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Topical 0·3% ciprofloxacin, norfloxacin, and ofloxacin in treatment of bacterial keratitis: a new method for comparative evaluation of ocular drug penetration

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Abstract

***Aims*—This study was designed to assess the relative corneal penetration of topical drops of three antibiotics and to relate those levels to minimum inhibitory concentrations for organisms associated with bacterial keratitis.**

***Methods*—Four drops of each of ciprofloxacin, norfloxacin, and ofloxacin (0·3% topical ophthalmic preparations) were given to 12 patients undergoing corneal transplantation. After the recipient tissue was removed, corneal drug penetration was measured using high performance liquid chromatography.**

***Results*—Intracorneal concentrations of ofloxacin (geometric mean 0·81 mg kg^{-1}) were significantly higher than both ciprofloxacin (0·60 mg kg^{-1}; p=0·048) and norfloxacin (0·54 mg kg^{-1}; p=0·012). Ciprofloxacin and norfloxacin concentrations did not differ significantly (p=0·33).**

***Conclusions*—Review of the minimum inhibitory concentrations of the fluoroquinolones against ocular pathogens reveals that ciprofloxacin is more potent than ofloxacin against many bacteria; ofloxacin is in turn more potent than norfloxacin. These data favour the selection of ciprofloxacin and ofloxacin rather than norfloxacin for the empirical treatment of corneal infection. The greater potency of ciprofloxacin offsets the superior penetration of ofloxacin. There is a need for improved clinical trial data concerning the use of fluoroquinolone eyedrops in ulcerative keratitis; some encouraging data are available for ciprofloxacin but not (in humans) for norfloxacin or ofloxacin.**

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Bacterial keratitis may arise secondary to corneal epithelial breakdown associated with dry eye, contact lens use, trauma, or the presence of a persistent corneal suture. Subsequent long term visual loss occurs as a consequence of corneal scarring affecting the visual axis. The extent of scarring may be limited if the infection is identified early and treated adequately. Optimal management requires an attempt to isolate the causative organism in order to ensure appropriate antibiotic treatment. However, the aetiological agent is rarely known in time to guide initial antibiotic treatment and frequently is never identified. Thus, successful treatment of a presumed bacterial corneal abscess is often dependent on empirical selection of an effective broad spectrum antibiotic regimen. For many years the most widely accepted option has been the instillation of a combination of a β lactam and an aminoglycoside, both in high concentration solutions requiring local formulation.

The fluoroquinolones are broad spectrum bactericidal agents with activity against many of the important corneal pathogens including staphylococci, *Neisseria gonorrhoea*, *Haemophilus influenzae*, Enterobacteriaceae, and *Pseudomonas aeruginosa*.[1 2] Three fluoroquinolone antibiotics have been formulated as 0·3% topical ophthalmic drops: ciprofloxacin (Alcon), norfloxacin (Merck, Sharpe and Dohme), and ofloxacin (Allergan). While these antibiotics have similar spectra of activity, they are not equally potent against many organisms and vary in their pharmacokinetic properties.[3] The success of an antibiotic depends on its ability to penetrate the site of infection in concentrations effective against the causative bacteria. Therefore, this project was designed to assess the relative corneal penetration of topical drops of these antibiotics and to relate those levels to minimum inhibitory concentrations for organisms commonly associated with bacterial keratitis.

Materials and methods

Twelve patients due to undergo routine penetrating keratoplasty (with or without cataract extraction) under general anaesthesia at the Bristol Eye Hospital were enrolled. Exclusion criteria included the presence of a broken corneal epithelium, active corneal inflammatory disease, topical antibiotic use during 1 week before enrolment, or a history of renal disease. Patients were given an information sheet pertaining to the trial before giving their informed consent. The trial was approved by the local ethics committee.

