Successful moxifloxacin prophylaxis against experimental streptococcal aortic valve endocarditis

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Objectives: Studies related to the prophylactic efficacy of fluoroquinolones against infective endocarditis are scarce. The aim of this study was to evaluate the efficacy of moxifloxacin, a quinolone active in vitro against Gram-positive cocci, in preventing streptococcal aortic valve endocarditis.

Methods: Non-bacterial thrombotic endocarditis of the aortic valve was induced by the insertion of a polyethylene catheter. Twenty-four hours later, rabbits were randomly assigned to a control group, and groups receiving either two doses of ampicillin (40 mg/kg, intravenously), 2 h apart, or a single dose of moxifloxacin (15 mg/kg, intravenously). Ampicillin and moxifloxacin were administered 0.5 and 1 h, respectively, prior to the intravenous inoculation of $10^7$ cfu of Streptococcus oralis.

Results: Eighty-nine percent of the control animals developed infected vegetations. In rabbits challenged with this very high inoculum, moxifloxacin and ampicillin prevented endocarditis in 80% ($P < 0.001$ versus controls) and in 50% ($P = 0.022$ versus controls) of animals, respectively. The difference between ampicillin and moxifloxacin was not statistically significant ($P = 0.128$).

Conclusions: Moxifloxacin was at least as effective as ampicillin in preventing streptococcal endocarditis.

Keywords: Moxifloxacin, streptococci, fluoroquinolones, rabbits, ampicillin

Introduction

Gram-positive cocci are the most frequent aetiologic agents, responsible for up to 80–90% of the cases, of infective endocarditis (IE). Amoxicillin and newer macrolides are the primary prophylactic regimens for most high risk patients.1 However, the frequency of resistance or reduced susceptibility to amoxicillin (11%),2 and resistance to macrolides (up to 55%) among some species of viridans group streptococci,3 raise concerns regarding their use as prophylactic agents for infections caused by these organisms. Moreover, the need for parenteral administration of glycopeptides is the main disadvantage of their use as prophylactic agents. Thus, new compounds (i) exhibiting no cross-allergy to 8-lactam antibiotics, (ii) having a low level of toxicity, (iii) being effective against streptococci and other Gram-positive cocci as well, and (iv) having an oral route of administration and a prolonged half-life in the serum, are warranted.

Moxifloxacin is active in vitro against Gram-positive bacteria. It is also exhibits a long half-life (9–15 h), and good oral bioavailability while it is well tolerated when administered orally as a single dose in adults.4 Thus, it could be eligible as a prophylactic agent against IE.

This study was designed to evaluate the prophylactic efficacy of moxifloxacin against a strain of Streptococcus oralis, by applying the rabbit model.

Materials and methods

Microorganism

The strain of S. oralis used in this study was isolated from the blood of a patient with endocarditis and was identified by standard methods. The bacteria were stored at –80°C in skimmed milk and were subcultured on blood agar plates (BAPs) 3 days before each experiment.

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Moxifloxacin as endocarditis prophylaxis

Susceptibility testing

The MICs of penicillin, ampicillin and moxifloxacin were determined by a microdilution technique in volumes of 0.1 mL, by using logarithmic-growth-phase inocula of *S. oralis* in Todd–Hewitt broth (BBL Microbiology Systems, Cockeysville, MD, USA) adjusted to a final inoculum of ~5 × 10⁷ cfu/mL. The MIC was defined as the lowest concentration causing no visible turbidity after incubation for 18 h at 37°C. The MBC of moxifloxacin was determined by subculture of 0.1 mL from each well well onto BAPs and was defined as the lowest concentration that reduced the number of organisms of the initial inoculum, by ≥99.9%.

