

A Mucoadhesive Polymer Extracted from Tamarind Seed Improves the Intraocular Penetration and Efficacy of Rufloxacin in Topical Treatment of Experimental Bacterial Keratitis

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Bacterial keratitis is a serious infectious ocular disease requiring prompt treatment to prevent frequent and severe visual disabilities. Standard treatment of bacterial keratitis includes topical administration of concentrated antibiotic solutions repeated at frequent intervals in order to reach sufficiently high drug levels in the corneal tissue to inhibit bacterial growth. However, this regimen has been associated with toxicity to the corneal epithelium and requires patient hospitalization. In the present study, a mucoadhesive polymer extracted from tamarind seeds was used for ocular delivery of 0.3% rufloxacin in the treatment of experimental *Pseudomonas aeruginosa* and *Staphylococcus aureus* keratitis in rabbits. The polysaccharide significantly increased the intra-aqueous penetration of rufloxacin in both infected and uninfected eyes. Rufloxacin delivered by the polysaccharide reduced *P. aeruginosa* and *S. aureus* in the cornea at a higher rate than that obtained by rufloxacin alone. In particular, use of the polysaccharide allowed a substantial reduction of *S. aureus* in the cornea to be achieved even when the time interval between drug administrations was extended. These results suggest that the tamarind seed polysaccharide prolongs the precorneal residence times of antibiotics and enhances drug accumulation in the cornea, probably by reducing the washout of topically administered drugs. The tamarind seed polysaccharide appears to be a promising candidate as a vehicle for the topical treatment of bacterial keratitis.

The eye is a unique organ that is virtually impermeable to most environmental agents. Continuous tear flow, aided by the blink reflex, mechanically washes substances from the ocular surface and prevents the accumulation of microorganisms. In addition, lysozyme, lactoferrin, secretory immunoglobulins, and defensins are present at high levels in tears and can specifically reduce bacterial colonization of the ocular surface (16, 23).

Since most pathogens cannot penetrate the intact corneal layer, corneal infections derive essentially from a failure of the protective mechanisms that maintain ocular surface integrity. Defects in the tear film, chemical or foreign body trauma, allergic hypersensitivity reactions, and overuse of contact lenses, as well as complications after laser in situ keratomileusis, can result in injury to the ocular surface and predispose the cornea to infection (14, 21).

Because of its high incidence and potential complications, bacterial keratitis is one of the most threatening ocular infections. *Pseudomonas aeruginosa* and *Staphylococcus aureus* frequently cause severe keratitis that may lead to progressive destruction of the corneal epithelium and stroma (2, 3). Infectious keratitis due to these organisms often causes corneal scarring, corneal perforation, and blindness if aggressive and appropriate therapy is not promptly initiated (7, 18).

Successful therapy of bacterial keratitis must be able to rapidly attain high drug concentrations at the site of infection. Since the cornea is not vascularized, it is not readily permeated by systemically administered drugs, which are therefore generally not used for the treatment of keratitis (7). On the other hand, topical treatment may fail to achieve therapeutically active drug levels in the cornea, as continuous tear flow reduces the bioavailability of topically applied antibiotics and the corneal epithelium acts as a barrier against drug penetration. For this reason, standard treatment of severe bacterial keratitis requires administration at frequent intervals (every 15 to 60 min for 48 to 72 h) of eyedrops often containing fortified (more concentrated than commercially available solutions) solutions of fluoroquinolones or multiple antibiotics, usually a cephalosporin and an aminoglycoside (7, 13, 28, 29). However, this regimen not only is disruptive to the patient and usually necessitates hospitalization, but it has also been associated with in vitro toxicity to the corneal epithelium (9, 22). Efforts are now directed to testing new antimicrobials that better permeate the cornea and to developing systems capable of prolonging the contact time between antibiotics and the corneal tissue, thereby potentially enhancing intracorneal delivery of ophthalmic medicaments.