Table 1 Six quinolone antibiotic administration regimens showing the sequence in which patients received single drops of topical 0·3% ciprofloxacin, norfloxacin, and ofloxacin, every 20 minutes for 1 hour

Sequence	*Start (0 min)*	*+20 min*	*+40 min*	*+60 min*
A1/B1	Nor/Cip/Oflo	Oflo/Nor/Cip	Cip/Oflo/Nor	Nor/Cip/Oflo
A2/B2	Oflo/Nor/Cip	Cip/Oflo/Nor	Nor/Cip/Oflo	Oflo/Nor/Cip
A3/B3	Cip/Oflo/Nor	Nor/Cip/Oflo	Oflo/Nor/Cip	Cip/Oflo/Nor
A4/B4	Nor/Oflo/Cip	Cip/Nor/Oflo	Oflo/Cip/Nor	Nor/Oflo/Cip
A5/B5	Cip/Nor/Oflo	Oflo/Cip/Nor	Nor/Oflo/Cip	Cip/Nor/Oflo
A6/B6	Oflo/Cip/Nor	Nor/Oflo/Cip	Cip/Nor/Oflo	Oflo/Cip/Nor

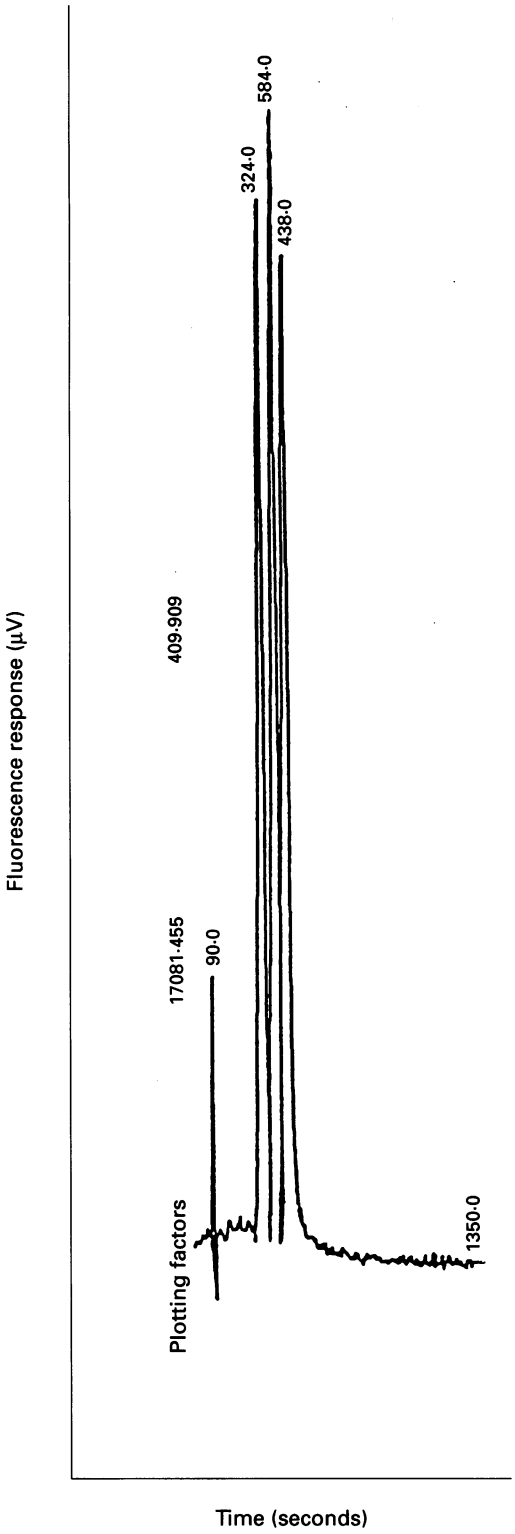

Figure 1 High performance liquid chromatogram of fluoroquinolones. Elution order ofloxacin, norfloxacin, ciprofloxacin.

Topical preparations of 0·3% ciprofloxacin, norfloxacin, and ofloxacin were obtained from their manufacturers and rebottled to ensure that the same drop volume was applied in each case. Patients received four drops of each drug over a 60 minute period as shown in Table 1. To avoid bias resulting from their order of presentation the three antibiotics were administered in one of six sequence permutations. The first patient enrolled was randomly allocated an administration regimen and thereafter, patients received the next regimen in the sequence. Drops were administered at 2 minute intervals to avoid overspill. Patients received concurrent surgical preparation with unpreserved miotic/mydriatic drops and surgery was commenced within 60 minutes after instillation of the last antibiotic drop. The host corneal button was snap frozen in liquid nitrogen and stored at −70°C awaiting corneal drug assay.

