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Intraocular Penetration of Norfloxacin Eye Drops

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Summary. **A 0.3% ophthalmic solution of norfloxacin (NFLX), one of the new quinolone compounds, was instilled into the rabbit eye 5 times every 5 min, and concentrations of NFLX in both cul-de-sac and the ocular tissues were measured. The following were noted:**

1. NFLX concentrations exceeding the MIC90 against various bacteria were consistently demonstrated in the cul-de-sac up to 6 h after a single instillation in the normal eye.

2. On 5 instillations every 5 min, high NFLX concentrations were demonstrated in the outer parts of the normal eye. In the inflamed eye, the penetration of NFLX at high concentrations was noted in the outer and inner parts of the eye.

3. In the inflamed eye, the penetrating concentrations were higher than in the normal eye, especially in the aqueous humor, cornea, eyelid, and iris and ciliary body.

INTRODUCTION

In recent years, new quinolone compounds have been intensively evaluated as an addition to currently available antibiotics. Based on the results of clinical evaluations, the role of these compounds in the future therapy of bacterial infections has been receiving widespread attention.

Norfloxacin (NFLX) is one of the new quinolone compounds which was synthesized in Kyorin Central Research Laboratory.[1)] This drug has a broad spectrum of antibacterial activity covering not only common gram negative rods but also *Staphylococci*. Its distribution into tissue is also excellent, proving higher tissue concentrations in the lung, pancreas, spleen, adrenal, lymphnode and bone than concentrations in the blood.[2)]

The present studies were carried out to evaluate the usefulness of local application as eye drops, and we examined NFLX concentrations in the ocular tissue after the instillation of NFLX ophthalmic solution.

MATERIALS AND METHODS

1. Drug

The NFLX ophthalmic solution used in the present study was a colorless, clear aqueous solution containing 3.0 mg/ml (potency) of NFLX. The solution was isotonic to normal saline and the pH range was 5.0-5.6.

2. Animals

Normal adult New Zealand white rabbits weighing 2-3 kg were used.

3. NFLX levels in cul-de-sac

After single instillation of 2 drops of NFLX ophthalmic solution, the tear fluid in the cul-de-sac was taken out with a micropipette at 1/12 1/4, 1/2, 1, 2, 3 and 6 h. Thereafter, 1 ml of 1/15 M phosphate-buffered solution (pH 7.0) was added to 5 μl of each sample. Each result of NFLX levels in the cul-de-sac was shown as the mean value of 6 eyes.

4. Induction of inflamed eye

Eye inflammation was induced using the following

procedure. A silicon sponge tube containing 10% NaOH solution was placed on the center of the cornea of the rabbit eye for about 5 sec, and washed out with physiological saline for about 30 sec.

5. Concentration of NFLX in ocular tissue

NFLX ophthalmic solution was instilled 5 times every 5 min into the normal and inflamed eyes for 12 to 16 h after the induction of the inflammation. Eye balls were removed at 1/4, 1/2, 1, 2 and 6 h after the end of instillation (25 min after the first instillation) and the concentrations of NFLX in ocular tissue and serum were determined. The drug levels were determined on 2-3 eyes each time.

6. Assay methods

Concentrations of NFLX in the cul-de-sac and in the ocular tissue were measured with high performance liquid chromatography using a fluorescence detector according to the procedures and conditions described by Ozaki et al.[3]

RESULTS

1. NFLX levels in cul-de-sac

The NFLX levels in cul-de-sac after instillation are shown in Table 1 and Fig. 1.

The concentration of NFLX was revealed to be the highest, 1340±791 μg/ml at 5 min, and decreasing to about half value at 15 min, and to one forth at 30 min. Thereafter, the NFLX level decreased relatively rapidly in 6 h.

2. Concentrations in the ocular tissue

1) Normal eye (Fig. 2)

In the outer parts of the eye, a relatively high penetration was observed in various ocular tissues at 1/4 h after instillation. At 1/2 h after instillation, all the drug levels except for that in the eyelid showed a tendency to decrease. At 1 h after instillation, tissue levels decreased to a half or one fifth of the peak levels observed at 1/4 or 1/2 h.

Thereafter, all the drug levels decreased gradually over 6 h except for that in the eyelid. In general, the drug level was highest in the cornea among all tissues, and the drug level in the sclera was the lowest.