A mucoadhesive polymer extracted from tamarind seeds (xyloglucan, or tamarind seed polysaccharide [TSP]) has been described as a viscosity enhancer showing mucomimetic, mucoadhesive, and bioadhesive activities (M. F. Saettone, S. Burgalassi, E. Boldrini, P. Bianchini, and G. Luciani, 1997, international patent application PCT/IT97/00026). Several features make TSP an attractive candidate as a vehicle for ophthalmic

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medicaments, since it (i) is completely devoid of ocular toxicity (4; Saettone et al., patent application); (ii) has recently been put on the market (TSP; Farmigea S.p.A., Pisa, Italy) as a tear fluid substitute because of its activity in preventing alterations of the corneal surface known as keratoconjunctivitis sicca (4); (iii) increases the corneal-wound healing rate (5); (vi) reduces the in vitro toxicity exerted by timolol, methiolate, and fluoroquinolones on human conjunctival cells (27); and (v) significantly increases the corneal accumulation and intraocular penetration of gentamicin and ofloxacin when administered topically to healthy rabbits (15).

In this study, we used TSP for ocular delivery of rifloxacin, a broad-spectrum monofluorinated quinolone that has never been tested for its effectiveness in the topical treatment of bacterial keratitis. Rifloxacin is administered orally and displays a long half-life in plasma (28 to 30 h), consistently high bactericidal concentrations at the site of infection, and good penetration into infected tissues and cerebrospinal fluid (24, 31). To evaluate the intraocular penetration and therapeutic benefit of rifloxacin, whether delivered by TSP or not, an experimental model of *P. aeruginosa* and *S. aureus* keratitis in rabbits was developed; the drug efficacy was then compared with that of ofloxacin, which is commonly used for the topical therapy of this ocular pathology (13, 26, 29).

MATERIALS AND METHODS

Strains used and preparation of inocula. *P. aeruginosa* ($n = 10$) and *S. aureus* ($n = 10$) clinical isolates, recovered from patients with ocular infections, were identified using the Vitek2 system (BioMérieux, Paris, France). The strains were used to calculate the MICs of rifloxacin and ofloxacin, which were compared to those obtained with *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213. The last two strains were used to induce experimental bacterial keratitis in rabbits. To this end, inocula for each organism were prepared by propagating cultures in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 5 h. Bacteria were suspended in phosphate-buffered saline (PBS; pH 7.4) to a concentration of $\sim 1.5 \times 10^4$ CFU/ml. Retrospective quantitation of inocula was done by plating triplicate serial 10-fold dilutions on tryptic soy agar (Difco Laboratories). *Bacillus subtilis* ATCC 6633 was used as the indicator organism for determining rifloxacin and ofloxacin concentrations in biological samples by an agar diffusion assay (15). *B. subtilis* spores were prepared in 0.1 M PBS, pH 8.0, and stored at 4°C as sterile suspensions.

Drug formulations. Sterile water solutions of rifloxacin (Mediolanum Farmaceutici, Milan, Italy) or ofloxacin (Sigma, St. Louis, Mo.) contained mannitol (50 mg/ml; Sigma), benzalkonium chloride (0.05 mg/ml; A.C.E.F., Milan, Italy), and rifloxacin or ofloxacin (3 mg/ml). The viscous preparation of rifloxacin (TSP-rifloxacin) was obtained by adding 10 mg of TSP (Farmigea S.p.A./ml) to the rifloxacin-water solution. A 10-mg/ml TSP solution containing the excipients (50 mg of mannitol/ml and 0.05 mg of benzalkonium chloride/ml) was used as a control.

