CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
21-199

PHARMACOLOGY/TOXICOLOGY REVIEW

Review and Evaluation of Pharmacology/Toxicology Data

Key words: Conjunctivitis, Levo-isomer, and Ofloxacin

Reviewer Name: Asoke Mukherjee

Division Name: Analgesic, Anti-inflammatory and Ophthalmic drug products.
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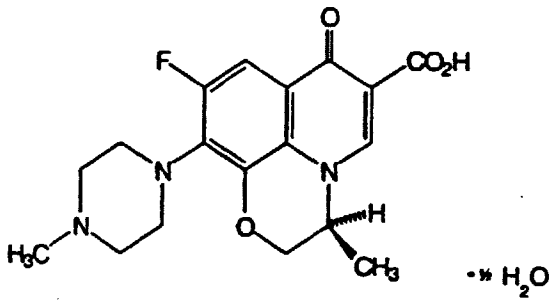
IND/NDA Number: 21-199
Serial Number/date/type of submission: 000, 505-(b) (1)
Information to the sponsor: Yes ( ) No (X)
Sponsor: Santen Inc., California 94558.
Manufacturer of Drug Substance: Daiichi Pharmaceutical Co. Ltd.
Drug: Levofloxacin
Code Name: DR-3355
Generic Name: Levofloxacin Ophthalmic solution 0.5%
Trade Name: Quixin
Chemical Name: (S)-9-fluoro-2,3-dihydro-3-3-methyl-10-(4-methyl-1-piperizineyl)-7-oxo-7H-pyrido[1,2,3-de]1,4-benzoxazine-6-carboxylic acid hemihydrate.
CAS registry number: 138199-71-0
Molecular formula/Molecular Weight: $C_{18}H_{20}FN_3O_4$,1/2H_2O; 370.38

Structure:



Relevant IND/NDA/DMF: NDA 20-634, NDA 20-635, [redacted]
Drug Class: Antimicrobial agent
Indication: Bacterial conjunctivitis
Clinical formulation:

Levofloxacin hemihydrate, 5.12 mg/ml
Benzalkonium chloride, 0.05 mg/ml
Sodium Chloride [redacted]
Hydrochloric acid and sodium hydroxide [redacted]
Purified water [redacted]

Route of administration: Ophthalmic drops

Proposed clinical use:

The NDA is submitted for the approval of levofloxacin ophthalmic solution for the treatment of bacterial conjunctivitis against Gram positive and Gram negative bacteria. The proposed dose is one to two drops per eye every 2 hours up to 8 times per day on days 1-2. During days 3-5, one or two drops per eye every 4 hours up to 4 times a day. The sponsor stated that each drop of 0.5% ophthalmic solution of LVFX is about 350 µg (page 10, vol 8).

Previous clinical experience, introduction and drug history:

Levofloxacin is the levo isomer of the fluoroquinolone antibiotic ofloxacin.

Levofloxacin (LVX) is a broad-spectrum antibiotics and L -isomer of ofloxacin, an approved drug. LVX is already approved for systemic administration as tablets and parenteral formulations. The recommended dose for Levofloxacin is 500 mg/day/oral or slow IV injections. The sponsor submitted letter to cross-refer the approved NDAs of oral and IV levofloxacin. The sponsor stated that preservative free levofloxacin ophthalmic solution (0.5%) is approved in Japan. The racemic ofloxacin ophthalmic drops (0.3%) are also approved in the US for the treatment of conjunctivitis.

Studies reviewed within this submission:

A 26-week ocular toxicity study of levofloxacin (DR-3355) ophthalmic solutions in pigmented rabbits. Page 11-002 and vol 11.

Maximization test of DR-3355 in guinea pigs. Page 123, vol 11.

Determination of the minimum erythema dose (MED) and phototoxicity of levofloxacin in guinea pigs: A supplementary study. Page 156, vol 11.

Skin photosensitization study of levofloxacin in guinea pigs. Page 185, vol 11.

Pharmacokinetics of levofloxacin, a new fluoroquinolone agent, after instillation of 0.5% ^{14}C-levofloxacin ophthalmic solution to pigmented rats. Page 271, vol 11.

Ocular pharmacokinetics of levofloxacin (LVFX) after instillation to pigmented rabbit eyes. Page 293, vol 11.

Determination of levofloxacin in tear fluid after instillation of levofloxacin ophthalmic solutions in rabbits. Page 307, vol 11.

Melanin affinity in vitro study of levofloxacin in comparison with various drugs. Page 324, vol 11:

30-day repeat dose ocular toxicity study of levofloxacin ophthalmic solution in pigmented rabbits. Page 004, vol 10.

Ocular toxicity study of levofloxacin (DR-3355) ophthalmic solution after 2-week administration in white rabbits. Page 180, vol 9

One-day ocular irritation study of a topical ophthalmic anti-infective solution in rabbits. Page 1, vol 9.

One day ocular irritation study of levofloxacin (DR-3355) ophthalmic solution in rabbits-after 10 times instillation in a single day. Page 224, vol 8:

Treatment of experimental Staphylococcus aureus endophthalmitis with topical levofloxacin. J. Eye, 1995, 12 (5), 795-798. Page 179, vol 8.

Prophylaxis using levofloxacin ophthalmic solution in experimental S. aureus endophthalmitis. J. Eye, 1997, 14, 107-112. Page 191, vol 8.

Prophylaxis using levofloxacin ophthalmic solution and ointment in experimental P. aeruginosa keratitis. Page 208, vol 08.

Studies not reviewed within this submission:

Following publications were read but a written review is not presented.

Ophthalmotoxicity and ototoxicity of new quinolone antibacterial agent levofloxacin in [redacted] rats. Page 216, vol 11.

ESR detection of free radical and active oxygen species generated during photolysis of fluoroquinolones. Page 222, vol 11.

Participation of reactive oxygen species in phototoxicity induced by quinolone antibacterial agents. Page 228, vol 11.
Stimulation of prostaglandin production by quinolone phototoxicity in Balb/c 313-mouse fibroblast cells in vitro. Page 235, vol 11.

Involvement of reactive oxygen species, protein kinase C, and tyrosine kinase in prostaglandin E_2 production in Balb/c 3T3 mouse fibroblast cells by quinolone phototoxicity. Page 241, vol 11.

Fluoroquinolone toxicity profiles: A review focusing on newer agents. Page 247, vol 11.

Sparfloxacin but not levofloxacin prolongs cardiac repolarization in rabbit purkinje fibers. Page 260, vol 11.

Fluoroquinolone antibiotics block neuromuscular transmission. Page 267, vol 11.

Pharmacology:

Levofloxacin is the levo isomer of fluoroquinolone antibiotic ofloxacin. Its mode of action is possibly inhibition of bacterial replication by the inhibition of transcription of DNA. It is also known to be an inhibitor of DNA topoisomerase. Levofloxacin inhibits both gram positive and gram negative bacteria and is an effective broad spectrum antibiotic.

The sponsor submitted published reports of several studies to substantiate the efficacy of levofloxacin as the antibacterial agent of ocular infections and corneal ulcers. The reports are discussed briefly as follows:

1. Treatment of experimental Staphylococcus aureus endophthalmitis with topical levofloxacin. J. Eye, 1995, 12 (5), 795-798. Page 179, vol 8.

In the Japanese white rabbit model effects of 0.3% levofloxacin and ofloxacin were compared for the inflammatory changes in the eye. The dose was one drop three times a day for 3 days. Results show that 0.3% levofloxacin is more effective than ofloxacin for the treatment of infectious endophthalmitis.

2. Prophylaxis using levofloxacin ophthalmic solution in experimental S. aureus endophthalmitis. J. Eye, 1997, 14, 107-112. Page 191, vol 8.

S.aureus was injected into the vitreous in Japanese white rabbits to induce endophthalmitis. Effect of 3% LVFX at 2 drops x6 times per day for 5 days was examined. The control group received saline. Ocular inflammation was reduced in the LVFX treated eyes compared to the saline treated eyes.

3. Prophylaxis using levofloxacin ophthalmic solution and ointment in experimental P. aeruginosa keratitis. J. Eye, 1996, 13, 249-253. Page 208, vol 08.

The efficacy of the LVFX 0.5% ophthalmic solution and 0.5% ophthalmic ointment was compared to the respective vehicle in bacterial keratitis in the Japanese white model. The dose was one drop of solution or 1 cm of the ointment in each eye six times daily for 3 days. Results of the experiment suggest that 0.5% LVFX solution or ointment was effective in the prevention of corneal infection. Histopathological examination of cornea following necropsy showed that the vehicle treated eyes developed detachment of corneal epithelium, hypertrophy of the corneal stroma and infiltration of neutrophils. The treated eyes showed structurally normal cornea. However, inflammatory cell infiltration in the corneal stroma was reported. Data suggest that 0.5% LVFX solution or ointment was effective for the treatment of experimental keratitis in the rabbit model.

The sponsor also stated that 1.5% LVFX solution was effective in bacterial ulcerative keratitis.

Summary:

Levofloxacin showed antibacterial property in in vivo studies at 0.3 to 3.0% concentrations in the albino rabbit model. The sponsor has also submitted minimum effective concentration profiles of several organisms that are commonly present in ocular infections (page 134 vol 1).

Safety Pharmacology:

The sponsor submitted summary of data on safety pharmacology from the approved NDA in page 4 vol 8. Some of these data are shown in the following table.

System	Species	Dose, route	Findings
CNS	Mouse	600 mg/kg, po	CNS depression
		200 mg/kg, IV	Convulsions followed by CNS depression
	Rat	200 mg/kg IV	Tranquilizing effect
	Rabbit	200 mg/kg/oral	Decreased body Temp.
	Cat	30 mg/kg/IV	Seizures
ANS	Cat	20 mg/kg IV	Decreased Ganglion transmission
CVS	Dog	6 mg/kg IV, bolus	Decrease BP and respiration depth
GI	Mouse	200 mg/kg IV	Inhibition of GI motility
	Rat	200 mg/kg po	Decreased gastric secretion
Urinary	Rat	200 mg/kg po or IV	Decreased urinary vol

The proposed clinical dose is 16 drops per eye for days 1-2; 8 drops per eye for days 3-5. The maximum total dose will be 39.2 mg for 5 days. The average daily dose is 7.8 mg per 70 kg human subject or 0.11 mg/kg. The proposed human dose has large margin of safety compared to the data presented in the above table. Therefore, systemic safety concerns from the treatment in the eye are very little on the basis of the preclinical data.

