Effect of a novel mucoadhesive polysaccharide obtained from tamarind seeds on the intraocular penetration of gentamicin and ofloxacin in rabbits

E. Ghelardi\textsuperscript{a*}, A. Tavanti\textsuperscript{a}, F. Celandroni\textsuperscript{a}, A. Lupetti\textsuperscript{a}, C. Blandizzi\textsuperscript{b}, E. Boldrini\textsuperscript{c}, M. Campa\textsuperscript{a} and S. Senesi\textsuperscript{a}

\textsuperscript{a}Dipartimento di Patologia Sperimentale, Biotecnologie Mediche, Infettivologia ed Epidemiologia and \textsuperscript{b}Dipartimento di Oncologia, dei Trapianti e delle Nuove Tecnologie in Medicina, Università degli Studi di Pisa, Pisa, Italy; \textsuperscript{c}Farmigea S.p.A., Pisa, Italy

This report describes the efficacy of a novel mucoadhesive polymer, the tamarind seed polysaccharide, as a delivery system for the ocular administration of hydrophilic and hydrophobic antibiotics. Healthy rabbits were subjected to repeated ocular instillations with either conventional gentamicin or ofloxacin or these agents viscosified with the tamarind seed polysaccharide. Administration of viscosified preparations produced antibiotic concentrations both in the aqueous humour and cornea that were significantly higher than those achieved with the drugs alone. The increased drug absorption and the prolonged drug elimination phase obtained with the viscosified formulations indicate the usefulness of the tamarind seed polysaccharide as an ophthalmic delivery system for topical administration of antibiotics.

Introduction

The high frequency of ocular infections and the severity of endophthalmitis highlight the importance of establishing therapeutic regimens allowing the rapid attainment of high drug concentrations at the site of infection. Drop regimens may fail to produce therapeutically active drug levels in ocular compartments, since the continuous tear flow reduces the bioavailability of topically applied antibotics and the corneal epithelium acts as a barrier against drug penetration.\textsuperscript{1} Several systems have been developed to prolong the contact time between antimicrobial drugs and corneal tissues, thus potentially enhancing the intraocular delivery of ophthalmic medicaments. Among these, mucoadhesive polymers have been reported to improve the intraocular penetration of progesterone, pilocarpine and gentamicin.\textsuperscript{2,3}

Recently, a novel mucoadhesive polymer, consisting of a natural polysaccharide extracted from tamarind seeds and referred to as tamarind-gum polysaccharide (TGP), has been described as a viscosity enhancer with mucomimetic activity.\textsuperscript{4} Purified TGP is a high-molecular-weight, non-ionic, neutral, branched polysaccharide consisting of a cellulose-like backbone that carries xylose and galactoxylose substituents,\textsuperscript{4} chemical residues similar to that of mucin MUC-1 and episialin.\textsuperscript{5} Several properties make TGP an attractive candidate for use as a vehicle for ophthalmic medicaments: (i) it exhibits optimal performance as a tear fluid substitute;\textsuperscript{6} (ii) it prevents alterations of the corneal surface known as keratoconjunctivitis sicca;\textsuperscript{6} (iii) it reduces the \textit{in vitro} toxicity exerted by ofloxacin, rufloxacin, timolol and merthiolate on human conjunctival cells;\textsuperscript{7} (iv) it retains its properties when autoclaved;\textsuperscript{4} and (v) in contrast to other mucoadhesive polymers,\textsuperscript{3} it maintains its characteristics at neutral pH.\textsuperscript{4} The present study was thus designed to ascertain whether TGP could enhance the trans-corneal disposition of hydrophilic and hydrophobic antimicrobial drugs, such as gentamicin and ofloxacin, when administered topically to healthy rabbits by an ocular drop regimen.