Experimental keratitis and therapeutic regimen. Male New Zealand rabbits (body weight, 2.3 to 2.7 kg), free from preexisting corneal defects, were anesthetized by intramuscular injection of 35 mg of ketamine-HCl per kg of body weight and by topical application in each eye of 0.4% oxibuprocaine HCl (Novesina; Mipharm, Milan, Italy). Bacterial suspensions (20 μ l) were injected intrastromally in both eyes via a 30-gauge needle attached to a 1.0-ml syringe. Three animals were injected with 20 μ l of sterile PBS in one eye (surgical control), while the contralateral eye was left undisturbed (absolute control). Eye disease was evaluated by slit lamp biomicroscopy, and inflammation was scored as described by Johnson et al. (19). Briefly, conjunctival injection, conjunctival chemosis, iritis, fibrin in the anterior chamber, hypopyon, stromal infiltrate, and stromal edema were scored on a scale from 0 (absent) to 4 (severe). For each eye, individual parameter scores were summed to give a total slit lamp examination (SLE) score ranging from 0 (normal eye) to 28 (maximally inflamed eye). Infected animals were assigned randomly to the treatment groups or left untreated (five rabbits in each group). At 16 h postinfection, rabbits included in the treatment groups were subjected to instillation of 50- μ l drops of rifloxacin, TSP-rifloxacin, ofloxacin, or TSP solution in the lower conjunctival sacs of both

eyes. Animals infected with *P. aeruginosa* received one drop in the eye every 30 min for 6 h (treatment A). Three different treatment schedules were followed for rabbits infected with *S. aureus*: administration of one drop in the eye every 30 min for 6 h (treatment A), every 90 min for 6 h (treatment B), or every 90 min for 3 h (treatment C). To evaluate antibiotic penetration in the aqueous humors of uninfected eyes, groups of five healthy rabbits were subjected to instillation of rifloxacin, TSP-rifloxacin, or ofloxacin in both eyes following treatment A. At fixed time intervals after the last administration (1 h after treatment A; 2 h after treatment B or C), the infected and uninfected animals were killed by injecting an overdose of ethyl urethane into the marginal ear vein. Aqueous humor was recovered from the anterior chamber by paracentesis. The eyes were rapidly enucleated, trimmed of all adventitial tissue, and rinsed with PBS. The corneas were dissected away, weighed, suspended in PBS (25 mg of tissue/ml), and homogenized with 1.0-mm-diameter glass beads in a Mini-BeadBeater (Biospec Products, Bartlesville, Okla.) at 5,000 rpm for 30 s. The study was approved by the Ethics Committee of Pisa University Hospital.

Microbiological determinations. The MICs of rifloxacin, TSP-rifloxacin, and ofloxacin against *P. aeruginosa* and *S. aureus* strains were determined by the microdilution technique (25) with microtiter plates. In vitro susceptibility testing was performed five times for each strain, and modal MICs for each strain were calculated. Bacterial strains were also assayed for susceptibility to the TSP solution.

To determine rifloxacin and ofloxacin levels in aqueous humor samples, *B. subtilis* spores were incorporated into molten Antibiotic Medium No. 2 (Difco), and the assay was performed as described previously (15). Calibration curves were constructed by adding from 0.1 to 100 μ g of rifloxacin or ofloxacin/ml to pooled aqueous humor samples taken from untreated healthy rabbits. The assays were shown to be linear in the range of 1.6 to 15 μ g/ml for rifloxacin and 0.2 to 10 μ g/ml for ofloxacin. Each determination was performed in triplicate.

Bacterial CFU were quantified in corneal homogenates by plating triplicate serial 10-fold dilutions on tryptic soy agar and incubating them at 37°C for 18 h.

Statistical analysis. All values were expressed as the mean \pm standard error of the mean. Statistical analysis was performed using the two-tailed Student *t* test. A *P* value of ≤ 0.05 was considered significant.

