Sparfloxacin and clinafloxacin alone or in combination with gentamicin for therapy of experimental ampicillin-resistant enterococcal endocarditis in rabbits

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Sparfloxacin and clinafloxacin alone and in combination with gentamicin, were evaluated for the treatment of experimental endocarditis in rabbits caused by ampicillin-resistant enterococci. Clinafloxacin was tested against Enterococcus faecalis strain WH245, a β-lactamase producer lacking high-level gentamicin resistance (MIC 12.5 mg/L). Sparfloxacin was tested against Enterococcus faecium strain SF2149 a non-producer of β-lactamase (ampicillin MIC 400 mg/L, gentamicin MIC 12.5 mg/L). For strain WH245, clinafloxacin alone significantly reduced enterococcal counts in vegetations (7.7 log₁₀ cfu/g) and for strain SF2149, sparfloxacin significantly reduced counts (7.0 log₁₀ cfu/g) compared with untreated controls (WH245, 8.8 log₁₀ cfu/g and SF2149, 9.3 log₁₀ cfu) or treatment with ampicillin plus gentamicin (WH245, 9.7 log₁₀ cfu/g). The addition of gentamicin resulted in no further reduction of bacterial counts in vegetations but resulted in an increase in sterilization of blood for strain SF2149. These results suggest that sparfloxacin and clinafloxacin and may prove useful in the therapy of infections due to ampicillin-resistant enterococci.

Introduction

Enterococci are increasingly recognized as important human pathogens. They are responsible for urinary tract infections as well as bacteraemia and endocarditis and may account for as many as ten per cent of all nosocomial infections (Horan et al., 1986; Hoffman & Moellering, 1987). Treatment of enterococcal infections is problematical because of the intrinsic poor susceptibility of enterococci to aminoglycosides and pencillins. The bacteriostatic activity of penicillin alone is usually adequate for treatment of uncomplicated enterococcal infections in the absence of endocarditis, however, for serious infections such as bacteraemias and endocarditis, combination therapy with penicillin plus an aminoglycoside, is usual.

Recently, the treatment of enterococcal infections has been complicated by multiple-drug resistance including the emergence of β-lactamase-producing, glycopeptide-resistant and aminoglycoside-resistant strains. Pencillinase producing strains have been reported from a number of distinct geographical locations throughout the USA.

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including Boston (Rhinehart et al., 1990), Connecticut (Patterson & Zervos, 1989), Delaware, Virginia (Murray et al., 1991), Philadelphia (Murray et al., 1986), and Pittsburgh (Patterson & Zervos, 1989) and other countries including Lebanon and South America (Murray et al., 1991). Rapid dissemination, including widespread clonal dissemination (Murray et al., 1991), and an outbreak due to β-lactamase-producing strains (Rhinehart et al., 1990) have been identified. β-Lactamase producers inactivate penicillin in-vitro and show a very marked inoculum effect when in-vitro susceptibility is determined (Murray & Mederski-Samaroj, 1983; Patterson & Zervos, 1989). Penicillin resistance due to non-β-lactamase-mediated mechanisms has also been increasingly reported in hospitalized patients, with up to 9% of enterococci in some hospitals being ampicillin resistant (Oster et al., 1990; Chirugi et al., 1991).

Sparfloxacin and clinafloxacin have good in-vitro activity against enterococci including ampicillin-resistant strains (Perri, Chow & Zervos, 1993). To help assess their clinical potential we evaluated the efficacy of the investigational quinolones sparfloxacin and clinafloxacin alone or in combination with gentamicin for therapy of experimental enterococcal endocarditis in rabbits caused by two strains of ampicillin-resistant enterococci.

Materials and methods

Organism

Two enterococcus strains were used in this study. Their in-vitro susceptibility has been previously published (Patterson & Zervos, 1989; Perri et al., 1993) and is summarized in Table I. Enterococcus faecalis strain WH245 is a β-lactamase producer originally isolated from a patient at the West Haven Veterans Administration Medical Center, West Haven, CT (Patterson & Zervos, 1989). Enterococcus faecium strain SF2149 was isolated from a patient in the Veterans Administration Medical Center, Martinez, CA. It does not produce β-lactamase (Oster et al., 1990).