Pharmacokinetics/Toxicokinetics:

Pharmacokinetics of levofloxacin, a new fluoroquinolone agent, after instillation of 0.5% ^{14}C-levofloxacin ophthalmic solution to pigmented rats. Page 271, vol 11.

The data were published in Xenobiotic Metabolism and Disposition, 1997, 12(4): 281-288.
The pharmacokinetic of levofloxacin was investigated after single or repeated topical applications of 0.5% ^{14}C-LVFX ophthalmic solution to the pigmented rat eyes.

Ocular distribution, plasma levels of the radioactivity, levels in the systemic tissues and excretion of radioactivity in the urine and feces were reported.

The experiment was conducted in six weeks old brown Norway male rats. 0.5% ^{14}C-LVFX ophthalmic solution was prepared in physiological saline (specific activity 2.12 MBq/mg). One µL of the ophthalmic solution was instilled in each eye. The sponsor stated that the dose was 10 µg/rat. In separate experiments, rats were also injected 10 µg/rat in 0.2 ml volume intravenously into the tail vein. Blood samples were taken from the abdominal aorta under ether anesthesia at 5, 10, 15, 30 min, 1, 2, 4, 8, 24 and 168 hours after the test drug. Blood samples were also taken at 1, 3 and 6 months after the single dose either after the instillation into the eye or by IV route. Radioactivity of the plasma was determined.

Following tissue samples were taken after single instillation into the eye at 15 min, 30 min, 1, 4, 24, 168 hours, 1, 3 and 6 months after the administration.

Cerebrum, cerebellum, Harderian gland, heart, liver, lung, kidney, tongue, stomach, duodenum, ileum, and colon. Eye tissues and samples were aqueous humor, cornea, iris/ciliary body, vitreous, retina, retinal pigment epithelium/choroid and sclera. Radioactivity of the tissue samples was determined. The sponsor stated that systemic tissues were not sampled at 1, 3 and 6 months after instillation.

Animals were placed in the metabolism cage for 96 hours after single ophthalmic dosing for the collection of urine and feces. Urine and fecal samples were collected every 24 hour intervals. Radioactivity of the

plasma, feces and urine samples was determined by after appropriate treatment.

Plasma metabolites were separated and identified by TLC and HPLC methods at 10 minutes after dosing into the eye.

Frozen sections of whole animals were prepared at 15 min, 1, 24 and 168 hours after single dose in the eyes. Presence of radioactivity was shown by autoradiographic methods.

In another set of experiments, 10 µg of ^{14}C-LVFX was instilled into the eye in 1 µL volume. Treatment was given to both eyes, three times daily at 4-hour intervals for 14 days. Blood and ocular tissue samples were taken at 1, 4, 24 and 168 hours after day 7. Blood and ocular tissue samples were taken at 1, 4, 24, 168 hours, 1, 3, and 6 months after 14 days of the treatment.

Results:

Single dose:

The maximum plasma radioactivity (concentration) of 25.2 ng eq/ml was noted at 10 min post dose after ocular administration. The maximum radioactivity in the plasma was about 33.7 ng eq/ml after IV administration. The plasma levels over time curves following IV or ocular administration were almost similar. The radioactivity was not detectable after 8 hours. The concentration as ng eq/g or ml of the samples from ocular tissues after ocular dosing is shown in the following table.

Tissues	0.25 hr	0.5 hr	1 hr	4 hr	24 hr	168 hr	1 mth	3 mth	6 mth
Aq. Humor	531	517	161	6.7	Nd	nd	nd	nd	Nd
Cornea	5772	3615	1427	86	15	4	nd	Nd	Nd
Iris/ciliary body	6254	9860	16076	19143	13111	6568	3856	2137	558
Lens	19	10	7	5	1.7	nd	nd	Nd	Nd
Vitreous body	240	120	63	44	23	15	nd	Nd	Nd
Retina	43	29	19	7	Nd	nd	nd	Nd	Nd
Choroid and retinal Pigmented epithelium	2542	3294	3265	3192	2442	1594	942	351	77
Sclera	642	470	454	273	183	144	nd	Nd	Nd

Nd = not detected.

Maximum concentration was detected within 15 minutes in aqueous humor, cornea, lens, vitreous humor, retina and sclera. The elimination of the drug from iris was delayed due to the possible binding of the drug or its metabolites to the iris pigment. A similar observation was noted in the choroid/retinal-pigmented epithelium.

The concentration (ng eq/g or ml) in the systemic tissues after ocular delivery is shown in the following table.

Tissues	0.25 hr	0.5 hr	1 hr	4 hr	24 hr	168 hr	1 mth	3 mth	6 mth
Whole blood	22	13	10	0.8	Nd	nd	nd	Nd	Nd
Plasma	24	16	12	1.4	Nd	nd	nd	Nd	Nd
Cerebrum	1.3	1.5	1.0	Nd	Nd	nd			
Cerebellum	1.5	1.3	1.2	Nd	Nd	nd			
Harderian Gland	68	17	11	1.8	Nd	nd			
Heart	30	16	10	1.4	Nd	nd			
Liver	46	33	22	Nd	Nd	nd			
Lung	39	17	16	nd	Nd	nd			
Kidney	84	70	45	8.3	Nd	nd			
Tongue	38	22	20	3.0	Nd	nd			
Stomach	31	14	11	1.2	Nd	nd			
Duodenum	64	69	38	3.0	1.2	nd			
Ileum	21	84	69	8.4	1.8	Nd			
Colon	18	14	9.6	1.9	1.0	Nd			

As shown in the above table, systemic levels of the drug were smaller than several ocular tissues. Maximum concentrations were observed in the kidney that sustained up to one hour. The drug level was negligible at 4 hours in most of the systemic tissues. The data suggest that levofloxacin was cleared within 4 hours from the systemic tissues and blood in the rat model.

Radioluminographic study also showed the presence of detectable labels of radioactivity in the eyeglobe and gastrointestinal tract at 24 hours after single dose of the drug in eyes. Radioactivity was detected in the eyeglobe only at 168 hours post dose.

Cumulative (0-96 hrs) urinary and fecal excretion of radioactivity as % of the dose suggests 99.9% of the dose was excreted within 96 hours after single instillation into the eye. About 84% of the doses were excreted within 24 hours. Almost equal amount of the drug was excreted in the urine and feces.

Plasma metabolite analysis showed most of the radioactivity was due to levofloxacin at 10 minutes after the ocular dose. Another metabolite with faster retention time was present. However, the quantity and the nature of the metabolite were not identified in the study.

Repeated dose study:

Only traces of radioactivity were detected in the plasma or whole blood at four hours after last instillation of 19th or 40th doses. Data suggest that levofloxacin was not accumulated in the systemic circulation after repeated dosing. However, most of the radioactivity was detected up to 168 hours post dose in iris/ciliary body, pigmented epithelial cells of retina and sclera following repeated dosing. Therefore, levofloxacin binds and accumulates in the pigmented tissues of the eye.

Conclusion:

Levofloxacin is bioavailable to the ocular tissues in rats. It is not accumulated in the plasma or systemic tissues. However, it is retained in the pigmented ocular tissues up to six months after repeated dosing. Most of the radioactivity of ^{14}C levofloxacin was due to the parent compound. However, another radioactive peak with faster retention time was detected in the HPLC assay. Excretion of radioactivity in the urine and feces was 44.4 and 55.5% of the single dose, respectively, within 96 hours.

Ocular pharmacokinetics of levofloxacin (LVFX) after instillation to pigmented rabbit eyes.
Page 293, vol 11.

Pharmacokinetics of LVFX was determined following instillation of ^{14}C LVFX 0.5% ophthalmic solution into the rabbit eyes. Male Dutch belted rabbits weighing 2 kg were used in the study. Radioactivity was 1.1 MBq/ml. Each eye was treated with a single dose of 50 µL of the ophthalmic solution. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 24, 168 hours, 1, 3 and 6 months after dosing from auricular artery. Animals were anesthetized by sodium pentobarbital and exsanguinated. The following eye tissues were isolated:

Bulbar conjunctiva, palpebral conjunctiva, extraocular muscles, cornea, aqueous humor, iris/ciliary body, lens, vitreous body, retina, retinal pigment epithelium/choroid, sclera, lacrimal gland and Harderian gland. The radioactivity in the tissues was detected by a

Radioluminographic examination of the eyeglobe was conducted at 2 and 168 hours post dose. Eyeglobes were isolated and frozen in liquid nitrogen. Frozen sections of 20 µm thickness were prepared. Each section was exposed to photographic film and an image analyzer analyzed the image.

Microautoradiography of the retinochoroidal membrane and sclera were conducted at 12 hours post dose.

Results:

Ocular tissues, blood and serum concentrations of radioactivity in ng eq of LVFX per GM or ml are shown in the following table.

Tissue	.25 h	.5 h	1 h	2 h	4 h	8 h	24 h	168 h	1 mth	3 mth	6 mth
Serum	33	60	36	nd	nd	nd	nd	nd	Nd	nd	Nd
Whole blood	25	42	20	nd	nd	nd	nd	nd	Nd	nd	Nd
Bulbar conjunctiva	1433	636	152	110	38	38	nd	nd	Nd	nd	Nd
Palpebral conjunctiva	1058	695	154	61	26	nd	nd	nd	Nd	nd	Nd
Extra ocular muscles	1364	447	138	118	20	nd	nd	nd	Nd	nd	Nd
Cornea	6193	6839	3541	2839	671	274	54	nd	Nd	nd	Nd
Aq. Humor	539	842	673	624	nd	nd	nd	nd	Nd	nd	Nd
Iris/ciliary body	986	1705	4484	11514	5038	4844	3521	1804	641	105	Nd
Lens	Nd	nd	14	28	10	8	nd	nd	Nd	nd	Nd
Vitreous	3.8	6.5	nd	9.4	nd	nd	nd	nd	Nd	nd	Nd
Retina	Nd	100	nd	nd	nd	nd	nd	nd	Nd	nd	Nd
Retinal pigmented epithelium/Choroid	739	1448	1605	3269	1843	1766	1103	898	297	84	Nd
Sclera	173	198	260	166	nd	29	nd	nd	Nd	nd	Nd
Lacrimal gland	45	89	57	40	nd	nd	nd	nd	Nd	nd	Nd
Harderian gland	27	30	16.5	22.3	nd	nd	nd	nd	Nd	nd	Nd

Nd = not detected.