RESULTS

Antibacterial activity in vitro. Ofloxacin and rifloxacin were found to be similarly active against the *S. aureus* strains analyzed, showing a MIC range of 0.125 to 4 and 0.5 to 4 μ g/ml, respectively. In contrast, ofloxacin was more active than rifloxacin against *P. aeruginosa* in vitro, with respective MIC ranges of 1 to 4 and 4 to 32 μ g/ml. Modal MICs were 2 μ g of rifloxacin or ofloxacin per ml for *S. aureus* ATCC 29213 and 8 μ g of rifloxacin and 2 μ g of ofloxacin per ml for *P. aeruginosa* ATCC 27853. Identical MICs of rifloxacin and TSP-rifloxacin were obtained against all microorganisms tested, indicating that the drug maintains its activity in vitro when made viscous with TSP. The TSP solution exerted no inhibitory activity against any of the organisms tested.

Bacterial keratitis in rabbits. Experimental infections were initiated by injection of the following inocula (\log_{10}): *P. aeruginosa*, 2.51 ± 0.06 ; *S. aureus*, 2.49 ± 0.04 . Sham-injected and uninjected control eyes did not develop appreciable signs of inflammation. *S. aureus*-infected eyes showed moderate to severe chemosis and iritis, mild conjunctival injection, and trace amounts of fibrin in the anterior chamber (SLE score, 11.32 ± 0.60) 16 h postinfection. At a later stage (23 h postinfection), the eyes showed moderate conjunctival injection, mild stromal edema, severe chemosis and iritis, and moderate to severe fibrin accumulation in the anterior chamber (SLE score, 15.07 ± 0.85). Experimental *P. aeruginosa* keratitis appeared to evolve more slowly than that induced by *S. aureus*. Essentially, mild to moderate chemosis and iritis and trace to moderate conjunctival injection and stromal edema were observed at 16

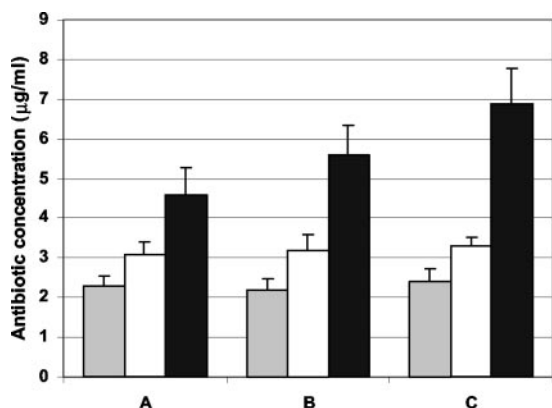


FIG. 1. Antibiotic concentrations in aqueous humor samples following topical administration of 50 µl of ofloxacin (shaded bars), rifloxacin (open bars), and TSP-rifloxacin (solid bars) every 30 min for 6 h in uninfected (A), *P. aeruginosa*-infected (B), and *S. aureus*-infected (C) rabbit eyes. The error bars indicate standard errors of the mean.

(SLE score, 7.43 ± 0.24) and 23 h (SLE score, 9.15 ± 0.97) postinfection.

Ocular penetration of antibiotics. Antibiotic concentrations were measured in the aqueous humors taken from both uninfected and infected eyes following treatment A (Fig. 1). When administered alone, rifloxacin tended to permeate the cornea better than ofloxacin; the average rifloxacin concentration in the aqueous humor was significantly higher than that of ofloxacin in uninfected (3.14 ± 0.32 versus 2.31 ± 0.26 µg/ml), *P. aeruginosa*-infected (3.25 ± 0.40 versus 2.24 ± 0.26 µg/ml), and *S. aureus*-infected (3.33 ± 0.21 versus 2.68 ± 0.33 µg/ml) eyes ($P < 0.05$). Administration of TSP-rifloxacin gave rise to intraocular drug levels significantly higher than those obtained with rifloxacin alone, as shown by values recorded in uninfected (4.62 ± 0.68 µg/ml; $P < 0.01$), *P. aeruginosa*-infected (5.63 ± 0.73 µg/ml; $P < 0.01$), and *S. aureus*-infected (6.91 ± 0.89 µg/ml; $P < 0.001$) eyes. In addition, it was considered of some relevance that, while comparable rifloxacin or ofloxacin levels were observed in the aqueous humors of infected and uninfected eyes treated with the drugs alone, a significantly higher concentration of rifloxacin was found in the aqueous humors of infected versus uninfected eyes when rifloxacin was administered in association with TSP (uninfected versus *P. aeruginosa*-infected eyes, $P < 0.05$; uninfected versus *S. aureus*-infected eyes, $P < 0.01$).

