PRODUCT MONOGRAPH

NOROXIN® Ophthalmic Solution

(norfloxacin ophthalmic solution)

0.3%

THERAPEUTIC CLASSIFICATION

Antibacterial Agent

MERCK FROSST CANADA LTD.
KIRKLAND, QUEBEC, CANADA

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ACTION AND CLINICAL PHARMACOLOGY

NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) is a fluoroquinolone carboxylic acid antibacterial agent for ocular administration. Norfloxacin inhibits bacterial deoxyribonucleic acid synthesis and is bactericidal.

At the molecular level three specific actions have been attributed to norfloxacin in the inhibition of *E. coli* cells:

1) inhibition of the ATP-dependent DNA supercoiling reaction catalyzed by DNA gyrase,

2) inhibition of the relaxation of supercoiled DNA,

3) promotion of double-stranded DNA breakage.

INDICATIONS AND CLINICAL USE

NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) is indicated in adults and children for the treatment of acute superficial infections of the eye and its adnexae (conjunctivitis, blepharoconjunctivitis and blepharitis) when caused by susceptible bacteria.

CONTRAINDICATIONS

NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) is contraindicated in patients with known hypersensitivity to any component of this product or any chemically related quinolone antibacterial agent.

PRECAUTIONS

General

As with other antibacterial preparations, prolonged use may result in overgrowth of non susceptible bacteria including fungi. If superinfection or resistance should develop, treatment with NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) should be discontinued and appropriate therapy instituted.

Information for the patient

Patients should be instructed of the following:

1. How to properly use this ophthalmic solution as this is a sterile solution;

2. To avoid contaminating the solution by preventing the dropper tip from touching any surface;

3. To consult a physician,

    a) unless otherwise informed, if there has been no improvement after 4 days of continuous treatment or if the infection seems to get worse

    b) if irritation or sensitization develops

    c) before wearing contact lenses.

    The preservative (benzalkonium chloride) in NOROXIN® Ophthalmic Solution may deposit in soft contact lenses. The lenses should be removed before application of the drops and not be reinserted earlier than 15 minutes after use. Ideally, patients with an eye infection should not wear their contact lenses until their treatment is finished.

Use in Obstetrics

NOROXIN® Ophthalmic Solution has not been studied in human pregnancy. Therefore, NOROXIN® Ophthalmic Solution should be given to a pregnant woman only if clearly needed.

Nursing Mothers

It is not known whether norfloxacin is excreted in human milk following ocular administration.

ADVERSE REACTIONS

The most frequently reported side effect was local burning or smarting (5.4%). Other drug-related side effects were conjunctival hyperemia (0.5%), chemosis (0.3%), photophobia (0.5%) and a bitter taste following instillation.

DOSAGE AND ADMINISTRATION

The recommended dose is one or two drops of NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) in the affected eye(s) four times daily. Depending on the severity of the infection, the dosage for the first day of therapy may be increased to one or two drops every two hours during the waking hours.

The usual course of treatment is 7 days.

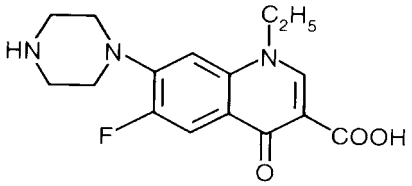
PHARMACEUTICAL INFORMATION

I. DRUG SUBSTANCE

Proper name: norfloxacin

Chemical name: 1-ethyl-6-fluoro-1,4 dihydro-4-oxo-7-(1-piperazinyl)-3 quinoline carboxylic acid.

Structural formula:



Molecular formula: $C_{16}H_{18}FN_30_3$

Molecular weight: 319.34

Description:

Norfloxacin is an odourless, white to pale yellow crystalline powder with a bitter taste and a melting point of 220°C-224°C. It is freely soluble in glacial acetic acid; sparingly soluble in dichloromethane; slightly soluble in acetone and chloroform; very slightly soluble in ethanol, methanol, ethyl acetate and benzene; and practically insoluble in water. Solubility in water is minimal between pH 6.0 to 10. Dissociation constants are: pKa_1 = 6.3 and pKa_2 = 8.8.