Data indicate that levofloxacin is bioavailable to the ocular tissues following a single dose. However, the clearance of the drug from pigmented tissues was slow. The sponsor stated that the elimination half-life in iris/ciliary body to be 20.8 days and that for retinal pigmented tissues to be 25.7 days.

Radioluminographic and microautoradiographic studies also confirmed that the levofloxacin is in the posterior chamber of the eye and the drug concentrates in the pigmented tissues.

Therefore, levofloxacin is not metabolized immediately after administration.

It is concluded that levofloxacin is bioavailable to the ocular tissues and blood after single ocular dosing in pigmented rabbit eyes. However, disappearance of the drug from the pigmented tissues of the eye was slower than other ocular tissues.

Determination of levofloxacin in tear fluid after instillation of levofloxacin ophthalmic solutions in rabbits. Page 307, vol 11.

Dutch pigmented and New Zealand albino rabbits were used in the study. The sponsor has not mentioned the sex of the animals used in the study. Several concentrations of ophthalmic solution at pH 6.5 were used in the study. The lot numbers and concentrations are shown in the following table.

0.15% LVFX ophthalmic solution	Lot # DR930213-2
0.3% LVFX ophthalmic solution	Lot # DR930222-1
0.5% LVFX ophthalmic solution	Lot # DR930222-2
3.0% LVFX ophthalmic solution	Lot # DR940902-1

Single dose of 50 µL of the test solution was applied into each eye. Tear fluids were sampled at 5, 10, 15, 20, 25, 30, 60, 120, 180, 300 and 480 minutes using [redacted] Levofloxacin was extracted and assayed by HPLC.

Results of the experiment suggest that excretion of LVFX in the tear fluids increased with the dose. Within one hour after the application, tear concentration of the drug was above 1 µg/ml at 0.3-3% LVFX. The sponsor stated that the concentration was effective in killing most of the E.coli and can ensure antibacterial activity in the conjunctiva. The sponsor did not identify the differences in the level of levofloxacin in the tear fluids of pigmented and albino eyes.

Melanin affinity, in vitro study of levofloxacin in comparison with various drugs. Page 324, vol 11:

Melanin pigment was isolated from bovine eyes. Several test substances were investigated for the melanin binding potential including that of levofloxacin. The drug substance was dissolved in 5 ml of 0.02M-phosphate buffer at pH 7.4 and 5 mg of melanin was added to the buffer. The final concentration of the test substance was 1 or 0.1 mM. The mixture was incubated for 24 hours at 37C. [redacted]

The following drug substances were used for the melanin binding assays.

Ofloxacin, levofloxacin, norfloxacin, ciprofloxacin, lomefloxacin, chloroquine, chlorpromazine, timolol, befunolol, micronomycin, dibekacin, sisomicin.

Results:

The affinity (%) to bovine eye melanin at 1 mM and 0.1 mM concentrations is shown in the following table.

Drug	1 mM	0.1 mM
Ofloxacin	29	68
Levofloxacin	27	66
Lomefloxacin	39	69
Norfloxacin	60	91
Ciprofloxacin		83
	46	99
	55	98
	78	98
	61	91
Timolol	28	51
Chlorpromazine		97
Chloroquine	85	99

Data suggest that the affinity of drugs was lower at the higher concentration. Fluoroquinolones except norfloxacin showed almost equal affinity to the bovine melanin. Dissociation of the drug from the binding sites over 168 hours suggest that fluoroquinolones dissociate slowly from the melanin binding sites.

Comments and conclusions of PK/TK studies:

Distribution and levels of ^{14}C levofloxacin in the ocular tissues, plasma and selected systemic tissues were investigated in brown Norway rats after a single ophthalmic dose of 10 µg/rat and repeated doses at 10 µg/rat for 14 days. The drug did not accumulate in the blood or systemic tissues after repeated dosing. It is distributed to the anterior and posterior chambers of the eye. Levofloxacin binds to the pigmented tissues of the eye and the drug was detected 3-6 months after the dosing in rats. A similar observation was noted in Dutch rabbits at 500 µg/rabbit doses into the eyes. Half-life of the drug in iris/ciliary body was about 20 days and that for the pigmented epithelium of retina was 25 days in the rabbit model.

Assay of the drug in tear fluids in pigmented and non-pigmented rabbits at 0.3-3.0% ophthalmic solutions showed the tear level was higher than 1 µg/ml. The sponsor stated that the levels were sufficient for the antibacterial activity of the drug in eyes for E.Coli.

The binding of the drug in bovine melanin in vitro confirmed that levofloxacin dissociated slowly from the melanin binding sites. The data support the accumulation of the drug in the pigmented eye tissues following single and multiple doses into the eye.

Toxicology:

ID# 939403

Title: One-day ocular irritation study of levofloxacin (DR-3355) ophthalmic solution in rabbits-after 10 times instillation in a single day:

Page 224, vol 8:

Conducting Laboratory: Santen Pharmaceutical Laboratory, Osaka, Japan

Date of study initiation: Feb 12, 1993

GLP compliance: Yes

QA report: Yes

Methods:

Species: Male Japanese white rabbits

#/group: 5

Age: 8-9 weeks

Weight: 1.91-2.38 kg

Dosage groups in administered unit:

Group	Left eye	Right eye
1.	Vehicle	Untreated
2.	0.3%	Untreated
3.	1.0%	Untreated
4.	3.0%	Untreated
5.	10%	Untreated
6.	25%	Untreated

Route and volume:

Ophthalmic solutions were instilled into the left eye. One drop was applied ten times within 24 hours at 30 min intervals starting 9 AM in the morning. Right eye served as the untreated control. The sponsor has not indicated the volume per drop. However, each drop is generally considered to be about 30-50 µL. The sponsor stated that 0.3% ophthalmic solution is chosen as the minimum concentration because ofloxacin is approved as 0.3%.

Drug Lot #:

Lot numbers of the formulations are shown in the following table.

Formulation	Lot number	pH	% of labeled amount
1. Vehicle	DR930316-6	5.77	-
2. 0.3% LVFX	DR930316-1	6.49	99.1
3. 1.0% LVFX	DR930316-2	6.50	99.5
4. 3.0% LVFX	DR930316-3	6.52	100.5
5. 10% LVFX	DR930316-4	6.58	97
6. 25% LVFX	DR930316-5	6.58	97.4

Formulation and the vehicle:

The sponsor has not provided the formulation. However, for the clinical batches, levofloxacin was dissolved in water as the vehicle. The pH was adjusted with NaOH or HCl.

General conditions and clinical signs:

General conditions and body weight were recorded on the day before the treatment. Clinical conditions were checked before the first instillation and 20 hours after the last instillation.

Ophthalmic examinations:

The blinking frequency was recorded in the treated eye before and after the first application of the drug or vehicle.

Ocular irritation was monitored one day before the treatment, immediately before the treatment and at 1, 3, 5 and 20 hours after the last instillation according to the method of Draize (already described in page 16).

Any changes in the anterior chamber of the eye were examined by the slit lamp biomicroscopy.

Slit lamp examinations were conducted one day before the treatment and 1,3 5, 20 hours after the last dose. If abnormalities were found, the examination was continued until recovery.

Corneal damage: Cornea was stained by fluorescein dye five hours after the final instillation and examined for corneal damage by slit lamp. Following scoring system assessed the damage to the cornea.

Grade of staining	Score
No staining	0
Visible by slit lamp	0.5
Slightly visible macroscopically and stained spot observed only at the upper portion of the corneal epithelium by slit lamp examinations	1.0
Visible without magnification	2.0
Corneal staining higher than 25% and less than 50%	3.0
Corneal staining greater than 50%	4.0

Results:

No abnormalities in the general conditions of the cornea were recorded in the study. Signs of discomfort, characterized by rubbing the treated eye was observed dose dependently with 10 and 25% solutions.

Ophthalmic examinations:

Blinking frequencies with 25% ophthalmic solution were increased compared to the vehicle control. The data are shown in the following table.

Group	Before instillation	After instillation
Vehicle	0.6	2.0
0.3%	0.2	1.2
1.0%	0.8	1.6
3.0%	0.8	1.8
10%	1.2	1.0
25%	0	7.4*

*Statistically significant.

Slit lamp examinations for irritation and flare in the anterior chamber:

Iris:

Iritis in the treated eye was observed with the 25% ophthalmic solution that persisted between 1 to 20 hours after the last dose. The average score ranged from 0.4 to 0.8. The criteria were 0= normal, 1= abnormal but reacts to light; 2= no reaction to light and hemorrhage. Based on the criteria, iritis observed in the group is considered to be slight.

Palpebral conjunctiva:

Redness and edema were observed in the palpebral conjunctiva with 10 % ophthalmic solutions between 1 to 20 hours after the last dose. However, statistically significant changes were observed only at 25% LVFX between 1 hour to 5 days after the last dose. The redness score was between 0.3-2.6 when 0 to 3 scale of score was used. Accordingly, redness in the palpebral conjunctiva with the 25% ophthalmic solution is considered to be moderate in severity.

Edema and swelling in the palpebral conjunctiva was observed with the 10% ophthalmic solution between 1-20 hours after the last dose. The score was between 0.4-0.7 in 0-4 scale. The severity is considered to be slight and statistically not significant compared to the vehicle control group. However, edema and swelling were observed in the palpebral conjunctiva with the 25% ophthalmic solution that lasted almost 2 days after the last dose Statistically significant changes from the vehicle control group were observed up to 20 hours post dose. The average score was between 1.6-2.6 out of 0-4 scale that was considered to be above normal.