Corneal antibiotic concentrations were assayed by high performance liquid chromatography (HPLC) using methods based on those of McDermott[4] and Gau.[5] Corneal buttons were weighed, cut into small pieces with a scalpel, and homogenised in 500 μl of water in a ground glass Griffiths tube. The bulk of the first homogenate was removed and a second 500 μl of water was added. After further homogenisation the solutions were pooled. After brief centrifugation the clear supernatant was analysed by HPLC on Spherisorb 5 ODS II (4×300 mm) (HPLC Technology, Macclesfield) at 50°C with a mobile phase of Water (1 litre) plus phosphoric acid (1·6 ml) adjusted to pH 3·0 with tetrabutylammonium hydroxide and mixed with acetonitrile (50 ml). Flow rate was 1 ml min^{-1} and detection was by fluorescence (excitation 310 nm, emission 467 nm) using a Perkin-Elmer LC 240 detector. External calibration was performed with an aqueous solution containing ciprofloxacin (1·67 mg l^{-1}), norfloxacin (1·73 mg l^{-1}), and ofloxacin (1·67 mg l^{-1}). Twenty μl of calibrator solution were processed in the same manner as the corneal samples. The drug concentration in each corneal sample was determined by taking the dilution factor of the calibrator as 20/1020 and the dilution factor of the tests as $x/1000+x$ where x is the weight of the corneal plug in mg. Preliminary experiments (data not shown) indicated >95% recovery of calibrator solution processed in the presence of drug free cornea. Typical within assay reproducibility was plus or minus 16% or better.

A brief literature review was conducted to identify the bacterial species most commonly implicated in keratitis and their sensitivity to the fluoroquinolones studied. Where possible data were selected from publications in which the sensitivities of bacteria of interest to all three antimicrobials were compared. For each fluoroquinolone the mean intracorneal concentration was expressed as a proportion of the minimum concentration required to inhibit the growth of 90% of isolates of these species (MIC_{90}). The resultant coefficients were used to assess the likely in vivo potency of each agent.

Results

Nine (75·0%) female and three (25·0%) male subjects were recruited; the mean age was 49 (range 7–87) years. Each drug administration permutation listed in Table 1 was used for two patients. The three fluoroquinolones were all baseline separated in the HPLC analysis. A typical chromatogram is shown in Figure 1.

The antibiotic concentrations found in the corneal buttons are given in Table 2. The geometric mean values (and 95% confidence limits) were: ciprofloxacin 0·60 mg kg^{-1} (0·38–0·95 mg kg^{-1}), norfloxacin 0·54 mg kg^{-1} (0·36–0·95 mg kg^{-1}), and ofloxacin 0·81 mg kg^{-1} (0·51–1·28 mg kg^{-1}). The difference

Table 2 Corneal antibiotic concentration (mg/kg) for 12 patients receiving four topical drops of 0·3% ciprofloxacin, norfloxacin, and ofloxacin over 60 minutes before undergoing penetrating keratoplasty

Patient code	Ciprofloxacin	Norfloxacin	Ofloxacin
A1	3·33	2·38	3·73
A2	0·56	0·48	0·60
A3	0·63	0·76	1·57
A4	0·79	0·44	0·53
A5	0·53	0·66	0·48
A6	0·19	0·24	0·32
B1	0·37	0·49	0·47
B2	0·89	0·72	0·62
B3	1·07	1·10	2·24
B4	0·58	0·27	0·74
B5	0·34	0·37	0·63
B6	0·32	0·27	0·93
Geometric mean	0·60	0·54	0·81

between ofloxacin and ciprofloxacin ($p=0·048$, paired t test) and ofloxacin and norfloxacin ($p=0·012$) was significant, while ciprofloxacin and norfloxacin concentrations did not differ significantly ($p=0·33$).

Table 3 lists 19 bacterial species associated with bacterial keratitis, published MIC_{90} data for these species, and mean corneal fluoroquinolone concentrations expressed as a proportion of the MIC_{90} values. All three antibiotics achieved corneal levels above or close to the MIC values for most of the Gram negative organisms listed, with the exceptions of *Acinetobacter* spp and, notably, *Pseudomonas aeruginosa*, for which only ciprofloxacin achieved corneal concentrations above the MIC_{90} values. However, corneal concentrations of norfloxacin were below the MIC_{90} values for all the Gram positive bacteria and none of the fluoroquinolones achieved corneal concentration equivalent to the MIC_{90} values for streptococci or *Enterococcus faecalis*.