II. COMPOSITION

Each mL of NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) contains 3 mg norfloxacin. Non-medicinal ingredients: disodium edetate, sodium acetate, sodium chloride, and hydrochloric acid (to adjust pH). Benzalkonium chloride 0.0025% is added as preservative.

III. STABILITY AND STORAGE RECOMMENDATIONS

Protect from light. Store at room temperature, below 30°C.

AVAILABILITY OF DOSAGE FORMS

NOROXIN® Ophthalmic Solution (norfloxacin ophthalmic solution) is a sterile, clear, colourless to light yellow solution.

NOROXIN® Ophthalmic Solution, 0.3% is supplied in a 5 mL white, opaque, plastic OCUMETER® Ophthalmic dispenser with a controlled drop tip.

MICROBIOLOGY

In Vitro Studies

The *in vitro* activity of norfloxacin against clinical isolates of gram-positive and gram-negative aerobic bacteria is shown in Table 1. Susceptibility was determined by both agar and broth dilution tests, pH 7.1-7.4, using inoculum sizes ranging from 10^4 to 10^5 colony-forming units (cfu) per mL. Norfloxacin lacks useful activity against *Actinomyces* spp., *Fusobacterium* spp., *Bacteroides* spp., and *Clostridium* spp., other than *C. perfringens*.

TABLE 1

CUMULATIVE PERCENT OF STRAINS INHIBITED AT THE INDICATED CONCENTRATIONS OF NORFLOXACIN

		(mg/L)															
Organism	**No./Strains**	**>0.004**	**0.008**	**0.016**	**0.03**	**0.06**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
Achromobacter xylosoxidans	(30)										3	7	13		37	60	73
Acinetobacter calcoaceticus	(122)							1	7	20	38	62	91	96	100		
Alcaligenes faecalis	(23)										4		9	22	70	91	
Citrobacter freundii	(27)				26	48	74	93	96	100							
Citrobacter spp.	(15)					60		80	87		100						
Enterobacter cloacae	(76)				3	28	50	92	96		97	100					
Enterobacter spp.	(67)					33	55	87	91	96	97	100					
Escherichia coli	(417)				0.5	47	77	93	95	97	99	99	100				
Haemophilus influenzae, H. aegyptius (Koch-Weeks Bacillus)	(21)				48	86	100										
Klebsiella pneumoniae	(50)					6	30	64	84	88	94	98	100				
Klebsiella spp.	(138)					24	56	77	84	95	99	99	100				
Moraxella species	(22)			4	23	41	68	73	82	95	100						
Morganella morganii	(52)				2	25	79	87		94	100						
Neisseria gonorrhoeae	(11)	18	45	73	91	100											
Proteus mirabilis	(115)					51	63	74	93	100							
Proteus vulgaris	(58)				10	26	67	93	98		100						
Proteus spp., indole[+]	(10)					90	100										
Proteus spp.	(27)						52	93	100								
Providencia rettgeri	(111)				10	23	43	66	74	80	85	87	93	100			
Providencia stuartii	(16)			6	32	63	76	82	88		94			100			
Providencia spp.	(20)						80	85	95	95	100						
Pseudomonas aeruginosa	(245)						1	8	36	64	84	92	98		100		
Pseudomonas cepacia	(17)											6		71	100		
Pseudomonas maltophilia	(43)											2	9	49	84	95	100
Pseudomonas spp.	(112)						10	12	58	81	86	90	96	99	100		
Salmonella spp.	(11)					55		91		100							
Serratia marcescens	(87)						6	44	67	79	85	90	93	99	100		
Serratia spp.	(20)					15	95	100									
Shigella spp.	(3)							100[x]									
Streptococcus Spp Group A & B	(37)									11	32	40	89	95	100		
Streptococcus pneumoniae	(15)								7		27	60	93	100			
Staphylococcus aureus	(111)						1	2	8	42	76	95	99	100			
Staphylococcus epidermidis	(75)						3	15	41	81	95	97	100				
Staphylococcus saprophyticus	(15)							13	13	87	100						
Streptococcus agalactiae	(10)									10	90	100					
Streptococcus bovis	(15)							20	20	33	53	80	87	100			
Streptococcus faecalis	(67)									3	52	91	100				
Ureaplasma urealyticum	(20)											15	50	90	100		

[x] Only three strains.