Bulbar conjunctiva:

Dose dependent increase in the conjunctival redness was observed with 10 and 25% LVFX up to 20 hours post dose. The changes were statistically significant with the 25% ophthalmic solution up to 5 hours and the score ranged from 1.6-1.8 out of 0-2 scale. It is concluded that the bulbar conjunctival changes were marked with the 25% solution.

Nictitating membrane:

A tendency of swelling was observed with the 10% solution up to 20 hours post dose. However, marked congestion and dilatation of nictitating membrane were observed for two days after the last dose with 25% LVFX that ranged from 0.9-2.0 out of 0-2 scale.

Conjunctival discharge:

Conjunctival discharge was observed with 25% LVFX up to 20 hours post dose. The score ranged from 0.8-1.6 in 0-3 scale. Therefore, conjunctival discharge was moderate in the severity with the 25% ophthalmic solution.

The slit lamp scores with 10 and 25% ophthalmic solutions of LVFX in the treated eyes are shown in the following table.

Observations	1 hr	3 hr	5 hr	20 hr	2 days	3 days after last dose
Iritis						
10%	0	0.2	0.2	0.2	0	0
25%	0.8*	0.4	0.4	0.4	0.2	0
Palpebral conjunctiva, redness						
10%	1.0	1.1	1.1*	1.1	0.7	0.1
25%	2.6*	1.8*	1.6*	2.5*	1.5*	0.9
Palpebral conjunctiva, edema						
10%	0.4	0.5	0.5	0.7	0.1	0
25%	1.3*	2.1*	2.6*	1.6*	0.3	0
Bulbar conjunctiva, redness						
10%	0.9	0.8	0.9	0.9	0	0
25%	1.8*	1.4*	1.6*	1.3	0.7	0.1
Nictitating membrane, swelling						
10%	0.5	0.6*	0.6	0.8	0.2	0
25%	1.5*	1.1*	2.0*	1.7*	0.9*	0.6
Conjunctival discharge						
10%	0	0	0	0.8	0	0
25%	1.6*	0.6	0.8*	0.8	0	0

* Statistically significant

Cornea:

Corneal edema was noted in one rabbit with the 10% solution during 20 hours to 2 days after the last dose. The changes in the cornea became normal on third day post dose. Corneal epithelial damage was also noted 5 hours post dose at slight to mild intensity. However, corneal edema was observed in all five animals with 25% LVFX in the treated eye. The severity varied from mild to severe.

Key study observation:

Ten drops of 10% and 25% LVFX ophthalmic solutions over 5 hours showed inflammatory changes in the iris and conjunctiva. In addition, corneal edema was also present. Most of the above changes were observed within one hour after the last dose. The 25% LVFX ophthalmic solution also induced discomfort to the eye. Preservative free levofloxacin ophthalmic solution at 0.3-3.0% is safe for acute use up to ten drops per day in the rabbit model.

Study ID# M036-96

One-day ocular irritation study of a topical ophthalmic anti-infective solution in rabbits. Page 1, vol 9.

Conducting laboratory:

Date of study initiation: Aug 7, 1996

GLP compliance: None

QA Report: No

Methods:

Species: White New Zealand rabbits

#/sex/group: 3

Age; 12-13 weeks

Weight: Male 2.6-3.0 kg ; Female 2.6-3.1 kg

Dosage groups in administered units:

Group	Drug Dose
1. Untreated control	0
2. Vehicle 1	0.001% BAK
3. Vehicle 2	0.0025% BAK
4. Vehicle 3	0.005% BAK
5. Formulation A	0.3% LVFX and 0.001% BAK
6. Formulation B	0.3% LVFX and 0.0025% BAK
7. Formulation C	0.5% LVFX and 0.001% BAK
8. Formulation D	0.5% LVFX and 0.0025% BAK
9. Formulation E	1.0% LVFX and 0.001% BAK
10. Formulation F	1% LVFX and 0.0025% BAK

LVFX= Levofloxacin; BAK Benzalkonium chloride

Route, form and volume:

Animals were treated with eye drops applied to the cornea. One drop given 10 times at 30 min intervals into the right eye. The left eye served as the untreated control. Animals were observed for 24 hours after the last dose for ocular irritation except one male rabbit that was observed up to 96 hours. The average volume per drop has not been mentioned in the report.

Formulation /vehicle: Not mentioned in the report. However, formulations used in other studies had levofloxacin, benzalkonium chloride, NaOH or HCl as needed for pH adjustment and purified water.

Clinical signs: Clinical observations were made two to four hours after the last dose.

Body weight: Body weights were recorded one day prior to dosing.

Eye examinations:

The cornea, iris and conjunctiva were observed at pretest, prior to each dose and 1, 3, 5 and 24 hours after last dose using a penlight according to the method of Draize (Jr. Pharmacol. Exptl. Therapy, 82, 377-390, 1944). Blinking frequency was determined prior to dosing for 3-minute interval and after the first dose.

Slit lamp biomicroscopic examination was conducted at pretest and at 1, 3, 5 and 14 hours after the last dose. Corneal, iridal and conjunctival scores were recorded according to the Draize scale. Pupillary diameter was determined at pretest and 1, 3, 5 and 24-hour post dose. Corneal damage was evaluated by fluorescein dye at pretest and 5 hours after the last dose.

The ranges for the Draize scores are shown below:

Corneal opacity 0-4, Area of cornea 0-25%=1; 25-50%=2; 50-70%=3; 75% and above 4.

Iris reaction to light 0-2; conjunctival redness 0-3; chemosis of the eyelids and nictitating membranes 0-4; discharges from eye 0-3.

Gross pathology and necropsy: None, histopathology was not conducted.

Animals were sacrificed by overdose of IV sodium pentobarbital at the end of the experiment.

Results:

Clinical signs and mortality: no adverse clinical signs and mortality were recorded in the study.

Ophthalmic examinations:

Macroscopic:

Male rabbits at 0.3% LVFX and 0.0025% BAK showed average conjunctival scores (for redness, chemosis and discharge) that ranged from 2-4 between 8th dose to 3 hour post dose. However, no treatment-related effects on the conjunctival score at other doses were reported.

Female rabbits at 0.3% LVFX and 0.001% BAK also showed average conjunctival scores of 2.7 to 3.3 between 8th dose and 1 hour post dose. However, the scores at other doses were similar to the untreated control.

Macroscopic examinations with a penlight suggest that there was no treatment-related irritation potential in the eye.

Blinking frequency data showed higher frequency in male and female rabbits at 0.5% LVFX/BAK0.001% and 1.0% LVFX/BAK 0.0025%. Data do not indicate there was a treatment-related trend in the discomfort to the eyes due to the presence of isolated incidences of changes in the blinking frequencies.

Slit lamp examinations: The sponsor has not reported any damage to the cornea.

Slit lamp examinations showed no abnormalities in the treated animals except iritis (3.3 out of maximum 10) and conjunctivitis (3.3 out of maximum 20) one hour after at 0.3% LVFX/BAK 0.0025% ophthalmic solution in the male rabbits. The sponsor stated that animal #31 and 32 (gr 6) showed slight iridal irritation.

Female rabbits showed slight conjunctival irritation in the untreated and treated groups mostly 1-5 hours after the last dose.

Animal # 32 (male, gr 6) at 0.3% LVFX/0.0025% BAK showed swelling that appeared like a mass in the nictitating membrane that became normal within 96 hours.

Slit lamp examinations revealed that slight iritis and conjunctivitis were observed in some animals treated with levofloxacin ophthalmic formulation up to 5 hour post dose. It is not possible to confirm that the changes are treatment related because untreated animals also showed slight irritation in the eye during ophthalmic examinations.

Pupillary diameter:

Pupillary diameter of the treated right and untreated left eyes did not show any change compared to the control.

Key observations:

Results of the study do not indicate treatment-related irritation in female rabbits up to 1.0 % LVFX/0.0025% BAK given one drop ten times during 24 hours in nonpigmented rabbit eyes. However, iritis and conjunctivitis were noted one hour after 0.3% LVFX/BAK 0.0025% ophthalmic solution in male rabbits.

ID # 939405

Ocular toxicity study of levofloxacin (DR-3355) ophthalmic solution after 2-week administration in white rabbits.

Page 180, vol 9

Conducting laboratory: Santen Pharmaceutical company, Osaka, Japan

Date of study initiation: March 2, 1993

GLP compliance: Yes

QA report: Yes

Dosing information:

Species: Japanese white rabbits, male

#/sex/group: 8

Age and weight: 8-9 weeks old, 1.97-2.37 kg

Dosage groups:

Group	Left eye	Right eye
1	Vehicle	Untreated
2	0.3%	Untreated
3	0.5%	Untreated
4	1.0%	Untreated

Route form volume:

Ophthalmic solution was instilled into the left eye. One drop was instilled four times daily starting 9 AM at four-hour intervals. The right eye was left untreated.

Drug Lot #:

The lot number and % of purity are shown in the following table.

Solution	Lot Number	PH	% of the label
Vehicle	DR930303-4	5.50	
0.3% LVFX	DR930303-1	6.50	98.7
0.5% LVFX	DR930303-2	6.50	97.6
1.0% LVFX	DR930303-3	6.49	98.1

The preparation is stable for 19 days in room temperature.

Clinical sign: general condition was assessed on day 0 (one day before the dosing started) before instillation and after last instillation each day.

Body weight: Body weight was measured on days 0, 4, 8 and 15.

Ophthalmic examinations:

On days 0, 4, 8 and 15 the number of eye blinkings per minute was determined.

Eye irritation was determined on day 0 before dosing and on days 4, 8 and 15 approximately one hour after the last dose according to the method of Draize. The score ranges are given below.

Corneal opacity, 0-4, size of opacity 1-4, Iris 0-2, Conjunctival redness 0-3, chemosis 0-4, redness of bulbar conjunctiva 0-2, nictitating membrane 0-2 and conjunctival discharge 0-3.