Animal studies

Experimental endocarditis was produced in New Zealand white rabbits (Langshaw Farms, August, USA) weighing 1.8-3.2 kg as described by (Kaatz et al. (1987) with the following modifications: After the rabbits were anaesthetized with xylazine 10 mg/kg im plus ketamine hydrochloride 35 mg/kg im a catheter was inserted into the left carotid artery and positioned across the aortic valve.

Rabbits were inoculated through a peripheral ear vein with 1 mL of a culture of WH245 or SF2149 (10¹¹ cfu/L) suspended in brain heart infusion broth (Difco, Detroit, USA) 72 h after catheter placement. Sparfloxacin (AT-4140) and clinafloxacin (CI-960, PD 127391) were supplied by Parke-Davis Pharmaceutical Division, Warner-Lambert Co., Ann Arbor, MI, USA. Treatment was begun 24 h post infection. For WH245 rabbits were randomized into one of the following groups: control (no treatment) 22 rabbits; gentamicin 3 mg/kg im bd, 12 rabbits; ampicillin 100 mg/kg im tds plus gentamicin 3 mg/kg im bd, ten rabbits; clinafloxacin 50 mg/kg im bd, 17 rabbits; clinafloxacin 50 mg/kg im bd plus gentamicin 3 mg/kg im bd, 17 rabbits; sparfloxacin was not tested. For SF2149, rabbits were randomized into the following groups: control (no treatment) 30 rabbits; sparfloxacin, 50 mg/kg im bd, 18 rabbits; sparfloxacin 50 mg/kg im bd plus gentamicin 3 mg/kg im bd, 17 rabbits; clinafloxacin was not
<table>
<thead>
<tr>
<th>Strain inoculum</th>
<th>MIC/MBC (mg/L)</th>
<th>penicillin</th>
<th>Ampicillin</th>
<th>Sparfloxacin</th>
<th>Clinafloxacin</th>
<th>Gentamicin</th>
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<tr>
<td></td>
<td>$10^5$</td>
<td>$10^7$</td>
<td>$10^9$</td>
<td>$10^7$</td>
<td></td>
<td></td>
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<td>$E.~faecalis$ WH245</td>
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<td>$E.~faecium$ SF2149</td>
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<td></td>
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<td></td>
<td></td>
<td>$&gt;12/1.2$</td>
<td>$0.5/0.5$</td>
<td>$12.5/ND$</td>
</tr>
</tbody>
</table>

Table I. In-vitro susceptibility of the strains of enterococcus used in this study.

Data from Patterson & Zervos (1989) and Perri et al. (1993).

ND, Not determined.
All animal studies were conducted with the approval of the William Beaumont Hospital animal review board; animals were examined daily by a veterinarian for signs of distress or suffering but post-operative analgesics were not deemed necessary. Treatment was administered for three days and animals were killed 12 h after the last dose by administration of sodium pentobarbital 71 mg/kg iv. Control rabbits were killed 24 h after inoculation or at the end of 3 days; only animals surviving to these endpoints were included in the statistical analysis. Autopsies were performed immediately and a sample of blood was taken for blood culture. Animals were included in the study only if vegetations were present although vegetations were found in all animals in whom the catheter was correctly positioned across the aortic valve. Vegetations were removed, weighed and homogenized in 1 mL of sterile saline. Homogenates were serially diluted and inoculated on to agar plates to determine the number of cfu present. Results were expressed as the log_{10} cfu/g of vegetation. Brain heart infusion agar plates were streaked with 100 μL of blood and incubated for 3 days. Tubes containing 5 mL of thioglycollate broth with X and V Enitor supplement (Difco, Detroit, USA) were inoculated with 1 mL of blood and incubated for 5 days. On day 5, the thioglycollate tubes were subcultured on to plates and incubated an additional 2 days. Results of blood cultures were recorded as positive or negative depending on the presence or absence of enterococcal growth on the plates.

