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 bacterialidal 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 bacterialidal 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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E. faecalis LC40 is a clinical blood culture isolate with high-level gentamicin resistance (gentamicin MIC > 2,000 μg/ml). The primer pairs 5'-GAGCAATAAGGGCATACCCAAAAAT C-3' and 5'-CCGTGCTTGGCTTTAAAAACGTG-3' were used to confirm the presence of aac(6')-Ie-aph(2')-Ia in E. faecalis LC40 by PCR, as previously described (18). The absence of the aph(2')-Ic and aph(2')-Ia gentamicin resistance genes in LC40 was also confirmed by PCR, as previously described (18). Ampicillin and arbekacin MICs were determined by broth microdilution using standard methods (11). The combination of ampicillin plus arbekacin produced synergistic killing of E. faecalis LC40 in a previous in vitro study (9). Experimental aortic-valve endocarditis was established in New Zealand White female rabbits according to the method described by Perlman and Freedman (13). A catheter was placed across the aortic valve after introduction from the internal carotid artery and remained in place throughout the study period. Twenty-four hours after placement of the catheter, the rabbits were infected with 10⁸ CFU of E. faecalis LC40 per ml. Treatment was started 20 h later with ampicillin alone (100 mg/kg of body weight intramuscularly [i.m.] three times a day), ampicillin plus gentamicin (3 mg/kg i.m. twice a day), or ampicillin plus arbekacin (5 mg/kg i.m. twice a day). Untreated animals were sacrificed 3 days (instead of 5 days) later due to our institutional review board’s concerns that the animals may have endured prolonged suffering if left untreated for 5 days. The treated animals received the antibiotic(s) for 5 days and were sacrificed 12 h after the last antibiotic dose. Blood cultures were drawn from the animals immediately prior to sacrifice. The aortic-valve vegetations were harvested, weighed, homogenized in saline, and quantitatively cultured onto blood agar plates. After incubation for 24 to 48 h at 37°C, the colonies were counted and the results were expressed in log₃ CFU per gram. Ampicillin at 100 mg/kg i.m. is the same dosage that we have used in past experiments, and it provides a peak ampicillin level in serum of 58.0 ± 14.7 μg/ml (15, 17). The gentamicin dosage of 3 mg/kg i.m. was based on previously published rabbit data that showed a 1-h postdose concentration in serum of 6.2 ± 1.2 μg/ml at 2.5 mg/kg i.m. (14) and a concentration of 5.9 ± 1.8 μg/ml or 3.8 ± 0.6 μg/ml at 3 mg/kg i.m. (1, 15). The arbekacin dosage of 5 mg/kg i.m. was based on unpublished rabbit data (from Meiji Seika Kaisha Ltd., Tokyo, Japan) that showed a 1-h postdose concentration in serum of 18 ± 0.46 μg/ml at 5 mg/kg i.m. In humans, arbekacin given at a dose of 200 mg intravenously every 12 h or in a single daily
then they have been reported worldwide and have become
tance to gentamicin were reported in France in 1979 (6). Since
was 14.62
the ampicillin-only and ampicillin-plus-arbekacin groups were
in the untreated group and 1 of 10 animals in the ampicillin-
groups had a lower mean colony count per gram of vegetation
in the detection limit of 0.4
gene is still by far the most prevalent gentamicin
-Ia
0
0
-streptomycin (3). Thus, use of the classic synergistic combina-
tion therapy with a cell wall-active agent plus an aminoglyco-
sis resistant to streptomycin. In some centers, all isolates with high-
level gentamicin resistance are also resistant to
-aph(2)-Id. While aac(6')-Ie-aph(2')-Ia does not encode streptomyein resis-
tance, many gentamicin-resistant enterococci are also resis-
tant to streptomycin. In some centers, all isolates with high-
level gentamicin resistance are also highly resistant to
streptomyein (3). Thus, use of the classic synergistic combina-
tion therapy with a cell wall-active agent plus an aminoglyco-
side 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 com-
bination of ampicillin and arbekacin may prove to be a thera-
peutic 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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TABLE 1. Vegetation counts in experimental rabbit endocarditis
cauised by high-level gentamicin-resistant E. faecalis LC40

<table>
<thead>
<tr>
<th>Antimicrobial regimen</th>
<th>No. of rabbits</th>
<th>Log10 CFU of bacteria/g of vegetation</th>
<th>Mean (range)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antimicrobial therapy</td>
<td>10</td>
<td>9.44 (8.30–10.05)</td>
<td>±0.59</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>8</td>
<td>6.29 (3.60–7.92)</td>
<td>±0.31</td>
<td></td>
</tr>
<tr>
<td>Ampicillin + gentamicin</td>
<td>10</td>
<td>5.98 (3.81–8.13)</td>
<td>±0.32</td>
<td></td>
</tr>
<tr>
<td>Ampicillin + arbekacin</td>
<td>10</td>
<td>4.82 (2.70–6.36)</td>
<td>±0.19</td>
<td></td>
</tr>
</tbody>
</table>