Irritation of the anterior chamber was examined by slit lamp biomicroscopy on days 0, 4, 8 and 15. Following initial examinations, corneal damage was further assessed usin and staining techniques. Corneal staining by was assessed using the score of 0-4. A score from 0-3 assessed corneal staining by . Slit lamp examination was performed with or without a to observe the distribution, intensity, and area of stained spot. Two animals from each group (animals # 2 and 8) were not assessed for corneal damage by the staining method on days 15. These animals were assessed by electron microscopy for corneal damage.

Histopathology:

After the second instillation on day 16, animals were anesthetized, exsanguinated and the eyeglobes were dissected. Eyeglobes and conjunctiva were fixed in 2.5% formalin and 3% glutaraldehyde solution. The nasal-side bulbar conjunctiva and inferior-side fornicial conjunctiva were embedded in paraffin for cutting sections. Sections were stained with hematoxylin and eosin for histopathological examinations. Goblet cell counts per mm of conjunctiva were determined following staining. Histological examinations of the following tissues were conducted:

Cornea, bulbar conjunctiva, iris, lens, retina, palpebral conjunctiva, nictitating membrane, lacrimal gland and Harder's gland.

Results:

Clinical sign:

No abnormal clinical observations were reported that could be due to the treatment. One animal at 1% showed diarrhea on day 12. However, the animal recovered and it was considered to be unrelated to the treatment.

Body weight:

The average body weight on day 0 and 30 are shown in the following table.

Group	Day 0	Day 15
Vehicle	2.19 (kg)	2.39
0.3%	2.20	2.43
0.5%	2.21	2.46
1.0%	2.20	2.42

Data indicate that the body weight gain was not affected by the treatment.

Ophthalmological examination:

Blinking frequency of the treated groups was not statistically different from the vehicle control group. However, the blinking frequency was increased in all groups after the treatment.

Results of the Draize test show redness in the treated eye and untreated eyes in weeks 4, 8 and 15. However, it was not related to the treatment because the untreated eye also showed similar changes.

Slit lamp biomicroscopy did not show any abnormality due to the treatment. Staining with
did not show treatment related corneal damage.

Histopathological examinations:

No abnormality due to the treatment has been reported.

Electron microscopic changes in the cornea:

No abnormality of the corneal epithelium and endothelium was reported.

Key study information:

Male Japanese white rabbits treated up to 1.0% levofloxacin ophthalmic solution at 4 drops per day for 14 days did not show any ocular toxicity.

Sponsor's ID# 6776-100

Title: 30-day repeat dose ocular toxicity study of levofloxacin ophthalmic solution in pigmented rabbits.

Page 004, vol 10.

Conducting laboratory: [redacted]

Date of study initiation: October 8, 1996

GLP compliance: Yes

QA report: Yes

Dosing Information:

Species: New Zealand cross/Dutch belted rabbits

#/sex/group: Five

Age and weight: 5-6 months old, 2.3 to 3.0 kg body weights

Dosage groups in administered units:

Group	LVFX (%)	benzylalkonium chloride (ppm)	PH
1 (vehicle control)	0	50	6.65
2 (low)	0.5	15	6.77
3 (mid)	0.5	25	6.75
4 (high)	0.5	50 (0.005%)	6.77

Route, form and volume:

Doses were administered as ophthalmic drops. Two drops per dose were instilled into the right eye. The left eye served as the untreated sham control. Six doses were administered on days one and two (12 drops/eye). Thereafter, from days 3-day of necropsy, four doses (8 drops/eye) were instilled into the eye. Minimum two hours of time elapsed between the doses. The sponsor stated that the drug concentrations were selected so as to determine the safety of the possible clinical formulation when rabbits were given twice the duration of the expected clinical uses. The sponsor has not indicated the volume of each drop. The reviewer considered it to be about 50μL per drop. Accordingly, about 400 μg/day (8 drops) doses of levofloxacin were given to rabbits.

Drug, lot number and purity:

Following formulations were used.

0.5% LVFX and 15 ppm benzylalkonium chloride (BAK): Lot NH 71004A
0.5% LVFX and 25 ppm BAK: Lot NH 71003A
0.5% LVFX and 50 ppm BAK: Lot NH 71002A
Placebo: 500 ppm BAK, Lot NH 71005A

Formulations in mg/ml:

Ingredient	Placebo, 50ppm BAK	0.5% LVFX and 15 ppm BAK	0.5% LVFX and 25 ppm BAK	0.5% LVFX and 50 ppm BAK
Levofloxacin	0	5.0	5.0	5.0
Benzalkonium Chloride	0.05	0.015	0.025	0.05
Sodium Chloride				
NaOH or HCl				
Purified water q.s				

The formulations of 0.5% LVFX were stable for more than 4 months. The pH of the placebo was reduced from [redacted] at the end of six months.

Times at which observations were made:

Clinical signs: Rabbits were observed twice daily for mortality and moribund conditions. Food and water consumption was recorded daily.

Body weight: Body weights were recorded on day 1 and once every week thereafter.

Ophthalmological examinations: Eyes were examined macroscopically before dosing and daily during the treatment before the first dose. Both eyes were examined for irritation and scored according to the Draize system.

Indirect ophthalmoscopic and slit lamp examinations were conducted on both eyes on day –3, 8, 15, 22, and 30. Eyes were dilated by 1% Mydriacyl. [redacted] was used for staining. Ocular irritation was scored according to a scoring system given below.

Conjunctival congestion: 0-3; Conjunctival chemosis and swelling: 0-4; Conjunctival discharge: 0-3; Aqueous flare in anterior chamber (Tyndall effect): 0-3; Light reflex: 0-2; Iritis: 0-4; Cornea: 0-4; % area of corneal opacity 0-4; corneal vascularization: 0-2; Corneal staining: 0-4; % area of corneal staining 0-4; Retina 0-2; Lens: 0-1.

Hematology:

Blood samples were drawn before dosing (week -1) and before necropsy (week 5) from the ear artery. All animals were fasted overnight before blood sampling. The following parameters were assayed:

Differential leukocyte counts and cell morphology, erythrocyte count, hematocrit, hemoglobin, leukocyte count, mean cell volume, mean cell hemoglobin concentration, mean cell hemoglobin, platelet count, reticulocyte count, activated partial thromboplastin time and thrombin time.

Clinical chemistry: Serum chemistry tests were conducted at week –1 and week 5 on the following parameters:

Alanine aminotransferase, albumin, albumin/globulin ratio, alkaline phosphatase, aspartate aminotransferase, calcium, chloride, creatinine, gamma glutamyltransferase, globulin, glucose, inorganic phosphorus, sodium, potassium, total bilirubin, total cholesterol, total protein, triglycerides and urea nitrogen.

Urine analysis: Urine samples were obtained from the bladder at the time of necropsy. The following parameters were tested:

Appearance, bilirubin, glucose, ketones, microscopic examination of the sediment, occult blood, pH, protein, specific gravity, urobilinogen and total volume.

Gross pathology:

At the end of the treatment all animals were sacrificed by IV injections of sodium pentobarbital and exsanguinated. Macroscopical examinations of external surfaces of the body, cranial cavity, external surface of the brain, nasal cavity, paranasal sinuses, thoracic, abdominal and pelvic cavities and viscera were conducted.

Organ weights of following organs were recorded:

Adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and thymus.

Histopathology:

Each eye with optic nerve head, upper and lower eyelids were removed and fixed in [ ]fixative for 8 hours[ ]. The sponsor stated that information on the toxicity of levofloxacin already exists for other tissues and therefore other tissues were not collected at necropsy for histological examinations.

Histopathology of following tissues of the eye from animals in groups 1 and 4 was conducted.

Palpebral and bulbar conjunctiva, lids, cornea, anterior chamber, iris, ciliary body, lens, retina, optic nerve, posterior chamber and sclera. Tissues were stained with hematoxylin, eosin and examined under a light microscope.

Results:

There was no mortality reported in the study. No abnormal clinical signs were reported in the study.

Body weight:

Body weight gain (g) and standard deviation of the means between week 1 and 4 is shown in the following table.

Group	Male	Female
1	155 ± 59	245 ± 119
2	122 ± 68	229 ± 118
3	164 ± 60	301 ± 159
4	51 * ±61.7	205 ± 86

*Statistically significant

Data show that male animals in group 4 had statistically significant loss of weight gain. Female animals in group 4 also showed a loss of body weight gain when compared to the respective control. However, it was not statistically significant.

The decrease in the body weight in group 4 male rabbits was not treatment related. It was due to lower initial body weight. A similar change was also noted in a group 3 male as shown in the following table.

Animal #	Group	Sex	BW WK 1 (g)	BW WK 4 (g)	BW Gain (g)
E 54481	3	M	2580	2668	88
E 54489	4	M	2478	2522	44
E 54490	4	M	2536	2633	97

Ophthalmological examinations:

Microscopic observations in Draize test:

No abnormality in the right and left eyes was reported in the Draize test.

Ophthalmoscopic observations:

Ophthalmic examinations by the indirect ophthalmoscope did not show any treatment-related changes. On day 8, one male in group 4 showed [redacted] staining in the cornea in the right eye. However, similar changes were also observed in the untreated left eye. Therefore, the change was considered to be unrelated to the treatment. Corneal staining was also observed on days 15, 22 and 30 in treated and untreated eyes that was not related to the treatment.

Hematology:

There were no treatment-related changes in the hematological parameters in rabbits. Female rabbits in groups 2- 4 showed statistically significant changes in the prothrombin time. However, there were no differences in the mean values and they were considered to be unrelated to the treatment. Data for prothrombin time in sec for female rabbits are shown in the following table.

Group	Week -1	Week 5
1	6.2	6.1
2	6.4	6.3*
3	6.3	6.3*
4	6.3	6.3*

*Statistically significant.