Induction and prophylaxis of endocarditis

For the production of non-bacterial thrombotic endocarditis of the aortic valve, the model described by Perlman and Freedman was applied. Female white rabbits weighing 3.0 to 3.9 kg were anesthetized by intramuscular injection of ketamine hydrochloride (15 mg/kg of body weight). The left carotid artery was exposed in the neck and was cannulated with a polyethylene catheter (22Fr). The tip of the catheter was placed across the aortic valve into the left ventricle, and the proximal end was then secured in place in the neck for the duration of the experiment. Twenty-four hours after catheterization, the rabbits were randomly assigned to a control group, a group receiving moxifloxacin (kindly provided by Bayer Hellas) at a single dose of 15 mg per kg of body weight intravenously, and a group receiving two doses of 40 mg per kg of body weight of ampicillin (supplied by Bristol-Myers Squibb), intravenously, 2 h apart. The dose of moxifloxacin was chosen because in pilot studies the achieved peak serum levels in rabbits were similar to those in humans after a single oral dose of 400 mg. The dose of ampicillin was chosen because it has been used in previous studies in endocarditis prophylaxis. Animals treated with ampicillin or moxifloxacin were challenged 0.5 h or 1 h later, respectively, with an inoculum of ~10⁷ cfu of *S. oralis*. This inoculum was suspended in 1 mL of saline and injected via the marginal ear vein. The rabbits were sacrificed 3 days after bacterial challenge, by a rapid intravenous injection of 30 mg of sodium phenobarbital per kg, in order to avoid the carryover effect, since no detectable levels of moxifloxacin were expected to exist 72 h after their administration. Use of this time interval could also allow for the detection of any possible relapses due to the regrowth of persistent, viable bacteria in vegetation, after the complete elimination of antibiotics from the body. At the time of sacrifice, aortic valve vegetations were excised, weighed, homogenized in 1 mL of saline and quantitatively cultured in duplicate, onto BAPs, after eight dilutions with a 1 log inoculum difference between each dilution. The colonies were counted after incubation for 24 h at 37°C in room air with 5% CO₂. The results were expressed as the log₁₀ numbers of cfu per gram of vegetation. The macroscopic and/or bacteriological data obtained at the time of sacrifice provided confirmation of the successful induction of vegetative endocarditis. Rabbits with sterile vegetations were considered uninfected. The study received a permit from the veterinary directorate of the prefecture of Athens according to Greek legislation in conformance with the council directive of the EU.

Antibiotic concentrations in serum

Moxifloxacin levels were determined in serum samples obtained at 1, 2, 4, 8 and 24 h post-dosing. Ampicillin levels were determined in serum samples at 0.5, 1 and 2 h after the first dose and 2 h after the second dose. An agar well bioassay technique was applied. *Bacillus subtilis* ATCC 6633 was used as the test organism for moxifloxacin and *Micrococcus luteus* for ampicillin, and normal rabbit serum was used as the diluent. The lower limit of detection of this assay was 0.15 mg/L.

Statistical analysis

To compare the differences between sterile and non-sterile vegetations, the Fisher exact test for probabilities was used. To compare the differences between the mean log₁₀ cfu per gram of non-sterile vegetations, the Kruskal–Wallis test was used. A *P* value of <0.05 was considered significant.

Results

The MICs of penicillin, ampicillin and moxifloxacin for the strain of *S. oralis* were <0.03, 0.025 and <0.03 mg/L, respectively. The MBC of moxifloxacin was 0.03 mg/L.

The mean ± standard deviation (mean ± SD) serum concentrations of moxifloxacin at 1, 2, 4, 8 and 24 h post-dosing were: 2.8 ± 0.7 mg/L (*n* = 7), 2.1 ± 0.7 mg/L (*n* = 8), 1.1 ± 0.5 mg/L (*n* = 7), 0.3 ± 0.1 mg/L (*n* = 8) and undetectable, respectively. The mean ± SD (*n* = 15) concentrations of ampicillin in serum 0.5, 1 and 2 h after the first dose and 2 h after the second dose were: 9.56 ± 3.72 mg/L, 3.24 ± 1.19 mg/L, 0.58 ± 0.48 mg/L (in four animals the concentrations were below the level of detection) and 0.73 ± 0.47 mg/L, respectively.