*Correspondence address. Via S. Zeno 35–39, 56127 Pisa, Italy.
Tel: +39-050-836569; Fax: +39-050-836570; E-mail: ghelardi@biomed.unipi.it

© 2000 The British Society for Antimicrobial Chemotherapy
Materials and methods

Drug formulations
Sterile water solutions of gentamicin or ofloxacin contained mannitol 50 mg/mL (Sigma, St Louis, MO, USA), sodium merthiolate 0.02 mg/mL (CFM Oskar Tropitzsch, Marktredwitz, Germany), gentamicin sulphate 5 mg/mL (corresponding to 3 mg/mL gentamicin) (Sigma) or ofloxacin 3 mg/mL (Sigma). The viscosified solutions of antimicrobials contained TGP (Farmigea S.p.A., Pisa, Italy) at a concentration of 20 mg/mL. Farmigea S.p.A. kindly provided all the antibiotic formulations.

Experimental design
A total of 40 New Zealand male rabbits (2–2.5 kg) were used throughout the experiments and were subjected to an ocular drop regimen according to a treatment schedule described previously.1 In particular, treatment consisted of the instillation of gentamicin or ofloxacin (right eye) and the corresponding TGP formulations (left eye) into the lower conjunctival sac every 30 min for 6 h. Fifty microlitres of drug solution, corresponding to 150 μg gentamicin or ofloxacin, was applied with or without 1 mg TGP at each instillation. At fixed time intervals after the last administration (30, 60, 90, 120, 150 and 180 min), animals (five rabbits for each formulation and time analysed) were killed by injecting an overdose of ethyl urethane into the marginal ear vein. Examination of rabbit eyes during and at the end of treatment with antibiotics alone or with antibiotics viscosified with TGP did not reveal any signs of toxicity, including crusting, hyperaemia or lid swelling. Aqueous humour was aspirated by anterior chamber paracentesis using a 26-gauge needle. Corneas were taken from the animals killed 60 min after the last administration. For this purpose, eyes were rapidly enucleated, trimmed of all adventitial tissue and rinsed with sterile saline. Following excision, corneas were weighed, suspended (25 mg/mL) in phosphate-buffered saline (PBS) pH 7.2, homogenized, and centrifuged as described previously.8 Samples of aqueous fluid and corneal tissue were analysed immediately or stored at –30°C and examined within 1 month. The study was approved by the Ethics Committee of Pisa University Hospital.

Drug concentration assay
The antibiotic concentration in corneal and aqueous samples was measured by agar-diffusion bioassay using *Bacillus subtilis* ATCC 6633 as the indicator organism.1 *B. subtilis* spores were prepared in 0.1 M PBS pH 8.0 and stored at 4°C as sterile suspensions. For assays, 5 × 10⁸ spores/mL were incorporated into molten Antibiotic Medium No. 5 or No. 2 (Difco, Detroit, MI, USA) in order to measure gentamicin and ofloxacin concentrations, respectively. The assay plates (90 mm diameter) were pierced with six holes (7 mm diameter) which were filled with 100 μL of two-fold dilutions of aqueous or corneal samples. After incubation at 37°C for 24 h, the diameter of growth inhibition halos was taken as the measure of drug concentration, by reference to calibration curves constructed by adding known amounts of gentamicin (from 0.01 to 5 mg/L) or ofloxacin (from 0.05 to 10.0 mg/L) to pooled aqueous humour or homogenized corneas taken from untreated animals. Assays were shown to be linear in the range 0.02–3.5 mg/L for gentamicin and 0.2–10.0 mg/L for ofloxacin. Each determination was performed in triplicate and the mean value was calculated; inter-assay and inter-day variations corresponding to one dilution step were observed in no more than 3% of the experiments. No growth inhibition zones were detected when wells were filled with 100 μL of TGP, either alone or in combination with the excipients (mannitol and sodium merthiolate).