Clinical chemistry:

Male and female rabbits showed higher AST activity in the serum. The changes are not levofloxacin related because animals in group1 (placebo treated) also showed similar effect. Data do not predict the role of benzalkonium chloride for changes in the AST because the sponsor has not included untreated animals in the study. Similarly, male and female rabbits in group 4 showed an elevation of calcium that was statistically significant. Female animals showed an increase in the phosphate levels on week 5 in groups 3 and 4. Female rabbits showed higher levels of urea nitrogen on week 5 in groups 1-4. However, it was not statistically significant. The changes in AST, calcium and inorganic phosphorus are shown in the following table.

Gr	AST (week-1)		AST (week 5)		Calcium (week-1)		Calcium (week 5)		Phos (wk-1)		Phos (wk5)	
	M	F	M	F	M	F	M	F	M	F	M	F
1	12	10	23	20	12.9	12.2	16	15.9	5.0	4.9	6.2	6.1
2	11	11	20	23	12.5	12.2	15.3	16.1	5.1	4.6	6.0	6.1
3	11	12	19	19	12.8	12.6	15.8	14.4*	5.0	4.8	5.9	5.3*
4	12	12	23	21	12.4	12.5	14.2*	14.2*	4.9	4.6	5.5	5.3*

The individual values of the calcium and inorganic phosphorus for groups 3 and 4 on week 5 overlaps that of groups 1 and 2. Therefore, the statistical changes compared to the control group are due to biological variations and not due to the treatment with levofloxacin. It is concluded that levofloxacin treatment has no adverse effect on the serum chemistry parameters. The sponsor stated that significantly lower mean values were observed for calcium and inorganic phosphorus. As shown in the data these values were increased on week 5 compared to week 1.

Serum creatinine values for group 3 and 4 female rabbits showed statistically significant increase compared to the control. However, the individual data for animals in group 3 and 4 were similar to the individual data for groups 1 and 2 animals. Therefore changes in the creatinine levels in groups 3 and 4 female animals are considered to be unrelated to the treatment. Data are shown in the following table.

Group	Week 1	Week 5
1	1.3	1.6
2	1.4	1.5
3	1.2	1.3*
4	1.3	1.4*

*Statistically significant

Urine chemistry:

The sponsor stated that urine chemistry data are unremarkable and levofloxacin had no adverse effect on the urine chemistry parameters.

Gross pathology:

There were no changes in the gross pathology and organ weight data.

Histology:

Histology data did not show any treatment-related changes in the eyes.

Key study observation:

Treatment with 0.5% levofloxacin with benzalkonium chloride did not show ocular toxicity in a 30-day study.

Sponsor's ID:

Title: A 26-week ocular toxicity study of levofloxacin (DR-3355) ophthalmic solutions in pigmented rabbits.

Page 11-002 and vol 11.

Conducting Laboratory:

Date of study Initiation: May 28, 1993

GLP Compliance: Yes

QA report: Yes

Methods:

Species and strain: Male Dutch rabbits, Kbt

#/sex/group: Five animals per group. Animals were used in another study # 939406 a two-week ocular safety studies for 1% levofloxacin. However, after second instillation the study was terminated because the safety of the drug was already examined in another two-week study # 939405. Following eight week of recession animals were enrolled into the present study. The sponsor stated that the previous exposure to the test substance would not affect the result of the present study. General conditions and eye examinations were conducted to ensure that animals with normal eyes were enrolled.

Age: About 13 weeks old at the procurement of the animals. Body weights were varied from 1.94 to 2.62 kg.

Dosage groups:

Group	Left eye	Right eye	Number of animals
1	Vehicle	Untreated	5
2	0.3%	Untreated	5
3	0.5%	Untreated	5
4	1.0%	Untreated	5
5	3.0%	Untreated	5

Route and forms:

Ophthalmic solutions or the placebo were instilled one drop four times a day at 3-hour intervals for 26 weeks. Ophthalmic drops were instilled into the conjunctival sac of left eyes and the right eye served as the untreated control. The doses were selected on the basis of the previous study # 939403 in which 10 times in a single day administration of the highest dose (0.3%) did not show ocular toxicity. Conjunctival edema and ocular discharges were noted at 10% and higher concentrations of levofloxacin in rabbits (page 225, vol 8).

Drug lot and purity:

Concentration	Date used	PH	% of the label	Lot Number
Vehicle	6/1993-9/1993	5.90		DR930607-5
Vehicle	9/1993-12/1993	5.46		DR930913-5
0.3%	6/1993-9/1993	6.50	99.8	DR930607-1
0.3%	9/1993-12/1993	6.50	98.5	DR930913-1
0.5%	6/1993-9/1993	6.50	101.3	DR930607-2
0.5%	9/1993-12/1993	6.50	98.7	DR930913-2
1.0%	6/1993-9/1993	6.50	100.3	DR930607-3
1.0%	9/1993-12/1993	6.50	97.7	DR930913-3
3.0%	6/1993-9/1993	6.50	102.4	DR930607-4
3.0%	9/1993-12/1993	6.57	100.8	DR930913-4

Vehicle and 0.3-1.0% solution contained 0.9% sodium chloride. The 3.0% solution contained 0.57% sodium chloride.

Clinical signs:

General conditions were observed daily from the day animals were randomized, before first instillation and after fourth instillation during the treatment period.

Body weights: Body weights were measured weekly from pretest (week -2, -1) through week 13. Body weights were recorded every four week intervals from week 14 to the end of dosing. The sponsor stated that week one of the study is defined as the first 7 days from the first day of drug administration. Although the sponsor stated that the drug was administered for 26 weeks, the body weight data show that the body weight was recorded for 28 weeks.

Food consumption:

The food consumption was recorded daily from the day of randomization.

Ophthalmic examinations:

Eye irritation to the drug was examined according to the modified Draize method at the time of randomization, and one-hour after the last daily dosing on the first day on weeks 4, 13 and 26.

Anterior chamber of the eye was examined by slit lamp for ocular flare.

Inflammation of the anterior chamber as measured by changes in the protein levels was examined and quantitated by laserflare photometry before dosing and during weeks 4, 13 and 26. The diameters of the pupil and light reflexes were also examined at pre study and on weeks 4, 13 and 26.

Lens and posterior chambers of the eye were examined by indirect ophthalmoscope at pretest and during week 26. Mydrin was given to induce mydriasis during the funduscopic examination.

Electroretinography was conducted at pretest and during weeks 26-27 before the end of the experiment.

Fluorescein elimination test:

During week 14 of the study a two-day fluorescein test (one day for each eye) was conducted. One drop of sodium fluorescein (1%) solution was instilled into the eye approximately one hour after the last dose of the day. The eye and nasal foramen were examined at 5 minutes intervals for one hour by slitlamp using a . The eye and nasal foramen were scored according to the following criteria.

1. No fluorescein admitted in conjunctival sac	0
2. Fluorescein is linearly remained in lower conjunctiva	1
3. Fluorescein is also remained in tear film on cornea	2

The sponsor stated that the time taken for the score to change to 0 in the eye is considered to be the elimination time of fluorescein from the eye. Similarly time taken for disappearing of outflow of fluorescein from nasal foramen was considered to be fluorescein excretion time.

Necropsy:

At the end of the dosing period, animals were anesthetized by the IV injections of pentobarbital. Glutaraldehyde 2.5% solution was instilled in the eye. Inferior nasal side fornicial membrane, nasal side conjunctival sclera and bulbar conjunctiva were dissected and preserved in 10% neutral buffered formalin. The larger half of the eyeglobe was cut in half and fixed in 2.5% glutaraldehyde for 4 hours. Optic nerve was obtained from the posterior half of the eyeglobe. Harder's gland, lacrimal gland, palpebral conjunctiva and nictitating membrane were fixed in 10% neutral buffered formalin.

Histopathology:

The sponsor stated that light microscopic examination of the eyeglobe (anterior hemisphere) was conducted in animals # 1, 3 and 4 from each group. However, microscopic examinations of posterior hemisphere, Harder's gland, lacrimal gland, palpebral conjunctiva, nictitating membrane and optic nerve were conducted in all animals.

The sponsor stated that paraffin embedded blocks of the isolated inferior fornicial conjunctiva and nasal side bulbar conjunctiva were sectioned into about 100 sections each 7 um thickness. One section out of every 10 sections was stained by [redacted] stain for counting goblet cells.

Optic nerve, Harder's gland, lacrimal gland, palpebral conjunctiva and nictitating membrane were subject to hematoxylin and eosin stains. After isolating the optic nerve, posterior half of the eyeglobe was vertically cut at nasal side of the optic nerve. The temporal side of the posterior eyeglobe was cut in parallel direction. The slides were fixed in glutaraldehyde solution and 1% osmic acid solution for dehydration. Slides were stained by toluidine and azure II dyes.

Electron microscopy (EM):

Animal # 2 and 5 from each group were chosen for the EM study of the eye tissues. Cornea, iris and ciliary body were isolated from the anterior hemisphere of the eyeglobe and fixed in 2.5% glutaraldehyde solution and 1% osmic acid solution. The posterior eyeglobe was also fixed in a similar way.

Cornea was dehydrated and dried before scanning electron microscopic examinations of the epithelium and endothelium. The sponsor stated that the scanning electron microscopic examinations were performed on animals in all groups.

Iris, ciliary body, retina and choroid were stained with uranyl acetate and lead nitrate. Slides were subjected to transmittance electron microscopic examinations. The sponsor stated that transmittance electron microscopic examination was performed on animals in the vehicle and 3% dose groups.

Results:

No mortality has been reported.

Clinical observation:

Lacrimation was observed in 1-2 animals out of 5 animals at 3.0% levofloxacin between week 3 and month 4. Both eyes showed lacrimation. However, lacrimation subsided towards the end of the dosing period.

Body weight:

Average body weight (kg) between week 1 and week 28 are shown in the following table.

Treatment	Week 1	Week 28	Gain/Loss
1. Vehicle	2.22	2.43	0.21
2. 0.3% Levofloxacin	2.21	2.41	0.20
3. 0.5% Levofloxacin	2.17	2.16	-0.01
4. 1.0 Levofloxacin	2.20	2.26	0.06
5. 3.0% Levofloxacin	2.25	2.43	0.18

Above data show that the body weight gain for animals in groups 3 and 4 was reduced compared to the control. However, animals in the high dose group did not show similar changes. There was no statistically significant change in the body weight is reported. Therefore, it is considered that the treatment did not affect the body weight gain in these animals.