Therapeutic treatment of *P. aeruginosa* keratitis. Based upon its in vitro activity, rifloxacin is regarded as an inappropriate antibiotic in the treatment of infections by some frequently encountered species of *Enterobacteriaceae* and *P. aeruginosa* (30). In our study, the MIC of rifloxacin (8 µg/ml) was considerably higher than that of ofloxacin (2 µg/ml) for *P. aeruginosa* ATCC 27853. The poor rifloxacin activity against *P. aeruginosa* was exploited to evaluate whether TSP would improve its therapeutic efficacy in the experimental model of *P. aeruginosa* keratitis. Animals infected with *P. aeruginosa* were subjected to ocular instillation of rifloxacin, TSP-rifloxacin, ofloxacin, or TSP solution following treatment A, and their corneas were removed 23 h after infection. At this time, the SLE scores for the eyes treated with rifloxacin (3.28 ± 0.66),

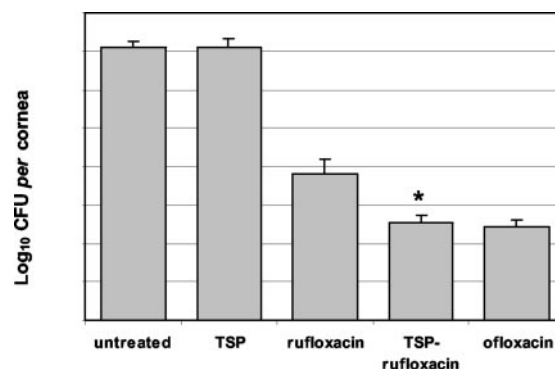


FIG. 2. Quantification of *P. aeruginosa* in the cornea following administration to infected eyes of 50 µl of TSP solution, rifloxacin, TSP-rifloxacin, and ofloxacin every 30 min for 6 h. Untreated control eyes did not receive any treatment. The error bars indicate standard errors of the mean. *, $P < 0.05$.

TSP-rifloxacin (2.91 ± 0.48), or ofloxacin (3.11 ± 0.75) were statistically lower than the scores for the *P. aeruginosa*-infected eyes that did not receive any treatment ($P < 0.05$) or that were treated with the TSP solution (8.72 ± 0.84) ($P < 0.05$). The untreated eyes harbored an average level of 1.3×10^7 CFU of *P. aeruginosa* in the cornea. A similar bacterial burden was recorded in corneas taken from animals treated with the TSP solution ($1.5 \times 10^7 \pm 0.8 \times 10^7$ CFU/cornea) (Fig. 2), indicating that the polysaccharide exerted no antibacterial activity in vivo. All the antibiotic-treated eyes exhibited a significant decrease in the corneal *P. aeruginosa* burden compared to the untreated eyes (Fig. 2). Administration of rifloxacin, TSP-rifloxacin, and ofloxacin produced a significant reduction in corneal bacteria compared to that in untreated eyes (rifloxacin versus control, $P < 0.01$; TSP-rifloxacin and ofloxacin versus control, $P < 0.001$). TSP-rifloxacin was significantly more active than rifloxacin in controlling bacterial proliferation in the cornea ($3.4 \times 10^2 \pm 2.2 \times 10^2$ versus $6.3 \times 10^3 \pm 9.4 \times 10^3$ CFU/cornea; $P < 0.05$). In contrast, no relevant difference was detected between treatments with TSP-rifloxacin and ofloxacin ($3.4 \times 10^2 \pm 2.2 \times 10^2$ versus $2.7 \times 10^2 \pm 1.4 \times 10^2$ CFU/cornea; $P > 0.05$). Therefore, administration of rifloxacin in association with TSP allows an intracorneal drug concentration to be achieved that is sufficiently high to inhibit *P. aeruginosa* proliferation as effectively as ofloxacin, even though the MIC of rifloxacin against *P. aeruginosa* ATCC 27853 is four times higher than that of ofloxacin.