The minimal inhibitory concentrations (MICs) of norfloxacin against aerobic bacteria are not significantly affected by culture medium composition or by changes in inoculum size in the range 10^3 to 10^6 cfu/spot (Table 2). In one study with 37 enterobacteria in Mueller Hinton agar, increases in MIC values of norfloxacin at 10^8 cfu/spot were 2- to 4-fold higher than at 10^6 cfu/spot.

MICs of 4- to 33-fold higher were seen when representative enterobacteria were tested simultaneously in Diagnostic Sensitivity Test (DST) agar at pH 5.5 (MIC range = 0.12 to 32 mg/L), and at pH 7.2 (MIC = 0.015 to 4). This reduction in antibacterial activity suggests a pH effect (Table 2). In pooled human urine agar at pH 6.5 the observed MICs ranged from 0.06 to 16 mg/L. The magnesium content of urine could account for the reduced activity of norfloxacin and other quinolones in pooled human urine agar.

Generally, minimal bactericidal concentrations (MBCs) for norfloxacin are 1 to 4 times the MICs against susceptible bacteria. At these concentrations, norfloxacin is bactericidal, even with bacteria in their stationary phase of growth.

TABLE 2
EFFECT OF CULTURE MEDIUM COMPOSITION, pH, AND INOCULUM SIZE ON ANTIBACTERIAL ACTIVITY OF NORFLOXACIN

		MIC (mg/L)										
		Culture Medium[a]				pH[b]			Inoculum Size (cfu)[c]			
Organism/Strain		**TSA**	**NA**	**MH**	**BHI**	**6**	**7**	**8**	**10^3**	**10^4**	**10^5**	**10^6**
Staphylococcus aureus	2868	0.125	1.0	0.5	2	4	2	1	2	2	2	2
Escherichia coli	4391	0.03	0.06	0.06	0.06	1	0.06	0.06	0.03	0.03	0.03	0.03
Klebsiella pneumoniae	4005	0.06	0.06	0.06	0.06	ND	ND	ND	0.03	0.06	0.06	0.06
Escherichia coli	4392	0.25	0.25	0.25	0.5	8	0.5	0.25	0.5	0.25	0.25	0.5
Proteus mirabilis	3125	0.125	0.25	0.125	0.25	2	0.5	0.125	0.03	0.125	0.03	0.125
Serratia marcescens	2854	0.25	0.5	0.125	0.25	ND	ND	ND	0.125	0.125	0.06	0.125
Pseudomonas aeruginosa	2835	1	1	1	2	4	1.0	0.5	1	1	0.5	0.5

[a]TSA = trypticase soy agar (BBL); NA = nutrient agar (Difco); MH = Mueller Hinton agar (BBL); BHI = brain heart infusion (Difco); 10^6 cfu, pH 7.3
[b]Trypticase soy broth, 10^5 cfu/mL; ND = not done
[c]TSA (BBL), pH 7.3

Development of Resistance

A progressive increase in MIC of norfloxacin was demonstrated in five bacterial isolates by daily passage in subinhibitory concentrations of drug (Table 3). Cross-resistance with other quinolone antibacterial agents has also been observed.

TABLE 3

COMPARATIVE NORFLOXACIN MIC OF PARENT AND DERIVATIVE STRAINS

		Norfloxacin MICs (mg/L)[x]		
Organism/Strain		**Parent Strain**	**R3**	**R5**
Escherichia coli	2891	0.25	0.25	0.5
Klebsiella pneumoniae	4354	0.25	4	8
Proteus vulgaris	2829	0.25	0.5	4
Pseudomonas aeruginosa	2835	0.5	2	8
Staphylococcus aureus	4310	1	4	32

[x] Broth dilution test, inoculum 10^6 cfu/mL, trypticase soy broth.
R 3 = parent strain after 3 transfers.
R 5 = parent strain after 5 transfers.