Ophthalmological examinations:

No irritation, corneal opacity, chemosis were noted in the anterior chamber of the eye. However, ocular discharge was noted in the treated and untreated eyes at 3% levofloxacin. Laser flare photometric examinations of the inflammatory changes in the anterior camber of the eye did not show inflammatory changes. The treatment had no effect on the light reflex and diameter of the pupil. [redacted] staining techniques did not show any damage to the cornea. The funduscopic examinations of the eye did not reveal any treatment-related changes in the lens and posterior chamber. Electroretinography also did not show treatment-related changes in the retina in response to the light.

During week 14, [redacted] elimination data suggest that treated and untreated animals eliminated the dye within 15 minutes from the eye. [redacted] excretion was noted within 5 minutes from the nasal foramen. One animal in the 3% dose group showed longer excretion time (60 min). The sponsor suggested that it resulted from lacrimation in the animal due to the treatment.

Light microscopy:

No treatment related abnormality in the anterior or posterior tissues of the eye was noted. Also goblet cell counts in the conjunctiva were not significantly different between the treated and untreated eyes.

Electron microscopy:

No treatment related changes in the cornea, endothelium, iris, ciliary body, retina and choroid were observed.

Key observation:

Treatment up to 3% preservative free ophthalmic solution of levofloxacin did not show treatment-related changes. The sponsor suggested that epiphora may have been present at 3% solution. One out of five animals at 3% solution showed prolonged excretion time of the [redacted] stain.

ID # 936302

Title: Maximization test of DR-3355 in guinea pigs.

Page 123, vol 11,

Conducting Laboratory: Santen Pharmaceuticals Co. Osaka, Japan

Date of the study initiation: Feb 12, 1993

GLP Compliance: Yes

QA Report: Yes

Methods:

Species: Female Hartley guinea pigs

#/group: 10

Age: Four weeks at procurement.

Weight: 329-358 g

Dosage Groups:

Group	Treatment	Number of Animal	Sensitization Dose Intracutaneous injection ; Patch Application	Challenge Dose
A	Negative Control	4	Water/FCA, 0.1 ml/sitex2 ; White petrolatum 200mg/site	White petrolatum 200mg/site at ARF
A	Negative Control	3	Water, 0.1 ml/sitex2 ; White petrolatum 200mg/site	25% DR-3355 ointment 100mg/site at PRF
A	Negative Control	3	Water/FCA, 0.1 ml/sitex2 ; White petrolatum 200mg/site	0.1% DNCB ointment 100mg/site at LF
B	DR-3355 Sensitization group	4	Water/FCA, 0.1 ml/sitex2 ; 25% DR-3355 ointment 200 mg/site	White petrolatum 100 mg/site at ARF
B	DR-3355 Sensitization group	3	5% DR-3355 solution 0.1 ml/sitex2; 25% DR-3355 ointment 200 mg/site	25% DR-3355 ointment 100 mg/site, PRF
B	DR-3355 Sensitization group	3	10% DR-3355 solution and FCA 0.1 ml/sitex2; 25% DR-3355 ointment 200 mg/site	25% DR-3355 ointment 100 mg/site, PRF
C	Positive Control Group	4	Water/FCA, 0.1 ml/sitex2; 1%DNCB ointment 200 mg/site	Petrolatum 100 mg/site, ARF
C	Positive Control Group	3	0.1%DNCB solution 0.1 ml/sitex2; 1%DNCB ointment 200 mg/site	0.1% DNCB ointment 100 mg/site, PRF
C	Positive Control Group	3	0.2%DNCB and FCA 0.1 ml/sitex2; 1%DNCB ointment 200 mg/site	0.1% DNCB ointment 100 mg/site, PRF

ARF= Anterior right flank; PRF= Posterior right flank; LF= Left flank; FCA= Freund's complete adjuvant; DNCB= 2,4 dinitrochlorobenzene.

Route: Initial induction was done by intracutaneous injections. A second induction was done by applying 25% petrolatum ointment of the test substance topically in a patch. Animals were challenged by topical application of 25% ointment of levofloxacin in petrolatum.

A preliminary study was conducted for finding the doses necessary for induction and challenge. 5% solution of levofloxacin did not show any irritation and 5-10% concentrations were chosen for induction in the main experiment. 25% ointment of levofloxacin was applied on the shaved skin in a filter paper and occluded by a tape for 24 hours. Skin reactions to the patch were examined after 24 hours. No skin irritation was observed at 25% ointment. Therefore, 25% ointment was used for the second induction and challenge in the main study.

Initial induction was achieved by intracutaneous injections of 5% or 10% levofloxacin emulsion in Freund's complete adjuvant (CFA). 0.1 or 0.2% DNCB emulsions in CFA was used as a positive control. After 7 days, The second induction was achieved by the application of 25% ointment of levofloxacin patch. 1-2% DNCB patches were used as the positive control. The occlusive patches were kept in place for 48 hours. The sponsor stated that 10% sodium lauryl sulfate ointment was applied to the test sites for negative control and levofloxacin treated groups immediately after shaving but day before the second challenge. The purpose of this application was to induce irritation. If the test substance is nonirritating, sodium lauryl sulfate is applied before the second challenge. After day 21, the test substance or the positive control as ointment was applied in the shaved flanks and occluded for 24 hours to determine sensitization. About 100 mg of petrolatum was applied as the negative control. At the end of 24 hours, the presence of erythema was scored according to the following criteria.

- \- No macroscopic change
- \+ Mild or scattered erythema
- ++ Moderate erythema
- +++ Severe erythema

Clinical signs: Clinical signs were recorded at pretest and on days 1, 8, 15 and 22 of the experiment.

Body weight: Body weights were recorded at pretest and on days 1, 8, 15 and 22 of the experiment.

Results:

Average body weight (g) data on days 1 and 22 are shown in the following table.

Group	Day 1	Day 22
Control	385.0	492.8
Levofloxacin	378.9	482.7
DNCB	386.3	505.8

The body weight gain between days 1 and 22 was 107, 104 and 119 g, for groups 1, 2 and 3, respectively. Data show that the treatment with levofloxacin did not affect the body weight gain.

Incidences of positive reaction after 24 and 48 hours are shown in the following table. Each group had 10 animals.

Hours	Control	Levofloxacin	DNCB
24	0	0	10
48	0	0	10

Data in the above table show that levofloxacin did not induce contact dermatitis in guinea pigs. Animals in the positive control group showed grade +++ erythema.

Study: 946315

Title: Determination of the minimum erythema dose (MED) and phototoxicity of levofloxacin in guinea pigs: A supplementary study.

Page 156, vol 11

Conducting Laboratory: Santen Pharmaceutical Co.Ltd., Osaka, Japan

Date of study initiation: June 29, 1994

GLP compliance: Yes

QA report: Yes

Methods:

The study schedule is shown in the following table.

Study schedule	Day 1	Day 2	Day 3	Day 4
Shaving	X			
Cutaneous application		X		
Irradiation		X		
Assessment			X	X

The minimum erythema dose (MED) was determined in the guinea pigs.

Back of the guinea pigs were shaved and depilated. Twenty-four hours after shaving, animals were exposed to UV A light at specified sites. A 10-hole plate covered back of each animal. Animals were exposed to UV B for 30 seconds. After the exposure, the holes were serially closed at 15-second intervals. The maximum exposure to UV B was 165 seconds. 24 hours after irradiation, sites exposed to UV A and UV B were examined. UV energy that caused slight erythema was calculated as MED on the basis of the time required to induce it and the intensity of UV lights.

Slight erythema was developed at 10.73J/cm^2 energy level when UV A was used for 1714 sec which is considered as MED for UV A.

Average energy necessary for developing slight erythema after UV B exposure was 187 mJ/cm2. About 70% of the energy required for MED, for example 130 mJ/cm^2 was used for the phototoxicity study.

Phototoxicity study:

Energy delivered for the phototoxicity experiments is shown in the following table.

Test	Actual intensity mW/cm^2)	Duration of irradiation (sec)	Energy delivered (J/cm^2)
1, UV-A	5.6	1875	10.50
2, UV-B	2.05	64	131.2
1, UV-A	5.75	1805	10.38
2,UV-B	2.14	61	130.5

Dosing information: Species: Hartley guinea pigs.

Sex/group: Female, three per group

Age: About 4weeks old

Weight: 334-372 g

Dosage groups:

	Cage Number	Animal number			Cutaneous application (0.05mlx2 sites)	UV irradiation
		Ear marking				
		Right	Left	Neither		
1. Negative control	1	1	2	3	Vehicle	UV A: 10-12J/cm2+ UV B 70% of MED with or without Al shielding
2. Levofloxacin	2	4	5	6	3% Levofloxacin	
3. Positive control	3	7	8	9		
4. MED determination	4	10	11	12		UV A:10-12 J/cm2 + UV B

MED = Maximum erythema dose.

Route: topical application on the skin as 3% solution. 0.05 ml was applied in two sites.

Drug, lot # and purity:

Levofloxacin DR 940516-1, vehicle DR 940516, MOP M3B6912 and ethanol V3R4297. Levofloxacin content in the solution was 106% of the control.

Formulation/vehicle: the sponsor has not provided the formulation in the report.

General condition: Each animal was observed for general condition at the time of grouping and daily during the experiment.

Body weight: The body weights were recorded at pretest and 48 hours after the application of levofloxacin.

Skin reaction to UV irradiation:

24 and 48 hours after the exposure to the UV lights, skin reactions were observed and scored according to the following scale. The irradiated skin reactions were compared to that of the non-UV-exposed site.