Statistical and pharmacokinetic analysis
Results were expressed as mean ± standard deviation (S.D.). Statistical analysis was performed using the two-tailed Student’s *t*-test. Pharmacokinetic analysis was carried out by fitting mean drug concentrations versus time data sets according to a one-compartment model. For this purpose a non-linear least-squares regression analysis was used (APO2PR software, MediWare, Groeningen, The Netherlands). Pharmacokinetic calculations were performed following standard methods.9 The peak concentration (Cₘₐₓ) and the time to reach peak concentration (Tₘₐₓ) values were identified from the inspection of drug concentration–time plots. The t₁/₂ for each elimination exponential (t₁/₂ₑ) was obtained from the equation t₁/₂ₑ = 0.693/Kₑ, where Kₑ (elimination constant) is the slope of the exponential. The area under the concentration–time curve (AUC) was calculated by the trapezoidal method for the area from the time 30 min (t₃₀) to the time of the last quantified drug concentration (tᵢₘᵢₐₓ). The mean residence time (MRT) was also estimated.

Results and discussion
Antibiotic concentrations in the aqueous humour varied significantly depending on whether rabbit eyes were treated topically with antibiotics alone or drug formulations viscosified with TGP. Administration of TGP–gentamicin and TGP–ofloxacin produced intraocular drug levels significantly higher than those obtained with the corresponding TGP-free formulations at each time point analysed (*P < 0.0001) (Figure). The highest concentrations of both ofloxacin (Figure, part a) and gentamicin (Figure, part b) were achieved 60 min after the last administration. The differences in the absolute concentration values detected for these antibiotics may be explained by taking into account
A new ophthalmic delivery system for antibiotics

the different permeability of corneal epithelium for gentamicin, which is hydrophilic, and ofloxacin, which is lipophilic. Indeed, the permeation of ofloxacin was consistently greater than that of gentamicin even when the drugs were delivered by TGP. It may be inferred that TGP improves significantly the intraocular penetration of topically administered hydrophobic and hydrophilic compounds without modifying the intrinsic solubility of the drugs. However, the greatest effect of TGP was on the intraocular penetration of the more hydrophilic gentamicin, as shown by the comparison of pharmacokinetic parameters calculated for each drug formulation examined (Table). While $C_{\text{max}}$ values indicate a three-fold increase in ofloxacin concentration when the drug was delivered by TGP ($9.54 \pm 1.75$ mg/L) compared with the drug alone ($3.24 \pm 0.62$ mg/L), a 4.5-fold increase in the level of TGP-delivered gentamicin ($2.11 \pm 0.26$ mg/L) was recorded compared with gentamicin alone ($0.47 \pm 0.14$ mg/L). Moreover, the AUC values for aqueous fluid show three-fold and six-fold increases in ofloxacin and gentamicin, respectively, when administered with TGP. These findings are consistent with both increased drug absorption and a prolonged elimination phase, since higher MRT and $t_{\frac{1}{2}}$ values were obtained for the TGP formulations. Moreover, in the presence of TGP, the gentamicin concentration declined more slowly than that of ofloxacin, as indicated by the lower $K_e$ value calculated for TGP–gentamicin (Table).

Figure. Concentration–time curves of ofloxacin (a) and gentamicin (b) in aqueous samples after the last topical administration (150 $\mu$g/installation every 30 min for 6 h) of drugs with or without TGP in healthy rabbits (○, ofloxacin; ▼, ofloxacin + TGP; ■, gentamicin; ▲, gentamicin + TGP). Each point represents the mean value ± S.D. (vertical bars) obtained from five rabbits.