In the inner parts of the eye, a low level of penetration was recognized. The aqueous levels were in the range of 0.16-0.68 μg/ml, and the iris and ciliary body levels from 0.08-0.68 μg/g. In other tissues such as the retina and choroid, optic nerve, vitreous body and lens, NFLX concentrations were 0.02-0.64 μg/g. A low NFLX concentration was noted in the serum.

2) Inflamed eye (Fig. 3)

In the outer parts of the eye, the drug level in the cornea at 1/4 h after instillation showed the peak value, 39.8 μg/g, and the levels in the other tissues were 14.7-1.99 μg/g. After 1/2 h, the highest level, 31.1 μg/g, was observed in the cornea. After 1 h, the drug level in the cornea showed the highest, 22.7 μg/g, and those in the other tissues were 21.5-1.15 μg/g. After 2 h, the highest level was maintained in the cornea, 12.7 μg/g, and the drug levels in other tissues were 6.59-0.94 μg/g. After 6 h, 1.30 μg/g of NFLX was detected in the cornea, and 1.28-0.23 μg/g was observed in other tissues.

In the inner parts of the eye, the highest level of the drug was observed in the aqueous humor among the other tissues, 23.8 μg/ml, at 1/4 h, the peak value of 41.5 μg/ml at 1 h, 14.8 μg/ml at 2 h and 0.35 μg/ml at 6 h. In the iris and ciliary body, levels of 5.85-0.16 μg/g were detected. In other tissues, levels of 0.10-1.13 μg/g were detected in the retina and choroid, and 0.46-1.39 μg/g in the optic nerve at 1/4 to 6 h. In the lens and vitreous body, a small amount of NFLX less than 1.0 μg/g was detected.

3. Comparison of the intraocular penetration between normal and inflamed eyes

The ratio of drug concentration in inflamed ocular

Table 1. Cul-de-sac concentration of 0.3% NFLX eye drops. Mean ±SD., n=6, μg/ml, rabbit eye, 2 drops

Time (h)	1/12	1/4	1/2	1	2	3	6
Concentration	1340±791	628±543	314±418	89.0±87.8	19.9±12.7	6.1±3.9	8.3±9.9