Therapeutic treatment of *S. aureus* keratitis. The comparable in vitro activities of rifloxacin and ofloxacin against *S. aureus* and the high frequency and severity of corneal infections due to the organism (2, 7) prompted us to examine whether TSP could improve the therapeutic efficacy of rifloxacin in an experimental model of *S. aureus* keratitis. At 16 h postinfection, *S. aureus*-infected eyes were subjected to instillation of rifloxacin, TSP-rifloxacin, ofloxacin, or TSP solution according to the treatment schedules A, B, and C. The animals were sacrificed, and their corneas were excised at 21 (treatment C), 23 (treatment A), and 24 h (treatment B). At these times, no difference between the SLE scores for untreated and TSP-treated eyes (mean values ranging from 13.5 to 15.7) was recorded. In contrast, at the end of treatment A, there was a

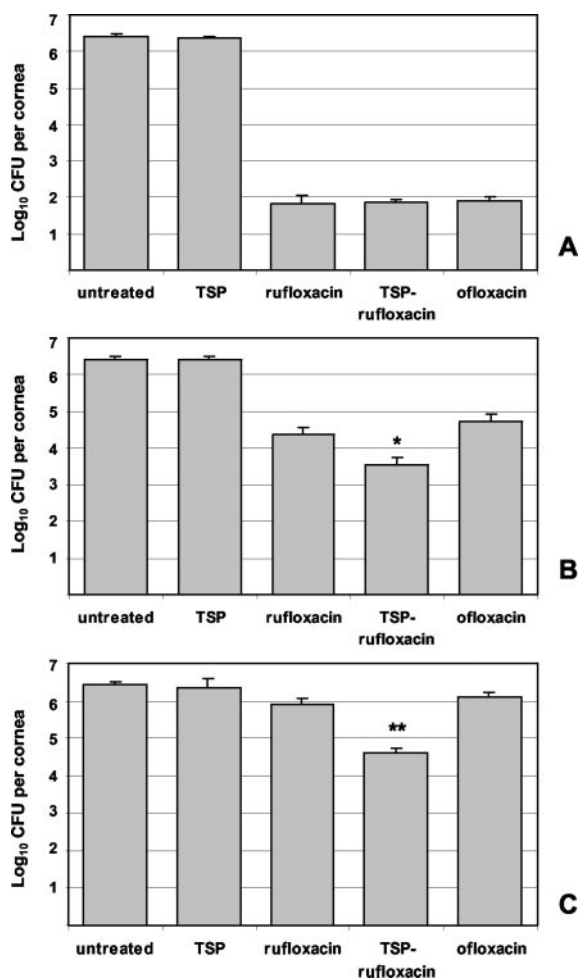


FIG. 3. Quantification of *S. aureus* in the cornea following administration to infected eyes of 50 μ l of TSP solution, rufloxacin, TSP-rufloxacin, and ofloxacin every 30 min for 6 h (A), every 90 min for 6 h (B), and every 90 min for 3 h (C). Untreated control eyes did not receive any treatment. The error bars indicate standard errors of the mean. *, $P < 0.05$; **, $P < 0.01$.

dramatic improvement in the clinical features of animals treated with rufloxacin (SLE score, 4.03 ± 0.43), TSP-rufloxacin (SLE score, 3.26 ± 0.24), or ofloxacin (SLE score, 4.51 ± 0.30) compared to untreated eyes ($P < 0.01$). Good improvement was also obtained following treatment B with rufloxacin (SLE score, 6.97 ± 0.60), TSP-rufloxacin (SLE score, 5.44 ± 0.24), and ofloxacin (SLE score, 7.52 ± 0.49) ($P < 0.05$). However, at the end of treatment C, only eyes treated with TSP-rufloxacin (SLE score, 7.42 ± 0.63) showed a significant reduction in the clinical signs of infection ($P < 0.05$).

