penicillin in the laboratory resulted in complete tolerance and lack of effective killing by penicillin. Thus, prior exposure to beta-lactam antimicrobials is the likely

explanation for the fact that virtually all enterococci currently isolated are "tolerant" to beta-lactams and other cell wall active agents. The basis for the intrinsic resistance of enterococci to the inhibitory effect of beta-lactams has been shown to be related to decreased affinity of key penicillin binding proteins (especially PBP5) for penicillin in enterococci.

Unfortunately, enterococci have proven remarkably adept at acquiring new mechanisms of resistance. Perhaps the most important of these mechanisms has been the acquisition of genes encoding a bifunctional enzyme that inactivates gentamicin (abrogating beta-lactam-gentamicin synergism), and the acquisition of high-level resistance to beta-lactams in E. faecium (which render beta-lactams useless in combination therapy). These later developments have clearly complicated our therapeutic options as are discussed in detail in this chapter.

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