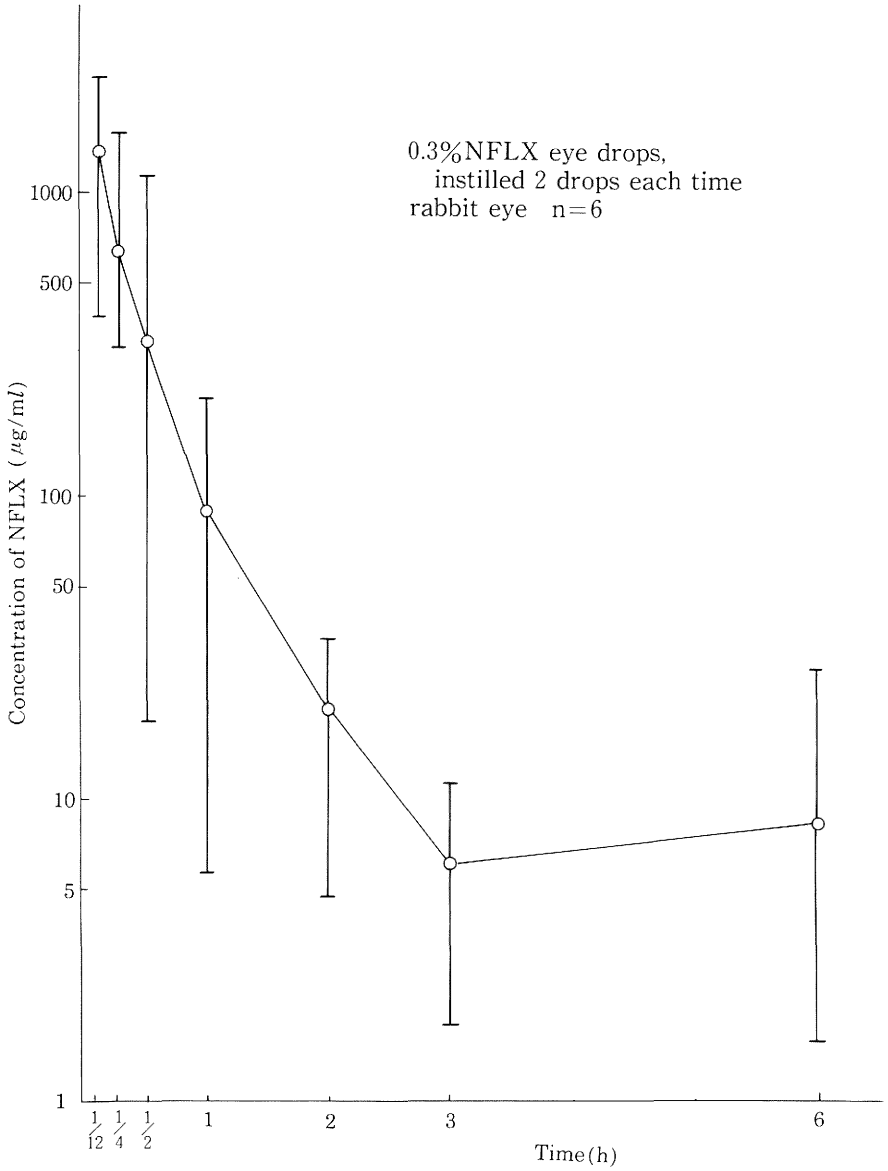

Fig. 1. Retention level of NFLX in the cul-de-sac

tissue to that in normal ocular tissue is shown in Table 2.

The highest ratio was recognized in the aqueous concentration with 2.1 to 259 times. The other tissue ratios were 2.5 to 24.0, 2.0 to 18.0, 1.3 to 10.5, 1.0 to 8.0 and 1.3 to 7.1, in the lens, the iris and ciliary body, the retina and choroid, the vitreous body and the cornea, respectively.

DISCUSSION

In the present study, the use of NFLX as an ophthalmic solution was evaluated. Studies were conducted on the dynamics of intraocular penetration upon instillation of an aqueous 0.3% solution of this compound.

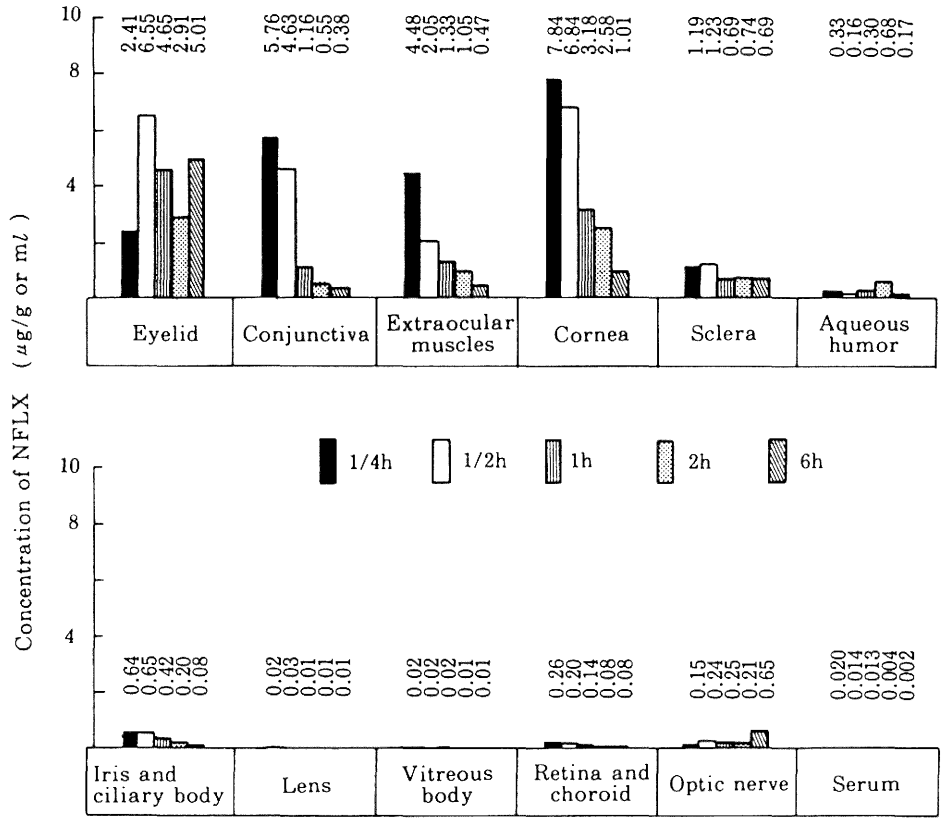

Fig. 2. Ocular tissue concentrations after instillation of 0.3% NFLX ophthalmic solution (normal eye)