The results of prophylaxis against the strain of *S. oralis* are presented in Table 1. Sixteen out of 18 (89%) of the untreated animals developed infected vegetations. Moxifloxacin prevented endocarditis in 12 out of 15 (80%) animals (*P* < 0.001 versus controls). Ampicillin prevented endocarditis in 7 out of 14 (50%) animals (*P* = 0.022 versus controls, *P* = 0.128 versus moxifloxacin). One animal in the ampicillin group was excluded because of incorrect placement of the catheter. The mean ± SD bacterial densities in vegetations from rabbits infected despite prophylaxis with moxifloxacin (8.38 ± 0.77 log₁₀ cfu/g) or ampicillin (7.78 ± 2.07 log₁₀ cfu/g) were not statistically different from those found in control animals (9.21 ± 0.94 log₁₀ cfu/g).

Discussion

The *in vivo* efficacy of the newer fluoroquinolones has been assessed in various experimental models. However, there are few experimental studies in which a fluoroquinolone was used successfully as prophylaxis against IE. Using a rat model, Voorn et al. studied ciprofloxacin against a cloxacillin-tolerant strain of *Staphylococcus aureus* and its non-tolerant variant at two different doses (6 and 30 mg/kg). The higher dose afforded almost full protection.

Table 1. Results of prophylaxis with moxifloxacin in rabbits challenged with *S. oralis*

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of vegetations [sterile/total (%)]</th>
<th>Log₁₀ cfu/g of non-sterile vegetations (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No prophylaxis</td>
<td>2/18 (11)</td>
<td>9.21 ± 0.94 (n = 16)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>7/14* (50)</td>
<td>7.78 ± 2.07 (n = 7)</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>12/15* (80)</td>
<td>8.38 ± 0.77 (n = 3)</td>
</tr>
</tbody>
</table>

*Significantly different from the value obtained for the control group (*P* = 0.022).

*Significantly different from the value obtained for the control group (*P* < 0.001).
protection against both strains, whereas the lower dose had a significantly lower protective effect. Katsarolis et al.\(^8\) reported successful prophylaxis (in 100% of the animals) with trovafloxacin, against experimental aortic valve endocarditis due to an ampicillin-tolerant but trovafloxacin-susceptible strain of \textit{S. oralis}. However, the necessity for a second dose of trovafloxacin, despite the fact that supra-MIC levels persisted in serum for close to 18 h after the administration of a single dose, points out substantial weaknesses of trovafloxacin as a potential prophylactic agent. Sparfloxacin has also been used successfully as a prophylactic agent against experimental staphylococcal and enterococcal endocarditis, but the results against streptococcal endocarditis were disappointing.\(^9\)

In this study, a single dose of moxifloxacin was effective as prophylaxis against experimental aortic valve endocarditis, in rabbits challenged with an inoculum of \textit{S. oralis} corresponding to the ID\(_{90}\). As stated by Moreillon and the Swiss Working Group for Endocarditis Prophylaxis,\(^10\) the duration of antibiotic presence in the serum is critical. Under experimental conditions, the drugs must remain above their MIC for the organisms for \(\geq 10\) h, to allow time for bacterial clearance from the valves. The concentrations of moxifloxacin in the serum were sustained at supra-MIC levels for more than 24 h after the administration of the drug, and this fact seems to be the most plausible explanation for the prophylactic efficacy of moxifloxacin.

In conclusion, in this study, moxifloxacin was effective as prophylaxis against endocarditis caused by a strain of \textit{S. oralis}. Its prophylactic efficacy was at least equivalent to that of ampicillin, and thus could be considered as an alternative to standard regimens for the prophylaxis of endocarditis, especially in patients with a history of allergy to \(\beta\)-lactams.

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Transparency declarations

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References