The frequency with which resistant mutants arise varies among genera (Table 4), is greatest for *P. aeruginosa* and least for *E. coli*. In concentrations within two dilutions of the MIC, mutants of *P. aeruginosa* can be isolated rather frequently (10^{-4} to 10^{-6}), whereas those of *E. coli* are relatively uncommon (10^{-8}). Increasing amounts of norfloxacin resulted in lower frequency of mutants (Table 4).

TABLE 4

FREQUENCY OF NORFLOXACIN-RESISTANT MUTANTS AMONG VARIOUS SPECIES OF *ENTEROBACTERIACEAE, PSEUDOMONAS AERUGINOSA*, AND *STAPHYLOCOCCUS AUREUS*

		Norfloxacin (mg/L)[x]		
Organism/Strain		**0.15**	**0.45**	**1.5**
Escherichia coli	3773	1.1×10^{-8}	ND	ND
Klebsiella oxytoca	4007	7.5×10^{-7}	1.5×10^{-7}	ND
Klebsiella pneumoniae	3972	2.2×10^{-7}	5.1×10^{-8}	ND
Enterobacter cloacae	301	2.3×10^{-8}	ND	ND
Serratia marcescens	1581	4.6×10^{-7}	2.8×10^{-7}	1.3×10^{-9}
Citrobacter freundii	2301	5.0×10^{-8}	ND	ND
Proteus vulgaris	J 17	1.2×10^{-6}	ND	ND
Morganella morganii	2481	1.3×10^{-7}	ND	ND

		Norfloxacin (mg/L)		
Organism/Strain		**2**	**8**	**24**
Pseudomonas aeruginosa	1404	8.9×10^{-5}	7.3×10^{-9}	ND
Pseudomonas aeruginosa	976	4.6×10^{-7}	ND	ND
Pseudomonas aeruginosa	526	1.3×10^{-6}	ND	ND
Pseudomonas aeruginosa	380	ND	ND	ND
Pseudomonas aeruginosa	133	4.6×10^{-4}	ND	ND
Pseudomonas aeruginosa	89	5.4×10^{-7}	ND	ND
Staphylococcus aureus	51	ND	ND	--
Staphylococcus aureus	105	ND	ND	--
Staphylococcus aureus	137	4.1×10^{-9}	ND	--

[x] Overnight cultures in trypticase soy broth (Oxoid) were plated in 10- and 100-fold dilutions on CLED agar (Oxoid) containing various concentrations of norfloxacin.

ND = Not detectable, frequency of resistant mutants < 10^{-10}.

Susceptibility Testing

The standard disc susceptibility test (using the 10 µg NOROXIN® disc) or dilution susceptibility should be used.

Organisms should be tested with NOROXIN® discs, since norfloxacin has been shown by *in vitro* tests to be active against genera and strains of bacteria known to be or determined to be resistant when nalidixic acid discs are used.

TABLE 5

INTERPRETATION OF SUSCEPTIBILITY CRITERIA OF NORFLOXACIN (FOR MILD OR MODERATE INFECTIONS OF THE URINARY TRACT)

	Zone Diameter (10µg norfloxacin disc) (mm)	Approximate MIC Correlation (mg/L)
Susceptible (susceptible to usual doses)	**≥13**	**≤16**
Resistant	**≤12**	**≥32**

Proposed control limits for monitoring susceptibility tests are given in Table 6.