Table 2. Comparison of the intraocular penetration between normal and inflamed eyes (ratio of normal/inflamed eye)

Tissues	Time (h)				
	1/4	1/2	1	2	6
Eyelid	6.1	2.0	4.6	2.3	0.3
Conjunctiva	0.6	0.4	1.0	1.7	1.0
Extraocular muscles	0.9	1.5	1.3	0.9	0.5
Cornea	5.1	4.5	7.1	4.9	1.3
Sclera	1.7	2.6	2.6	3.7	0.5
Aqueous humor	72.1	259	74.3	21.8	2.1
Iris and ciliary body	9.1	14.4	7.7	18.0	2.0
Lens	2.5	5.0	10.0	24.0	8.0
Vitreous body	5.0	4.5	2.5	8.0	1.0
Retina and choroid	3.8	5.7	6.7	10.5	1.3
Optic nerve	5.3	4.1	4.2	6.6	0.7
Serum	0.8	1.1	0.5	2.8	0.5

As to the retention time within the conjunctival sac, a concentration as high as 1340 ± 791 μg/ml was obtained 5 min after instillation in the normal rabbit eye. An ordinary dose requires the instillation of 0.05 ml (one drop) in each eye, making a total amount of 0.1 ml (two drops). Since 0.3% NFLX contains 3 mg NFLX in 1 ml, approximately 300 μg NFLX should be contained in two drops (corresponding to 3,000 μg/ml). Consequently, the instilled NFLX decreased to 1/2 the original amount 5 min after instillation. After 15 min, a further decrease to 1/2 occurred to reach 628 ± 543 μg/ml. The concentration was about 10% of the original level, 314 ± 418 μg/ml after 30 min. The subsequent decrease was relatively rapid, reaching 3% of the original level (89.0 ± 87.8 μg/ml) 1 h later, 0.6% (19.9 ± 12.7 μg/ml) 2 h later, and 0.2% (6.1 ± 3.9 μg/ml) 3 h later. Even after 6 h, 0.3% (8.3 ± 9.9 μg/ml) NFLX remained.