Peak serum concentrations

Animals were bled from an ear vein 1 h post dose and samples of serum were taken for peak serum determination of peak antibiotic concentrations. Serum concentrations of sparfloxacin and clinafloxacin were determined by Parke-Davis Warner Lambert Co using a validated HPLC procedure. Gentamicin concentrations in serum were determined by polarization fluoroimmunoassay (Jolley et al., 1981). Ampicillin concentrations were determined by HPLC (Rudrik & Bawdon, 1981).

Statistical analysis

The chi-squared test with the Yates correction was used to compare animal survival and sterility of vegetations. To compare the bacterial vegetation counts between treatment groups and controls, analysis of variance, Mann-Whitney U rank test corrected for multiple comparisons was used.

Results

The mean (± S.D.) peak serum concentrations were: ampicillin, 58.0±14.7 mg/L; gentamicin, 3.8±0.6 mg/L; sparfloxacin, 15.5 mg/L; and clinafloxacin, 4.9 mg/L.

Thirty-two rabbits were excluded from the analysis of the vegetation bacterial counts. Nine were excluded since catheters were not found across the aortic valve at necropsy. In addition eleven WH245-infected, and twelve SF2149-infected animals did not survive to endpoints as a result of systemic enterococcal infection. Only six antibiotic-treated rabbits died and none of these animals were believed to have died as a result of drug toxicity. Table II shows for E. faecalis WH245 the mean vegetation counts for untreated control rabbits and for the four treatment groups after 3 days of therapy. Compared with controls, statistically significant differences were observed.
Table II. A comparison of bacterial counts in vegetations and treatment regimens in experimental endocarditis in rabbits produced by \textit{E. faecalis} WH245

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. animals surviving to endpoint/total infected</th>
<th>Bacterial count in vegetations (mean ± S.D.) $\log_{10}$ cfu/g</th>
<th>Negative blood cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (24 h)$^a$</td>
<td>10/12</td>
<td>8.8 ± 1.0</td>
<td>0</td>
</tr>
<tr>
<td>Control (3 days)$^a$</td>
<td>5/10</td>
<td>10.1 ± 1.2</td>
<td>0</td>
</tr>
<tr>
<td>Clinafloxacin</td>
<td>16/17</td>
<td>7.1 ± 1.2$^a$</td>
<td>12</td>
</tr>
<tr>
<td>Clinafloxacin plus gentamicin</td>
<td>17/17</td>
<td>7.0 ± 1.1$^e$</td>
<td>13</td>
</tr>
<tr>
<td>Ampicillin plus gentamicin</td>
<td>9/10</td>
<td>9.7 ± 1.4$^c$</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10/12</td>
<td>10.0 ± 1.1$^b$</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a P < 0.01$ compared with controls; $^b P < 0.01$ compared with clinafloxacin or clinafloxacin plus gentamicin; $^c$ Control animals received no treatment and were killed 24 h or 3 days after infection.

with clinafloxacin alone and clinafloxacin plus gentamicin but there were no significant difference between these groups. Ampicillin plus gentamicin and gentamicin alone were significantly less effective than clinafloxacin in reducing enterococcal vegetation counts. Positive blood cultures occurred in 4/16 rabbits treated with clinafloxacin and in 3/16 rabbits treated with clinafloxacin plus gentamicin. For \textit{E. faecium} strain SF2149 the results are summarized in Table III. Sparfloxacin alone reduced bacterial counts significantly compared with untreated controls but there was no further significant reduction in enterococcal vegetation counts when sparfloxacin was combined with gentamicin. Positive blood occurred in 8/17 rabbits treated with sparfloxacin and 2/16 rabbits treated with sparfloxacin plus gentamicin. There were no instances when vegetations were sterile for either organism.