1. No erythema or edema, 0
2. Very slight erythema or edema, 1
3. Well defined erythema and slight edema, 2
4. Moderate to severe erythema and moderate edema, 3
5. Severe erythema or edema, 4

From the erythema and edema scores, fractional response (FR) and mean response (MR) were calculated according to the following formula:

FR= Number of positive animals/ Number of tested animals

MR= Number of animals with erythema and edema/total number of tested animals

Results:

Animals did not show any adverse clinical signs and the weight gain was normal. Both vehicle and 3% levofloxacin treated animals did not show any skin reaction to UV-A or UV-B irradiation. However, the positive control treated animals showed erythema score of 2-3 and edema score of 2-3 within 24 and 48 hours after the exposure. The MR for positive control was 5.3 and 3.7 at 24 and 48 hours, respectively. The FR for the positive control was 1 at 24 and 48 hours after the UV exposure.

Key study observation: 3% levofloxacin solution did not show phototoxicity.

Study # 946307

Title: Skin photosensitization study of levofloxacin in guinea pigs.
Page 185, vol 11

Conducting laboratory: Santen Pharmaceutical Co., Osaka, Japan.

GLP Compliance: yes

QA report: Yes

Methods:

Freund's adjuvant was injected intracutaneously in 0.1 ml volume at four corners of the square shaped shaved area of the skin. The superficial epidermis layer of the skin was removed by a Band-Aid so as to induce slight erythema. 3% LVFX or the vehicle or 2% tetrachlorosalicylanilide (TCSA) was applied cutaneously on the shaved skin. About 30 minutes later, the skin was exposed to UV-A radiation at approximately 10.5 J/cm^2. The procedure was repeated for 5 consecutive days. Three weeks after the photosensitization, animals were exposed to 0.02 ml of the test substance or active control or corresponding vehicles in the cutaneous surfaces. Half the exposed surface was covered with aluminum foil to shield the UV light. About 30 minutes later, animals were exposed to the UV-A light at 10.5 J/cm^2. The skin reactions to UV-A irradiated or non-irradiated surface were assessed at 24, 48 and 72 hours according to the scoring system described for study # 946315 already mentioned in the review. The fractional response (FR) and mean response (MR) were determined.

Dosing information:

Species: Female Hartley guinea pigs

#/group: Eight per group

Age: Four weeks

Weight: 350.7-403.5 g

Dosage groups:

	Sensitization for 5 days		Challenge	UV irradiation
	Cutaneous (0.1 ml)	UV irradiation	Cutaneous (0.1 ml)	
1. Negative control	Vehicle	10-12 J/cm²	Vehicle (cephalic side) 3% LVFX (caudal side)	Vehicle (cephalic side) 3% LVFX (caudal side)
2. Levofloxacin	3% Levofloxacin	10-12 J/cm²	Vehicle (cephalic side) 3% LVFX (caudal side)	Vehicle (cephalic side) 3% LVFX (caudal side)
3. Positive control	[redacted]	10-12 J/cm²	[redacted] (cephalic side) [redacted] (caudal site)	Vehicle (cephalic side) 3% LVFX (caudal side)

LVFX= Levofloxacin; [redacted]

Route: Topical

Drug, Lot #: 3% levofloxacin solution lot # DR 940516-1, assay of the levofloxacin content showed 106% of the labeled amount. Vehicle for levofloxacin, DR940516-2.

TCSA lot # 510E4055, acetone lot # V3G8992.

Formulation/vehicle: Not provided in the report.

Observations:

Clinical sign and body weight: Each animal was observed for clinical sign at pretest, and on days 1, 9, 16 and 23.Body weights were recorded at the same time.

Skin reaction: Erythema, edema and other changes in the skin were recorded at 24, 48 and 72 hours after irradiation following application of the challenging doses of the test substance or positive control. Skin reactions to UV exposed and non exposed skin was compared. The area that showed higher scores for erythema and edema compared to the non-irradiated skin was considered to be a positive response.

Results:

Data did not show any treatment-related changes in the general conditions of the animals or changes in the body weight gain. Levofloxacin and its vehicle did not show any erythema or edema following UV-A exposure after photosensitization. However, TCSA showed edema and erythema under similar experimental conditions at 24, 48 and 72 hours after the final exposure to the UV-A radiation.

Key study observation: Levofloxacin 3% ophthalmic solution did not induce photosensitization.

Overall toxicology summary:

Levofloxacin 10-25% ophthalmic solution 1 drop 10 times a day induced ocular discomfort, iritis, conjunctival swelling and corneal damage in acute ocular exposure in white rabbits. Preservative free solutions were used in the study. The data suggest that 3% LVFX solution without BAK does not produce ocular toxicities in non-pigmented white rabbits when applied one drop 10 times in one day. In a similar one day acute ocular safety study, 1% LVFX solution containing 0.0025% BAK did not show ocular toxicity at one drop 10 times a day for one day.

A two-week study at 1% LVFX without preservative did not show ocular toxicity at one drop x 4 times a day in non-pigmented eyes. The sponsor has conducted a four 4 week ocular safety study using the proposed clinical formulation (0.5% LVFX with 0.005% BAK as the preservative) in pigmented eyes of rabbits. No treatment related ocular toxicity was observed. The expected maximum duration of clinical use is about 14 days.

Levofloxacin 3% ophthalmic solution without preservative showed lacrimation in one out of five Dutch rabbits in a six-month safety study at one drop four times daily. However, a 1% solution did not show any ocular toxicity and discomfort.

Reports of above studies suggest that up to a 1% ophthalmic solution of levofloxacin did not show ocular toxicity in pigmented and non-pigmented eyes of rabbits. At a higher concentration e.g. 3% solution, slight lacrimation was observed in chronic uses.

Levofloxacin did not show contact dermatitis in the guinea-pig maximization test. No phototoxicity or photosensitivity was observed with a 3% levofloxacin solution.

No changes in blood chemistry, hematology, urine chemistry and gross pathology were reported in the 30-day ocular safety study. The sponsor submitted a summary of the approved NDA 20-634 for levofloxacin tablets and the package insert in volume 8 of this NDA.

Overall summary and evaluation:

The NDA is submitted for the approval of 0.5% levofloxacin ophthalmic solution with 0.005% BAK for the treatment of bacterial conjunctivitis in adults and children one year of age and older. The proposed dose is two drops in the treated eye for 8 times a day during first two days followed by two drops four times a day for another 3 days. The average daily dose (mg/kg) for a 70 kg adult is 0.11 mg/kg/day. The average daily dose for children over one year of age has not been indicated in the proposed label. The package insert for ofloxacin and the proposed insert for levofloxacin ophthalmic solution indicated the possibility of juvenile arthropathy in immature animals following oral dosing. However, the package insert for the approved racemic ofloxacin indicated that ocular administration of ofloxacin to immature animals has not shown any arthropathy. Since the clinical safety of ofloxacin in pediatric patients over one year of age is established by its uses in the population, the reviewer is not recommending additional ocular safety studies of levofloxacin in immature animals. The concentration of the approved racemic ofloxacin ophthalmic solution is 0.3%. Generally speaking, the pharmacological potency of the active isomer is greater than the racemic. However, in the present NDA the proposed concentration is greater than the racemic form.

Antibacterial property of levofloxacin is reported in several experimental models in albino rabbits at 0.3-3.0% ophthalmic formulations. Levofloxacin IV or oral route showed CNS, CVS, GI and urinary safety concerns in several animal species. The minimum systemic dose that showed safety concern in the NDA reports is that of cardiovascular effect at 6 mg/kg/IV bolus in dogs. The dose is equal to 3 mg/kg in human when normalized to the equal surface area. The average ophthalmic adult dose in human is about 0.11 mg/kg. The dose is about 28 folds lower than the dose projected for cardiovascular changes based on the

data in the dog model. Considering the margin of safety, clinical experience of ofloxacin and lower systemic bioavailability after ophthalmic doses, levofloxacin is considered to be less likely to produce adverse systemic effects.

The distribution of ^{14}C LVFX in ocular tissues and systemic bioavailability were studied in pigmented rat eyes using brown Norway rats. A dose of 10 µg/rat instilled into the eye did not show accumulation in the systemic circulation. Levofloxacin is distributed in the anterior and posterior chambers of the eye in rats. Levofloxacin binds to the iris, pigmented epithelium of retina and choroid for about 6 months in rats. A similar observation was also reported in Dutch rabbits. The half-life of the drug in iris/ciliary body is about 20 days and that for the pigmented epithelium of retina is about 25 days in the rabbit model. The binding of the drug to the pigmented bovine eye tissues has been demonstrated in the in vitro study. On the basis of the animal studies, it is concluded that levofloxacin is accumulated in the pigmented tissues of the eye.

The peak serum level of radioactivity (2 hours) at one drop (50 µL) of ^{14}C LVFX ophthalmic solution in pigmented rabbits is about 60 ng/ml. No ocular and systemic safety concern was reported in the study. The sponsor stated that the maximum plasma levels in humans were 2.25 ng/ml following ophthalmic doses at 0.5% LVFX. Based on the ratio of the systemic levels across species, it is concluded that levofloxacin has sufficient margin of safety against developing systemic toxicity.

Several ocular safety studies were conducted in pigmented and non-pigmented eyes in the rabbits. Levofloxacin 1% ophthalmic solution did not show ocular irritation, discomfort or other ophthalmoscopic changes at 10 times/ day dosing schedule or in 4 drops daily doses for six months. Based on the preclinical data, proposed clinical uses of 0.5% ophthalmic solution of levofloxacin for 5 days is considered to be safe.

Levofloxacin did not show contact sensitivity, phototoxicity and photosensitivity to skin in guinea pig models.

Conclusion:

On the basis of the preclinical efficacy and safety studies, 0.5% ophthalmic solution of levofloxacin is safe for the clinical uses. The NDA is approvable on the basis of the preclinical pharmacology and toxicology reports.

Recommendation:

Internal:

External: Nil

Labeling review:

[redacted]

Note: The dose ratio comparison between animal to human has been changed according to the policy established by the supervisory medical officer of ophthalmic team e.g. mg/kg and the average body weight of human which is 50 kg.

/S/

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/S/

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C.C:

Orig. NDA # 21-199
HFD-550/Div. File
HFD-550/Reviwere/A.Mukherjee
HFD-550/Medical Reviewer/W.Chambers
HFD-550/Chemist/S.Horshidi
HFD-550/CSO/M.Puglisi
HFD-345
R/D Init by:
F/T by:

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