Corneal antibiotic levels were measured at the sampling time corresponding to the maximum drug concentrations detected in the aqueous humour (60 min after the last administration). Each drug formulation produced higher corneal antibiotic concentrations than those detected in the aqueous humour, thus indicating that the cornea is a target site for the accumulation of topically administered drugs. The TGP-delivered drugs produced significantly higher intra-corneal levels than those achieved with the corresponding TGP-free formulations. The mean ofloxacin level obtained with the drug alone was $0.89 \pm 0.19$ mg/g, as compared with $2.84 \pm 0.70$ mg/g with TGP–ofloxacin ($P = 0.028$). Gentamicin concentration in the corneal tissue increased from $0.51 \pm 0.13$ mg/g to $1.46 \pm 0.25$ mg/g when the drug was delivered by TGP ($P = 0.01$). The significant increase in drug concentration obtained in corneal samples by using TGP as a delivery system suggests that this natural polysaccharide enhances corneal drug accumulation, probably because it reduces the wash-out of topically administered drugs. Although only one time-point was analysed for corneal samples, the ratio of corneal ofloxacin level to aqueous concentration was similar when the drug was delivered with or without TGP (7.44 and 6.87). In contrast, the ratio of corneal gentamicin level to aqueous concentration was reduced by about one-third for the TGP-delivered drug (17.31 compared with 26.90). This observation

<table>
<thead>
<tr>
<th>Drug formulation</th>
<th>$C_{\text{max}}$ (mg/L)</th>
<th>$T_{\text{max}}$ (min)</th>
<th>$K_e$ (per min × 100)</th>
<th>$t_{\frac{1}{2}}$ (min)</th>
<th>AUC (min·mg/L)</th>
<th>MRT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>3.24 ± 0.72</td>
<td>60</td>
<td>2.88</td>
<td>24</td>
<td>334.8</td>
<td>87.6</td>
</tr>
<tr>
<td>TGP–ofloxacin</td>
<td>9.54 ± 1.68</td>
<td>60</td>
<td>1.58</td>
<td>43.8</td>
<td>1044.0</td>
<td>115.8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.47 ± 0.13</td>
<td>60</td>
<td>2.31</td>
<td>30</td>
<td>35.4</td>
<td>74.58</td>
</tr>
<tr>
<td>TGP–gentamicin</td>
<td>2.11 ± 0.23</td>
<td>60</td>
<td>0.80</td>
<td>86.4</td>
<td>223.2</td>
<td>154.9</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$, peak concentration; $T_{\text{max}}$, time to reach peak concentration; $K_e$, elimination constant; $t_{\frac{1}{2}}$, elimination half-life; AUC, area under the concentration–time curve; MRT, mean residence time.
suggests that TGP especially favours the release of the more hydrophilic gentamicin from the cornea into the aqueous fluid. The potential utility of TGP as an ophthalmic delivery system for the prevention of ocular infections may be argued considering the known MIC values of gentamicin and ofloxacin for bacteria frequently causing such infections. Indeed, in contrast to ofloxacin alone, the use of TGP–ofloxacin in healthy rabbits led to the attainment of aqueous antibiotic concentrations (Figure, part a) consistently higher than the MIC values reported for Pseudomonas aeruginosa (MIC$_{90}$ = 3.1 mg/L), an organism often associated with severe intraocular infections. Similar conclusions can be drawn for gentamicin (Figure, part b), since in the absence of TGP, this antibiotic usually achieves aqueous drug levels lower than the MIC values (0.062–2 mg/L) reported frequently for common ocular pathogens including Staphylococcus aureus, Staphylococcus epidermidis and several P. aeruginosa clinical isolates. The remarkably high levels of ofloxacin and gentamicin obtained in the cornea after administration with TGP also indicate that this mucoadhesive polymer may be useful in the topical treatment of bacterial keratitis. Indeed, the concentrations of both drugs in the cornea greatly exceeded the MICs reported for most Pseudomonas, Streptococcus and Staphylococcus spp. strains frequently isolated from cases of keratoconjunctivitis.

The overall results obtained during this investigation suggest that the tamarind seed polysaccharide can be used successfully as an ophthalmic delivery system for antimicrobial drugs and possibly for other medicaments in the topical treatment of eye diseases.

Acknowledgement

This work was supported by University of Pisa, grant 1998–99, and by Farmigea S.p.A., Pisa, Italy.

References


Received 21 February 2000; returned 23 May 2000; revised 9 June 2000; accepted 10 July 2000