TABLE 6

CONTROL LIMITS FOR MONITORING NORFLOXACIN SUSCEPTIBILITY TESTS

Organism	Zone Diameter (mm)	MIC (mg/L)
E. coli ATCC* 25922	28 - 35	0.03 - 0.12
S. aureus ATCC* 25923	17 - 28	
S. aureus ATCC* 29213		0.5 - 2.0
S. faecalis ATCC* 29212		2.0 - 8.0
P. aeruginosa ATCC* 27853	22 - 29	1.0 - 4.0

* Trademark American Type Culture Collection

In Vivo Studies

Fourteen rabbits were subjected to experimental corneal ulceration and received 1% suspension of norfloxacin topically to both eyes four times a day for four days. The eyes in the control group received saline solution topically four times a day for four days. The results of this study are illustrated in Figure 1.

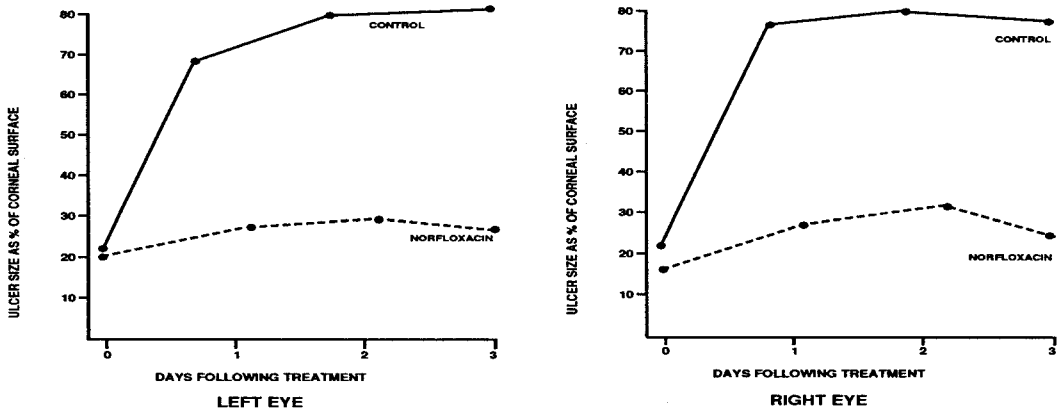


FIGURE 1

The Effect of Therapy With Various Antibacterial Agents on Corneal Ulcers Induced with *Pseudomonas aeruginosa*

Five treatment groups of 14 rabbits each, were subjected to experimental corneal ulceration, received norfloxacin 0, 0.15, 0.3, 0.6, or 1% topically to both eyes. Treatment was given at 90 minute intervals four times on Day 0 and six times on Day 1 and 2. As shown in Table 7, there is a dose-response relationship for norfloxacin in the reduction of colony counts of *S. aureus*.

TABLE 7

COLONY COUNTS OF *S. AUREUS* FROM CONJUNCTIVAL WASHINGS IN CONTROL AND TREATMENT EYES AT DAY 6

TREATMENT GROUPS	COLONY FORMING UNITS	CORNEAL INDEX RIGHT EYE	CORNEAL INDEX LEFT EYE
Control(0% norfloxacin)	252	6.8[a]	6.8[a]
0.15% norfloxacin	19	3.9[b,c]	5.5[a,b]
0.3% norfloxacin	8	3.5[b,c]	4.3[b]
0.6% norfloxacin	6	2.9[c]	3.8[b]
1% norfloxacin	2	2.9[c]	3.4[b]

a,b,c Means with a letter in common were not statistically different using honestly significant difference (HSD) test at 5% level of significance.

PHARMACOLOGY

Animal Pharmacology

Central Nervous System

Norfloxacin (0.1-1.0 g/kg p.o.) showed no significant influences on behavior or in various pharmacological tests of central nervous system activity in either mice or rats. Similarly, norfloxacin (10 mg/kg I.V.) produced no changes in the central nervous system of rabbits implanted with recording electrodes.

Peripheral Nervous System

Intravenous administration of norfloxacin (10 mg/kg) modestly reduced (30%) contractions of the cat nictitating membrane elicited by both pre- and post-ganglionic nerve stimulation. Nerve stimulated contractions of the rabbit tibialis muscle, however, were unaffected by similar doses of norfloxacin.