Untreated and TSP-treated animals harbored comparable average levels of *S. aureus* in the cornea at 21, 23, and 24 h postinfection (values ranging from 2.3×10^6 to 3.1×10^6 CFU/cornea). Following treatment A or B, administration of rufloxacin, TSP-rufloxacin, and ofloxacin produced significant reductions in corneal bacteria compared to untreated eyes (treatment A, $P < 0.001$; treatment B, $P < 0.01$) (Fig. 3A and B). However, while no difference among the therapeutic efficacies of the three compounds was observed following treat-

ment A, administration of TSP-rufloxacin produced a significant reduction in corneal bacteria ($3.5 \times 10^3 \pm 2.0 \times 10^3$ CFU/cornea) compared to both rufloxacin-treated ($2.3 \times 10^4 \pm 1.3 \times 10^4$ CFU/cornea) and ofloxacin-treated ($5.2 \times 10^4 \pm 3.1 \times 10^4$ CFU/cornea) eyes following treatment B ($P < 0.05$) (Fig. 3B). Even more evident was the difference in efficacy of TSP-rufloxacin compared with rufloxacin and ofloxacin after the end of treatment C (Fig. 3C). Indeed, while eyes treated with rufloxacin ($8.1 \times 10^5 \pm 3.5 \times 10^5$ CFU/cornea) and ofloxacin ($1.3 \times 10^6 \pm 0.4 \times 10^6$ CFU/cornea) displayed no appreciable reduction in corneal bacteria compared to untreated eyes, a significant reduction was obtained following administration of TSP-rufloxacin ($4.1 \times 10^4 \pm 1.5 \times 10^4$ CFU/cornea) ($P < 0.01$). These results indicate that all the antibiotic formulations can reach high drug concentrations at the site of infection and efficiently control bacterial growth when administered at very frequent time intervals and for a prolonged period of time (i.e., treatment A, every 30 min for 6 h). In contrast, with longer time intervals between treatments (i.e., treatment B, every 90 min for 6 h) and less prolonged treatments (i.e., treatment C, every 90 min for 3 h), the use of TSP allows a more efficacious antibacterial effect to be obtained, possibly improving the precorneal residence time of the antibiotic and enhancing drug accumulation in the cornea.

DISCUSSION

Successful treatment of bacterial keratitis requires multiple administrations and/or fortified solutions of antibacterial agents to maintain drug concentrations in the corneal tissue high enough and for a sufficient period of time to have a useful antibacterial effect. For this reason, the interest in both new antibiotics, able to reach high concentrations in the cornea, and ocular drug delivery systems has grown over recent years.

Fluoroquinolones (particularly ciprofloxacin and ofloxacin) have become widely used as antibacterial agents in the treatment of ocular infections, with topical, intravitreal, and systemic routes of administration being used. Therapeutic concentrations are achieved in the cornea after topical administration, so that fluoroquinolones have largely replaced combination therapy for treatment of bacterial keratitis (26, 29). Compared to ciprofloxacin, however, ofloxacin reaches higher concentrations in the cornea and aqueous humor and is considered the primary choice for treatment of this ocular infection (1, 12). In the present study, assessment of the intraocular penetration of rufloxacin following topical administration of a 0.3% solution demonstrated that the drug reaches significantly higher levels in the aqueous humor than ofloxacin in both infected and uninfected eyes. This finding suggests that rufloxacin can be at least as efficacious as ofloxacin in the topical therapy of ocular infections caused by bacteria that are similarly susceptible to the two drugs.