Discussion

Standard drug penetration studies are heavily influenced by uncontrolled interpatient variables – for example, presence of corneal disease, rate of blinking, tear flow, and time between drug administration and sampling. This tends to result in large intragroup variability; in this study the concentrations of each fluoroquinolone spanned a range of approximately tenfold (Table 2). In consequence, when comparative penetration studies are performed using two or more drugs given to two or more separate patient groups, large patient numbers are required to detect significant differences.

By giving all three antibiotics virtually simultaneously to each patient, any factor which promoted or inhibited penetration of one drug should have had that effect upon each agent. This phenomenon was indeed apparent, with a high degree of correlation of concentrations of the three agents observed (Table 2). The use of HPLC to assay three closely related drugs simultaneously in the same sample has thereby provided a powerful new technique for demonstrating significant differences in drug penetration without demanding large numbers.[13] Using the data from this study (concentration means and standard deviations) a power statistics calculation determined that 88 or 184 patients would be required to have an 80% probability of detecting (statistically) the difference between ofloxacin and ciprofloxacin and ofloxacin and norfloxacin respectively if paired comparisons were not possible.

There are some potential problems with this approach. When two or more drops are applied to the same eye the relative penetration of the second or subsequent drop may be increased, either because the second drop 'washes out' the first or because the first drug induces epithelial damage or otherwise enhances penetration of the second. Potential bias was minimised in this study by administering the drugs in each of six sequence permutations.

There have been a number of studies of corneal tissue penetration of topical quinolone antibiotics. McDermott *et al*[4] gave 14 drops of topical 0·3% ciprofloxacin over 11 hours to patients undergoing penetrating keratoplasty, demonstrating tissue concentrations averaging 5·28 mg kg^{-1} tissue. Pamel and Perl[14] used ciprofloxacin soaked collagen shields and

Table 3 Ciprofloxacin, norfloxacin, and ofloxacin MIC_{90} values and ratios of intracorneal concentrations: MIC_{90} values for bacteria associated with stromal keratitis

Organism	Ciprofloxacin		Norfloxacin		Ofloxacin	
	MIC_{90} (μg/ml)	Mean [corneal] ÷ MIC_{90}	MIC_{90} (μg/ml)	Mean [corneal] ÷ MIC_{90}	MIC_{90} (μg/ml)	Mean [corneal] ÷ MIC_{90}
Gram positives:						
Bacillus spp[8]	0·25[12]	2·4	3·1[10]	0·2	0·5[10 12]	1·6
Corynebacterium spp[8]	0·5[11]	1·2	4[11]	0·1	1[11]	0·8
Enterococcus faecalis[8]	2[1]	0·3	32[1]	0·02	4[1]	0·2
Staphylococcus aureus[6–8]	0·5[1 2]	1·2	2[1 2]	0·3	0·5[1 2]	1·6
Staphylococcus epidermidis/other coagulase −ve staphylococci[7 8]	0·25–0·5[1 2]	1·2–2·4	1[1 2]	0·5	0·5[1 2]	1·6
Streptococcus pneumoniae[6–8]	2[1 2]	0·3	16[1 2]	0·03	2–4[1 2]	0·2–0·4
Streptococcus pyogenes[6 8]	2[1]	0·3	16[1]	0·03	2[1]	0·4
Viridans streptococci[8]	4[1]	0·15	16[1]	0·03	4[1]	0·2
Gram negatives:						
Acinetobacter spp[8]	1[1]	0·6	16[1]	0·03	1[1]	0·8
Escherichia coli[8]	0·015[1]	40	0·25[1]	2·2	0·12[1]	6·5
Haemophilus influenzae[7]	0·008–0·03[1 2]	20–75	0·12[1 2]	4·3	0·03–0·12[1 2]	6·5–27
Klebsiella pneumoniae[6 8 9]	0·015[1]	40	0·5[1]	1·1	0·12[1]	6·5
Moraxella spp[6 8 9]	0·25[11]	2·4	0·5[11]	1·1	0·25[11]	3·2
Morganella morganii[7]	0·06[1]	10	0·25[1]	2·2	0·5[1]	1·6
Neisseria meningitidis[8]	0·008[2]	75	0·03[2]	18	0·015[2]	54
Neisseria gonorrhoeae[8]	0·004–0·008[1 2]	75–150	0·06–0·12[1 2]	4·3–9·0	0·03[1 2]	27
Proteus mirabilis[7 8]	0·12[1]	4·8	0·5[1]	1·1	1[1]	0·8
Pseudomonas aeruginosa[6–9]	0·25–0·5[1 2]	1·2–2·4	2–8[1 2]	0·07–0·3	2–4[1 2]	0·2–0·4
Serratia marcescens[7 8]	0·5[1]	1·2	0·5[1]	1·1	1[1]	0·8

