Efficacy of Ampicillin plus Arbekacin in Experimental Rabbit Endocarditis Caused by an Enterococcus faecalis Strain with High-Level Gentamicin Resistance

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Enterococcus faecalis LC40 is an ampicillin-susceptible clinical isolate with high-level gentamicin resistance due to the aac(6')-Ie-aph(2')-Ia aminoglycoside resistance gene. The combination of ampicillin plus arbekacin reduced mean bacterial vegetation counts significantly more than ampicillin alone or ampicillin plus gentamicin in a rabbit model of aortic-valve endocarditis caused by E. faecalis LC40.

Optimal therapy for severe enterococcal infections, especially infective endocarditis, consists of a synergistic bacterial combination of a cell wall-active agent, such as ampicillin or vancomycin, with an aminoglycoside. Enterococci intrinsically have low-level resistance to aminoglycosides (MICs ≤ 128 μg/ml). However, an increasing number of enterococci have acquired high-level resistance to aminoglycosides (MICs ≥ 2,000 μg/ml), thus enabling these isolates to become resistant to the synergistic bactericidal killing seen with combination therapy.

High-level gentamicin resistance in the vast majority of enterococci is associated with the presence of the bifunctional enzyme AAC(6')-APH(2'), which is encoded by the aac(6')-Ie-aph(2')-Ia gene (4). The presence of this enzyme eliminates the synergistic killing activity between cell wall-active agents and almost all the clinically available aminoglycosides (except streptomycin), including gentamicin, amikacin, kanamycin, tobramycin, netilmicin, and dibekacin (4). Arbekacin, a derivative of dibekacin, is a new aminoglycoside developed in Japan, where it is used to treat infections caused by gentamicin- and methicillin-resistant Staphylococcus aureus (7, 10, 12). Arbekacin is modified at a lower rate by the bifunctional enzyme AAC(6')-APH(2') than gentamicin is (8), which may explain why the majority of staphylococci that possess aac(6')-Ie-aph(2')-Ia remain susceptible to arbekacin in vitro (5, 18). The combination of ampicillin and arbekacin has produced synergistic killing of up to 40% of enterococcal isolates with high-level gentamicin resistance due to the aac(6')-Ie-aph(2')-Ia gene (9). The purpose of this study was to compare the efficacy of the combination of ampicillin and arbekacin with the efficacy of ampicillin alone in an experimental rabbit model of aortic-valve endocarditis caused by an Enterococcus faecalis isolate exhibiting high-level gentamicin resistance due to the aac(6')-Ie-aph(2')-Ia gene.

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Enterococcus faecalis LC40 is a clinical blood isolate with high-level gentamicin resistance (gentamicin MIC > 2,000 μg/ml).

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dose of 400 mg intravenously results in a peak level in serum of 16.56 or 27.77 µg/ml, respectively (12). Arbekacin levels in serum were obtained from 5 of the 10 animals in the ampicillin-plus-ARBekacin treatment group. Arbekacin concentrations were determined by fluorescence polarization immunoassay using the TDX kit (Dainabot Co., Ltd., Tokyo, Japan). Comparisons of mean bacterial counts (log_{10} CFU per gram of vegetation) between treatment groups were determined by Student’s t test. The untreated group was excluded from the statistical analysis in comparing treatment groups, since it was not strictly a control group (animals were sacrificed at day 3 instead of day 5). Statistical analysis was performed using the SAS system (release 6.12; SAS Institute Inc., Cary, N.C.).

The MICs of ampicillin and arbekacin for E. faecalis LC40 were 2.0 and 256 µg/ml, respectively. Table 1 shows data from the three antimicrobial treatment groups and the untreated group. One animal in the untreated group and two animals in the ampicillin-only group died after the initial surgery (before infection with E. faecalis LC40), resulting in a total of 37 animals available for analysis. The combination of ampicillin and arbekacin was more effective than ampicillin alone in decreasing the colony counts on vegetations from the treated rabbits (P = 0.02). Ampicillin plus arbekacin was also more effective than ampicillin plus gentamicin in decreasing the vegetation colony counts (P = 0.05). There was no significant difference in colony counts between the ampicillin-only and the ampicillin-plus-gentamicin groups (P = 0.63). All three treated groups had a lower mean colony count per gram of vegetation than the untreated group. Blood cultures from 8 of 9 animals in the untreated group and 1 of 10 animals in the ampicillin-plus-gentamicin group were negative. All blood cultures from the ampicillin-only and ampicillin-plus-ARBekacin groups were negative. The mean arbekacin concentration in serum at 1 h was 14.62 ± 4.56 µg/ml. In serum samples from all five rabbits, the trough concentrations of arbekacin were below the assay detection limit of 0.4 µg/ml.

The first clinical isolates of E. faecalis with high-level resistance to gentamicin were reported in France in 1979 (6). Since then, they have been reported worldwide and have become endemic in many U.S. hospitals (19). Although two other gentamicin resistance genes, aph(2’)-Ic and aph(2’)-Id, encode aminoglycoside-modifying enzymes that eliminate synergism between ampicillin and gentamicin, the bifunctional aac(6’)-Ie-aph(2’)-Ia gene is still by far the most prevalent gentamicin resistance gene found in clinical enterococcal isolates (2, 16). While aac(6’)-Ie-aph(2’)-Ia does not encode streptomycin resistance, many gentamicin-resistant enterococci are also resistant to streptomycin. In some centers, all isolates with high-level gentamicin resistance are also highly resistant to streptomycin (3). Thus, use of the classic synergistic combination therapy with a cell wall-active agent plus an aminoglycoside has been severely limited in many cases. Results from the present study are from only a single enterococcal strain. If these data are confirmed by more extensive studies, the combination of ampicillin and arbekacin may prove to be a therapeutic alternative in infections caused by ampicillin-susceptible strains with high-level gentamicin resistance caused by the aac(6’)-Ie-aph(2’)-Ia gene, provided in vitro synergism can be documented.

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