Experimental results of Mazuel/Clair are available

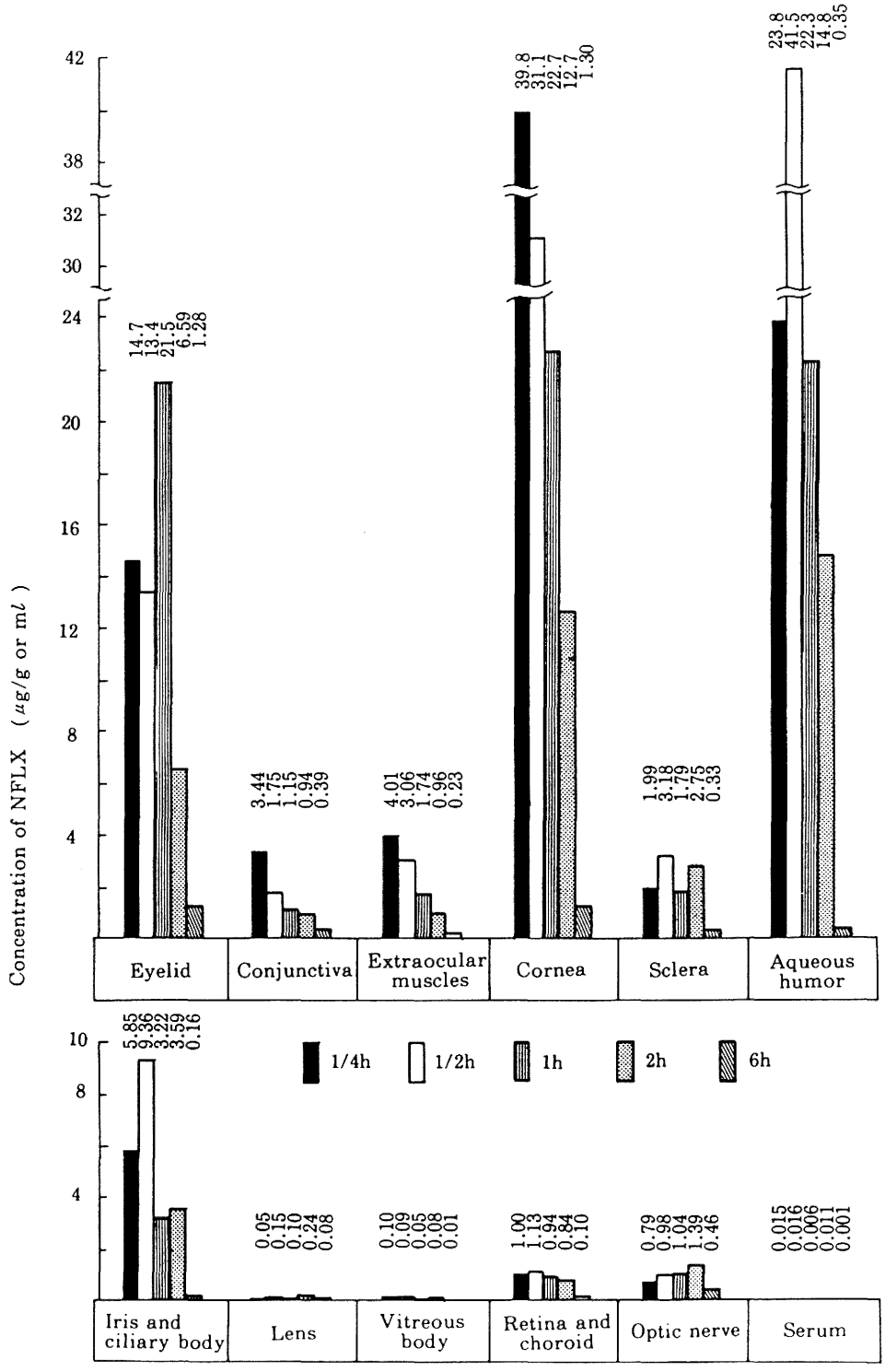

Fig. 3. Ocular tissue concentrations after instillation of 0.3% NFLX ophthalmic solution (inflamed eye)

regarding the concentration of NFLX in the cul-de-sac after instillation.[4] NFLX concentration was 355±136 μg/ml 15 min after a single instillation of 0.05 ml of 0.3% NFLX solution in the rabbit eye, 150 μg/ml at 30 min, 147±15 μg/ml at 1 h, 55±29 μg/ml at 2 h and 104±40 μg/ml at 3 h, followed by a decrease to 39±7 μg/ml at 4 h and 31±14 μg/ml at 6 h. These results are in accord with our results in finding the same concentration of NFLX in cul-de-sac 6 h after the instillation, despite some difference in the experimental method.

Ofloxacin (OFLX), a drug belonging to the same group as NFLX, was studied in a similar manner.[5] OFLX was detected in the cul-de-sac up to 30 min after the instillation of 2 drops of 0.3% solution in the rabbit eye, but only trace amounts were found after 1 h. It was possible to detect OFLX even 3 h after the instillation of 1 or 2 drops of 0.5% OFLX solution.

Consequently, the present results and those of Mazuel/Clair on the instillation of 0.3% NFLX solution suggest the possibility of a longer half life for NFLX in the cul-de-sac than OFLX after the instillation of 0.3% solution. While the reasons for this remain unknown, differences in the tissue affinity of the drugs or differential viscosity of the base may have contributed to it. Further studies are necessary to elucidate the mechanism. The long retention time of 0.3% NFLX ophthalmic solution indicates a long duration of action, suggesting clinical usefulness.

To test the premise of clinical usefulness, NFLX concentrations in the cul-de-sac will be evaluated regarding the required concentration for 90% inhibition of the growth of various bacteria (MIC 90). MIC90 against clinical isolates of *Pseudomonas aeruginosa* was 3.13 μg/ml, and those against *Klebsiella pneumoniae, E. coli,* Family *Enterobacteriaceae,* Genus *Proteus, Haemophilus influenzae, Staphylococcus aureus* and *coagulase negative Staphylococcus* were 0.78, 0.39, 1.56, 0.20, 6.25, 0.10, 3.13 and 3.13 μg/ml, respectively. NFLX retained within the cul-de-sac maintained concentrations above the MIC90 up to 6 h after instillation. Since the MIC90 against *Serratia* is 25 μg/ml, concentrations with 90% positive effect against *Serratia* were maintained up to 1 h following instillation.

