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Successful Trovafloxacin Prophylaxis against Experimental Streptococcal Aortic Valve Endocarditis

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Single-dose trovafloxacin (15 mg/kg given intravenously [i.v.]) and ampicillin (40 mg/kg given i.v.) protected 38 and 33% of animals challenged with an ampicillin-tolerant strain of *Streptococcus oralis*, respectively. As a double-dose regimen, trovafloxacin afforded total protection (100%; $P < 0.001$ versus controls). Trovafloxacin is the first fluoroquinolone effective in preventing experimental streptococcal endocarditis.

Trovafloxacin is highly active in vitro against a wide range of gram-positive bacteria (2, 3). It also exhibits a long half-life (11 h) and good oral bioavailability, and it is well tolerated when administered orally as a single dose (11). Thus, it could be eligible as a prophylactic agent against infective endocarditis (IE). This study was designed to evaluate the prophylactic efficacy of trovafloxacin against the most common etiologic agents responsible for the development of IE, namely, viridans group streptococci, by applying the rabbit model.

The strain of *Streptococcus oralis* used in this study was isolated from the blood of a patient with endocarditis and was identified by standard methods. MICs and MBCs of trovafloxacin (supplied by Pfizer Inc., New York, N.Y.) and ampicillin (supplied by the commercial route) were determined by a microdilution technique in a volume of 0.1 ml. Nonbacterial thrombotic endocarditis of the aortic valve was induced in female White rabbits weighing approximately 3.0 kg by using the model described by Perlman and Freedman, and the polyethylene catheter was left in place throughout the experiment (10). Twenty-four hours after catheterization, rabbits were randomly assigned to a control group, a group receiving trovafloxacin at a single dose of 15 mg/kg of body weight intravenously (i.v.), a group receiving two i.v. doses of trovafloxacin (15 mg/kg of body weight each) 7 h apart, and a group receiving ampicillin at a single dose of 40 mg/kg of body weight i.v. The dose of trovafloxacin was chosen because in pilot studies the peak levels achieved in the serum of rabbits were similar to those obtained in humans 1 h after administration of a single dose of 300 mg per os (11). This dose of ampicillin was chosen because it has been used in previous studies of endocarditis prophylaxis, as it mimics the peak levels in human serum (18 mg/liter) following administration of a 3-g oral dose of amoxicillin (1, 7, 8). Animals treated with trovafloxacin and animals treated with ampicillin were challenged 1 h and 0.5 h later, respectively, with an inoculum of $\sim 10^7$ CFU of *S. oralis*. The inocula were suspended in 1 ml of saline and injected via the marginal ear vein. The rabbits were sacrificed 3 days (72 h) after bacterial challenge. The processing of vegetations and criteria for IE were described previously (9). Trovafloxacin levels were determined in serum samples obtained at 1, 1.5, 3, 7, and 24 h postdosing. Additionally, for the animals receiving two doses of trovafloxacin, the levels in serum at 2 and 7 h after the infusion of the second dose were determined. Ampicillin levels were determined in serum samples obtained at 0.5 and 1 h postdosing. An agar well bioassay technique was applied. *Bacillus subtilis* ATCC 6633 was used as the test organism for trovafloxacin, and *Micrococcus luteus* was used for ampicillin. Normal rabbit serum was used as the diluent. The lower limit of detection of this assay was 0.1 μg/ml for both antibiotics. To compare the differences between sterile (successful prophylaxis) and nonsterile vegetations, the Fisher exact test for probabilities was used. To compare the differences between vegetations in the mean $\log_{10}$ number of CFU per gram, the Kruskal-Wallis test was applied. A P value of <0.05 was considered significant.

The MICs and MBCs of trovafloxacin and ampicillin and the results of prophylaxis against *S. oralis* are presented in Table 1. The strain used in this study was tolerant to ampicillin. The concentrations of trovafloxacin and ampicillin in rabbit serum, as well as pharmacokinetic data concerning trovafloxacin, are presented in Table 2. All of the control animals challenged with 10^7 CFU of *S. oralis* developed infected vegetations. In rabbits challenged with this very large inoculum, trovafloxacin administered twice completely prevented endocarditis ($P < 0.001$ versus controls). When administered as a single dose, it prevented endocarditis in 5 out of 13 rabbits (38.5%; $P = 0.039$ versus controls), a rate similar to that of ampicillin given as a single dose (33.3%; $P = 0.093$ versus controls). However, this dose of ampicillin is considered suboptimal since the time required to reach the MBC was less than 1 h. The mean number of CFU per gram of vegetation in the animals that failed to respond to the single-dose trovafloxacin or ampicillin prophylaxis regimen was lower than that of the control group ($P < 0.001$ and $P = 0.028$, respectively).

In the present study, trovafloxacin administered as a single or a double dose was evaluated as a chemoprophylactic agent against streptococcal endocarditis and was compared to ampicillin. Both failed to prevent IE as single-dose regimens. When trovafloxacin was administered in two doses, it displayed its best results.