Using *in vitro* smooth muscle preparations (guinea pig ileum and trachea), norfloxacin (100-300 mg/L) exhibited no anticholinergic, antiserotonergic or antihistaminergic activity. Likewise, norfloxacin did not alter the intrinsic muscle tone of the above preparations. In the isolated rat vas deferens, norfloxacin did

alter responses to norepinephrine (slightly enhancing low concentrations and reducing high concentrations) though no alteration in epinephrine responses were observed in the guinea pig ileum and trachea. Norfloxacin (100 mg/L) slightly reduced the amplitude of spontaneous contractions of the pregnant and nonpregnant rat uterus and rabbit ileum i*n vitro*. However, *in vivo* norfloxacin (10 mg/kg I.V.) was without significant effect on spontaneous contractions of the stomach, intestine, bladder and uterus of rabbits. Similarly, gastrointestinal motility in mice was unaffected by 1.0 g/kg p.o. of norfloxacin.

Cardiovascular and Respiratory Systems

Norfloxacin (1.0 mg bolus) slightly increased (10 ± 3%) coronary flow and decreased heart rate (7 ± 2%) while modestly depressing contractile force (45 ± 4%) of isolated, perfused guinea pig hearts. In the isolated rabbit ear artery, a similar 1 mg bolus of norfloxacin produced a weak, transient decrease followed by an increase in blood flow. *In vivo*, norfloxacin administered intravenously increased by about 20% the respiratory rate in urethane anesthetized rabbits (30 mg of norfloxacin/kg) and barbiturate anesthetized dogs (10 mg of norfloxacin/kg). In these animals blood pressure tended to be reduced without significant changes in heart rate. The decrease of blood pressure in rabbits (approximately 15%) and particularly dogs, was marked by considerable animal variation (3/6 dogs had decreases of 90-100 mmHg). Infusion of norfloxacin (180 mg/hour I.V.) produced an elevation in the respiratory rate (60% increase) with a decrease (about 10%) in blood pressure and no change in heart rate in dogs. In urethane anesthetized rats a reduction of about 20% in blood pressure was caused by norfloxacin (200 mg/kg I.V.). This reduction was inhibited by diphenhydramine but unaffected by atropine, propranolol or phentolamine.

Oral administration of norfloxacin (1.0 g/kg) did not alter the blood pressure of unanesthetized rats.

Other Systems

Norfloxacin (1.0 g/kg p.o.) did not change blood sugar levels or coagulation time in rats and did not exhibit any antiinflammatory activity in rats. Similarly, norfloxacin (300 mg/kg p.o.) was unable to prevent cold stress-induced ulcers in rats. Subcutaneous administration of norfloxacin (200 mg/kg) significantly reduced by about 90% gastric acid secretion while doses as high as 1.0 g/kg p.o. failed to alter bile secretion in rats. Norfloxacin (1.0 g/kg p.o.) significantly reduced urinary volume (30% decrease) over 24 hours and reduced urinary Na^+ excretion (ca. 25%) and significantly increased urinary K^+ excretion (ca. 30%) over this time period. None of these effects were observed at doses of 100 mg/kg p.o.

Four groups of three rabbits were given 50 µL of an ophthalmic solution containing 0.3% norfloxacin three times a day for a period of 48 hours. The tear fluid levels of norfloxacin showed no accumulation of drug after multiple dosing and aqueous humor levels were all below the detection limit of 0.2µg/mL.

Three rabbits lacking corneal epithelium were administered a single dose of 50 µL of a 0.3% ophthalmic solution of norfloxacin. A group of three rabbits with intact corneal epithelium served as control.

Aqueous humor levels of norfloxacin in rabbits lacking an intact corneal epithelium were substantially higher (about four to six times) than levels in animals of the control group. The results of this study are shown in Table 8.