Several systems have been developed for ocular delivery of antimicrobials (6, 8, 10, 11). Among these, bioadhesive polymers, usually consisting of macromolecular hydrocolloids with numerous hydrophilic functional groups, are known to adhere to the precorneal mucin layer via noncovalent bonds. Consequently, they rely on mucoadhesion as the primary mechanism for prolonging the residence of ocular dosage forms in the precorneal area (10). TSP is a high-molecular-weight, non-

ionic, neutral, branched polysaccharide consisting of a cellulose-like backbone that carries xylose and galactoxylose substituents (Saettone et al., patent application), chemical residues similar to those of mucin MUC-1 and episialin (17). Being similar to mucins, TSP is able to bind to the cell surface and intensify the contact between drugs and the adsorbing biological membrane (5). As previously reported for ocular delivery of ofloxacin and gentamicin (15), this study demonstrates that TSP enhances transcorneal disposition and intra-ocular penetration of rifloxacin in healthy rabbits when administered topically in a drop regimen. The increased aqueous concentration of rifloxacin due to TSP could be exploited for future treatment of intraocular microbial infections. It is worth pointing out, in this context, that the antibiotic levels we obtained in uninfected eyes approached the MIC at which 90% of isolates are inhibited (MIC₉₀) for most gram-negative bacteria and were greater than the MIC₉₀s of *Staphylococcus epidermidis* and *S. aureus* (30), the most common organisms in postoperative endophthalmitis. Another relevant finding was that administration of TSP-rifloxacin to eyes intracorneally infected with *P. aeruginosa* or *S. aureus* produced significantly higher aqueous drug concentrations than those obtained in uninfected eyes subjected to the same treatment, although the integrity of the corneal epithelial surface appeared to be conserved. This result may be explained by the fact that bacterial components, such as lipopolysaccharide and lipoteichoic acid, activate mucin production by epithelial cells (20) and can therefore increase the availability of substrates capable of adhering to TSP. A greater amount of mucins may have led to prolonged residence of TSP-rifloxacin on the corneal tissue, allowing increased penetration of rifloxacin into the aqueous humor.

The animal keratitis model developed in this study was reproducible in terms of the time course of pathological ocular changes and bacterial growth in vivo. Rifloxacin and ofloxacin significantly decreased *P. aeruginosa*- or *S. aureus*-induced ocular inflammation, indicating that neither of the drugs was toxic to rabbit eyes. The two drugs appeared to be similarly efficacious in the treatment of *S. aureus* keratitis, with bacterial growth in the cornea controlled at comparable levels. In contrast, as expected from the higher MIC of rifloxacin against the *P. aeruginosa* strain used for the experimental infection, rifloxacin was less active than ofloxacin in therapeutic studies of *P. aeruginosa* keratitis.

TSP-delivered rifloxacin reduced *P. aeruginosa* and *S. aureus* in the cornea at a higher rate than that achieved by rifloxacin alone. Use of TSP rendered the 0.3% rifloxacin solution as effective as ofloxacin in reducing *P. aeruginosa* in the cornea and more active than rifloxacin and ofloxacin in control of *S. aureus* keratitis when the time interval between two consecutive drug administrations was extended. These results indicate that rifloxacin bioavailability in the cornea is increased by TSP which, as a viscosity enhancer (Saettone et al., patent application), can prolong precorneal retention time compared to the reference solution. It may therefore be assumed that even scantily active antibiotics, when delivered by the polysaccharide, can reach adequate intracorneal concentrations and be effective in controlling bacterial replication in the cornea.

In conclusion, we suggest that TSP can be considered a

promising vehicle for topical ocular administration of antibiotics. Its application could possibly replace the use of fortified solutions of antimicrobials and reduce the necessity for repeated drug administration at frequent intervals, thereby potentially lowering corneal toxicity and increasing patient compliance.

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