The long duration of the effect of 0.3% NFLX eye drops is thus quite evident, promising extensive clinical usefulness. This is also related to the frequency of instillation, suggesting a possidglity of decreasing the frequency of instillation from 3 or 4 times to twice a day.

Concentrations of NFLX penetrating into the ocular tissue were then evaluated. Instillation was carried out 5 times every 5 min in the normal rabbit eye to measure NFLX concentrations in the outer parts of the eye at all times between 1/4 and 6 h. NFLX levels between 0.38 and 7.84 μg/g were obtained. The highest concentration of 1.01-7.84 μg/g was found in the cornea, followed by the eyelid, bulbar conjunctiva, extraocular musles and sclera. The concentration of penetration to the inner parts of the eye was quite low, always staying below 1.0 μg/g or ml.

In the inflamed eye, concentrations of the drug penetration to the outer parts of the eye were higher, 0.23-39.8 μg/g. The NFLX concentration in the cornea was the highest, 1.30-39.8 μg/g. Among the tissues in the inner parts of the eye, the penetration of a high concentration of 0.35-41.5 μg/ml to the aqueous humor was noted, followed by the iris and ciliary body at 0.16-9.36 μg/g. In other tissues, the concentration was rather low, around 1.0 μg/g or ml.

On comparison of the NFLX concentration between the inflamed and normal eyes, NFLX concentrations were higher in each tissue of the inflamed eye than in the corresponding tissue of the normal eye. Such a tendency was especially pronounced in the aqueous humor, cornea, eyelid and iris and ciliary body.

Facilitation of the intraocular penetration of NFLX in the inflamed eye showed exactly the same tendency as seen in the results obtained previously in our experiments using micronomicin eye drops[6] and sisomicin eye drops[7] in the rabbit eye. After injury to the corneal epithelium due to exposure to alkaline solution, the instilled drug was shown to penetrate readily from the cornea to the inside of the eye ball. This is especially advantageous in the treatment of ocular infections, especially corneal infection.

Concentrations of NFLX in the ocular tissue were then evaluated from the viewpoint of their antibacterial activity. As described above, the MIC90 of NFLX against various bacteria ranges between 0.20 and 25 μg/ml. In the outer parts of the normal eye, 90% effective concentrations were obtained for all the gram negative *bacilli*, except for *Serratia.* In the inflamed eye, 90% effective concentrations of NFLX were obtained in the outer parts of the eye, the aqueous humor, the iris and ciliary body. Especially in the cornea and aqueous humor, the concentrations of NFLX were higher than the MIC90 for *Serratia.* This would provide a marked advantage in the treatment of ocular infection due to *Pseudomonas aeruginosa* and *Serratia,* two important corneal pathogens. The concentration in the vitreous body was less than MIC90.

The 0.3% NFLX ophthalmic solution is thus

expected to provide effective clinical action on bacterial ocular infections, especially corneal infections caused by gram negative rods such as *Pseudomonas aeruginosa* and *Serratia*.

The continuance of effective drug concentration in the cul-de-sac for a long period will also aid in achieving a therapeutic effect.

REFERENCES

1) Ito A, Hirai K, Inoue H, Koga H, Suzue S, Irikura T, Mitsuhashi S: *In vitro* antibacterial activity of AM 715, a new nalidixic acid analog. *Antimicrob Agents Chemotherapy* **17**: 103-108, 1980.
2) Murayama S, Hirai K, Ito A, Abe Y, Irikura T: Studies on absorption, distribution and excretion of AM715 in animals by bioassay method. *Chemotherapy Jap* **29**(S-4): 98-104, 1981.
3) Ozaki T, Uchida H, Irikura T: Studies on metabolism of AM 715 in human by high performance liquid chromatography. *Chemotherapy Jap* **29**(S-4): 128-135, 1981.
4) Mazuel/Clair: Notebook MCPRL054 p 11-17, April, 1983.
5) Fukuda M, Tomii T, Tsuzuki H, Sasaki K: Measurement of Cephaloridine and Ofloxacin in Cul-de-sac. *Eye Jap* **2**: 1450-1453, 1985.
6) Ooishi M, Nishizuka K, Motoyama M, Ogawa T: Studies of DE 020 Eye drops. Report 1. Intraocular penetration. *Acta Soc Ophthalmol Jap* **85**: 405-409, 1979.
7) Ooishi M, Nagai S: Intraocular penetration of Sisomicin Eye drops. *Acta Med et Biol* **32**: 99-105, 1984.