reported much higher corneal stromal antibiotic concentrations (mean ciprofloxacin concentration 22·09 mg l^{-1}). To date there have been no comparative studies of corneal penetration of the different quinolone antibiotics.

Donnenfeld *et al*[15] performed a comparative study of aqueous humour penetration of ciprofloxacin, norfloxacin, and ofloxacin demonstrating significantly higher levels of ofloxacin than the other two agents (mean ciprofloxacin 0·072 mg l^{-1}, norfloxacin 0·057 mg l^{-1}, and ofloxacin 0·338 mg l^{-1}). Donnenfeld's study showed aqueous humour penetration of ofloxacin to be 4·7-fold greater than ciprofloxacin, while in this study the difference in corneal penetration was only 1·4-fold. Whether this discrepancy is real or reflects differences in protocol remains to be proved. Interestingly, in a previous comparative study of ciprofloxacin and norfloxacin penetration into aqueous humour after topical dosing (six drops in 6 hours) we found a larger difference between the two drugs (0·22 and 0·14 mg l^{-1} respectively; 1·6-fold difference)[16] than we found in this study (1·1-fold difference). It may be that differences in penetration are less apparent in corneal biopsies than in aqueous humour because of high concentrations of drug which are likely to accumulate in the most superficial layers of the cornea.

Penetration may be improved in patients with inflamed corneas and/or broken corneal epithelium. However, when one patient with a broken corneal epithelium was given preoperative quinolones corneal concentrations of 0·84 mg kg^{-1} ciprofloxacin, 0·45 mg kg^{-1} norfloxacin, and 1·11 mg kg^{-1} ofloxacin were recorded (data excluded from analyses), suggesting that the presence of a broken epithelium may not markedly increase drug levels. In this study corneas were harvested after application of just four drops of each agent given over a 1 hour period, a regimen which does not mirror common clinical practice. It is likely that changing the frequency of application and duration of therapy would affect the intracorneal quinolone concentrations achieved, as is suggested by the higher ciprofloxacin concentrations (5·28 mg kg^{-1}) recorded by McDermott *et al*[4] after applications of 14×0·3% drops in 11 hours. For these reasons some caution must be exercised when interpreting the data presented. A ratio of mean corneal concentration: MIC_{90} of 1 or above for a bacterium (see Table 3) can not be interpreted as a guarantee of successful therapy of corneal infection by members of that species, nor indeed does a ratio of below 1·0 necessarily predict treatment failure. The data can, however, be used to predict the relative in vivo potency of the fluoroquinolones and to identify species which are most likely to respond poorly to each agent and which therefore should be the particular focus of clinical trials. Ciprofloxacin and ofloxacin have a clear advantage over norfloxacin in terms of potency and/or ability to penetrate the cornea. The superior penetration of the cornea by ofloxacin is offset by the greater potency of ciprofloxacin against a number of species, most notably *P aeruginosa*. None of the fluoroquinolones tested is particularly active against streptococci, and this was reflected in our analyses.

There is a need for improved clinical trial data concerning the use of fluoroquinolone eyedrops in ulcerative keratitis. Ciprofloxacin has been the subject of a large multicentre comparison with more conventional treatment (mainly high concentration combinations of cefazolin with gentamicin or tobramycin) for the treatment of bacterial keratitis of various aetiologies.[17 18] Although treatment groups were not well matched, and were open, results were encouraging and suggested that ciprofloxacin eyedrops could be used as an empirical treatment for suspected bacterial keratitis. Although data have been published concerning the use of ofloxacin to treat corneal ulcers in rabbits,[19] the absence of trial data for ulcerative keratitis in humans legislates against its selection for this purpose as yet.

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