Discussion

To help evaluate the efficacy of sparfloxacin and clinafloxacin alone and in combination with gentamicin in treating serious infections caused by ampicillin-resistant

Table III. A comparison of bacterial counts in vegetations and treatment regimens in experimental endocarditis in rabbits produced by \textit{E. faecium} SF2149

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. animals surviving to endpoint/total infected</th>
<th>Bacterial count in vegetations (mean ± S.D.) $\log_{10}$ cfu/g</th>
<th>Negative blood cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (24 h)$^a$</td>
<td>13/15</td>
<td>9.3 ± 1.1</td>
<td>0</td>
</tr>
<tr>
<td>Control (3 days)$^a$</td>
<td>7/15</td>
<td>10.1 ± 1.4</td>
<td>0</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>17/18</td>
<td>7.7 ± 1.1$^a$</td>
<td>9$^a$</td>
</tr>
<tr>
<td>Sparfloxacin plus gentamicin</td>
<td>16/17</td>
<td>7.3 ± 1.2$^a$</td>
<td>14$^a$</td>
</tr>
</tbody>
</table>

$^a P < 0.01$ compared with controls; $^b P < 0.01$ compared with controls and with sparfloxacin plus gentamicin; $^c$ Control groups received no treatment and were killed 24 h or 3 days after infection.
enterococcal strains, we tested the efficacy of these drugs for treatment of experimental aortic valve endocarditis in rabbits produced by two ampicillin resistant strains of enterococci. Earlier investigations of experimental endocarditis (Ingerman et al., 1987; Hindes et al., 1989) showed that for a β-lactamase producing strain of *E. faecalis* with high-level gentamicin resistance, enterococcal counts in vegetations were significantly reduced only with penicillin plus clavulanate or with vancomycin. Strains of ampicillin-resistant *E. faecium* and β-lactamase-producing gentamicin-susceptible *E. faecalis* have not been tested.

*E. faecalis* WH245 used in this study is well characterized (Patterson & Zervos, 1989) and is similar to other reported isolates of β-lactamase-producing *E. faecalis* in that it inactivates penicillin *in vitro*, shows an inoculum effect with penicillin or ampicillin, and the inactivation and inoculum effects are reversed by the addition of the β-lactamase inhibitors sulbactam or clavulanate (Murray & Mederski-Samaroj, 1983; Patterson & Zervos, 1989). The β-lactamase is encoded by a plasmid distinct from those previously described. WH245 also lacks high-level gentamicin resistance having an MIC of 12.5 mg/L. In contrast *E. faecium* SF2149 exhibits high-level of ampicillin resistance that is not due to β-lactamase but is probably related to alterations in penicillin-binding proteins.

This study demonstrated that sparfloxacin or clinafloxacin alone have in-vivo activity against the strains tested. Sterilization of blood and a significant reduction in enterococcal vegetation counts was observed with sparfloxacin versus *E. faecium* and clinafloxacin versus *E. faecalis*. These results support the data showing excellent *in vitro* bactericidal activity of these antibiotics against the isolates (Patterson & Zervos, 1989; Perri et al., 1993). Despite *in vitro* synergy between gentamicin and sparfloxacin or clinafloxacin (Perri et al., 1993), there was no further reduction in enterococcal vegetation counts when combination therapy with gentamicin plus sparfloxacin or clinafloxacin was used. The addition of gentamicin did, however, result in a greater number of animals with negative blood cultures. These results are surprising but may reflect the excellent bactericidal activity of the quinolone alone. More prolonged therapy with these quinolones or, a larger dosage may have resulted in a further reduction in bacterial counts and sterilization of vegetations but this was not tested. Gentamicin alone and in combination with ampicillin had little activity against the *E. faecalis* WH245, with bacterial vegetation counts not significantly different from those in controls. The peak serum concentrations of ampicillin and gentamicin found were comparable to levels found in patients treated with normal dosage regimens. Little is known however, about achievable sparfloxacin and clinafloxacin concentrations in humans. The peak serum concentrations achieved in this study were above the MICs for test organisms used and are comparable to serum concentrations found with other quinolones.

A cell-wall active drug plus an aminoglycoside remains the treatment of choice for infections due to ampicillin sensitive strains of enterococcus lacking high-level aminoglycoside resistance. Therapy of serious enterococcal infections due to β-lactamase producing strains remains problematic, particularly since this trait is usually associated with high-level gentamicin resistance. Alternative agents with bactericidal activity against multi-resistant enterococci are needed. The results presented here suggest sparfloxacin and clinafloxacin may prove to be useful alternative agents for the treatment of serious infections due to ampicillin-resistant enterococcus and clinical trials will be needed to test this.
Experimental enterococcal endocarditis

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References


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