Previous observations have indicated that the number of bacteria adhering to the surface of vegetations after challenge is related to the size of the inoculum used. In such a case, sustained bacteriostatic levels in blood are required to successfully prevent endocarditis due to tolerant strains, probably by

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TABLE 1. Results of prophylaxis with ampicillin or trovafloxacin in rabbits challenged with *S. oralis*

Regimen	MIC/MBC (μg/ml)	No. of protected animals/ total no. (%)	Mean $\log_{10}$ no. of CFU/g of vegetation of infected animals ± SD[e]
Control		0/12 (0)	7.10 ± 1.14
Ampicillin (40 mg/kg, 1 dose)	0.25/8	4/12 (33.3)[a]	5.17 ± 2.61[d]
Trovafloxacin (15 mg/kg, 1 dose)	0.001/0.001	5/13 (38.5)[b]	4.19 ± 1.96[c]
Trovafloxacin (15 mg/kg, 2 doses)		10/10 (100)[c]	

[a] $P = 0.093$.
[b] $P = 0.039$.
[c] $P < 0.001$.
[d] $P = 0.028$.
[e] Lower limit of detection, 2 $\log_{10}$ CFU/g of vegetation.-

allowing adherent organisms to be cleared from the vegetations. The longer the duration of growth inhibition, the greater the likelihood of successful prophylaxis, even after challenge with inocula exceeding the 90% infective dose (ID_{90}) (7, 8).

In a pharmacokinetic study with rabbits and ^{18}F-labeled trovafloxacin (5), it was apparent that in most tissues, high accumulation was achieved within 20 min after the injection and decreased over the next 100 min. In normal and infected rabbits, the decline of the trovafloxacin level in blood was well described by biexponential functions. The same observations were also applicable to humans (6). In our study, trovafloxacin had to be administered in a double-dose regimen in order to protect all of the rabbits from endocarditis. In vitro, although bactericidal, trovafloxacin did not display a rapid killing rate (data not shown). Moreover, it was characterized by a rapid rate of elimination from rabbit serum and poor distribution in the vegetation at the time of inoculation, 7 h later (time of the trough levels in serum), and 1 h after administration of the second dose (data from our study not shown here but also reference 4). A single dose was not adequate, although for a long period after administration, supra-MBCs could be detected in serum. Presumably, after initial endovascular killing of an amount of challenging bacteria, sustained supra-MBCs had to be present in serum for a long period of time in order to sterilize vegetations from adhering bacteria. In preliminary studies of ours, trovafloxacin given as a single dose of 15 mg/kg was used in a model of endocarditis against an inoculum approximately 100 times the ID_{90}, where it failed to confer any protection at all and the bacterial densities of the infected vegetations were similar to those of control animals. When we lowered the administered dose to 10 mg/kg but preserved the inoculum size at the ID_{90}, the results were also disappointing. These two facts imply that the efficacy of trovafloxacin is most likely dose and inoculum dependent. In a therapeutic model of streptococcal endocarditis (4), trovafloxacin, at a dose simulating the levels in human serum following administration of an oral dose of 200 mg, significantly reduced bacterial counts in vegetations but was less effective than the control drug (ceftriaxone). The authors suggested that optimal treatment might require higher doses of the drug. This study stresses the fact that optimistic in vitro susceptibility test results do not always predict therapeutic results.

To our knowledge, this is the first experimental study in which a fluoroquinolone was used successfully in the prophylaxis of streptococcal endocarditis. There is only one study in

TABLE 2. Concentrations in serum and pharmacokinetic parameters of trovafloxacin or ampicillin in rabbits

Regimen	Mean concn (μg/ml) in serum ± SD (*n*) at indicated time (h) postdosing							Value of following pharmacokinetic parameters[d] in serum:			
	0.5	1	1.5	3	7	9[a]	14[b]	k_{el} (h^{-1})	$t_{1/2}$ (h)	$AUC_{0-\infty 24}$ (μg · h/ml)	*t*>MIC (h)
Trovafloxacin[c] (15 mg/kg i.v.)	ND[e]	5.17 ± 1.97 (17)	4.6 ± 1.26 (6)	1.06 ± 0.38 (5)	0.36 ± 0.26 (8)	4.44 ± 1.36 (4)	0.60 ± 0.36 (3)	0.45	1.54	10.32	~18
Ampicillin (40 mg/kg i.v.)	18.0 ± 6.4 (12)	6.2 ± 4.1 (5)	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] At 2 h after administration of a second dose of 15 mg/kg i.v.
[b] At 7 h after administration of a second dose of 15 mg/kg i.v.
[c] No trovafloxacin was detectable in serum 24 h after administration of a single dose of 15 mg/kg i.v.
[d] Maximum concentration of drug in serum = 5.17 μg/ml; time to maximum concentration of drug in serum = 1 h; k_{el}, elimination rate constant; $t_{1/2}$, half-life; $AUC_{0-\infty 24}$, area under the concentration-time curve from 0 to 24 h; *t*>MIC, time required to reach MIC.
[e] ND, not done.

the literature in which a fluoroquinolone (ciprofloxacin) was successfully used in the prophylaxis of endocarditis (12) due to a cloxacillin-tolerant strain of *Staphylococcus aureus* and a non-tolerant variant of it at two different doses (6 and 30 mg/kg). The larger dose afforded almost full protection against both strains, while the smaller dose had a significantly weaker protective effect.

In conclusion, trovafloxacin was proved effective in preventing streptococcal endocarditis. However, the restriction of its use due to severe hepatotoxicity and the necessity of a second dose, despite the fact that supra-MICs persisted in serum for close to 18 h after the administration of a single dose, point out substantial weaknesses of trovafloxacin as a potential prophylactic agent.

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