TABLE 8

CONCENTRATION OF NORFLOXACIN IN AQUEOUS HUMOR OF RABBITS LACKING CORNEAL EPITHELIUM AFTER A SINGLE INSTILLATION OF 50 µL OF A 0.3% SOLUTION

		DRUG CONCENTRATION
SAMPLING TIME IN MINUTES	**NUMBER OF EYES**	**IN AQUEOUS HUMOR µg/mL±(S.E.M.)**
20	6	4.2± 0.5
35	6	4.7± 0.4
65	6	3.3± 0.5
65[x]	6	0.8± 0.1

[x] Control group with intact corneal epithelium

TOXICOLOGY

Acute Toxicity

SPECIES	SEX	ROUTE	LD_{50}mg/kg (95% confidence limits)
Mouse	Male	p.o.	> 4,000
Mouse	Male	Subcutaneous	> 1,500
Mouse	Male	Intramuscular	470 (405-545)
Mouse	Male	Intravenous	220 (209-232)
Mouse	Female	p.o.	> 4,000
Mouse	Female	Subcutaneous	> 1,500
Mouse	Female	Intramuscular	480 (429-538)
Mouse	Female	Intravenous	237 (226-248)
Rat	Male	p.o.	> 4,000
Rat	Male	Subcutaneous	> 1,500
Rat	Male	Intramuscular	> 500
Rat	Male	Intravenous	270 (233-313)
Rat	Female	p.o.	> 4,000
Rat	Female	Subcutaneous	> 1,500
Rat	Female	Intramuscular	> 500
Rat	Female	Intravenous	245 (211-284)

The six metabolites were also tested orally for their acute toxicity in rats and

mice. The LD_{50} values were estimated to be greater than 2,000 mg/kg.

Subacute Toxicity Studies

One month oral toxicity studies were carried out in rats (250, 500 and 1,000 mg/kg/day, 10 males and 10 females per group), dogs (50, 100 and 200 mg/kg/day, one male and one female per group; or 25, 50 and 100 mg/kg, b.i.d., 6 days per week, 3 males and 3 females per group) and monkeys (25, 50, 100 mg/kg, b.i.d., 6 days per week, 2 males per group). In all studies similar size groups served as controls and received sterile distilled water.

A slight increase in urinary potassium excretion was seen in female rats dosed at 500 and 1,000 mg/kg/day. There was, however, no concurrent decrease in serum potassium level. A slight enlargement of cecum was observed in 3 of 10 male rats at 1000 mg/kg/day. Liver glucose-6-phosphatase activity was slightly increased at all dose levels in the dog study in which norfloxacin was given at doses of 25, 50 or 100 mg/kg b.i.d.

Chronic Toxicity Studies

Rat

A six-month oral toxicity study in rats at norfloxacin doses of 0, 125, 250 and 500 mg/kg/day (10 rats/sex/group) revealed only minimal effects as evidenced by slight body weight retardation in male rats in the 250 and 500 mg/kg/day groups.

A similar study was carried out in rats (15 rats/sex/group) at norfloxacin doses of 0, 50, 200 and 600 mg/kg/day orally. There was a slight, but not statistically significant, decrease in body weight gain at 600 mg/kg/day in males. In addition, crystalluria consisting of the dihydrate and the magnesium salt of norfloxacin occurred in rats from the 200 and 600 mg/kg/day groups. The crystalluria was most frequent in males given 600 mg/kg/day. A high incidence of cecal enlargement was seen at all dose levels. Gray feces were observed in all treated groups and a very slight but statistically significant decrease in

serum proteins were observed in males dosed at 600 mg/kg/day.

Dog

Four chronic oral toxicity studies were performed to evaluate the potential toxicity of norfloxacin in dogs following chronic administration for 20 weeks to 13 months. The doses used were: 0, 25, 50 and 100/200 mg/kg/day (the dose was increased due to lack of drug-induced effect, 6 days per week, 4 males and 4 females per group); 0, 50, 150 and 300 mg/kg/day (7 days per week, 3 males and 3 females per group); 0, 25, 50 and 100 mg/kg/day, (7 days per week, 1 male and 1 female per group); 0, 50, 100 and 150 mg/kg/day (7 days per week, 4 males and 4 females per group). Crystalluria was evident in one of these studies where dogs were given the drug at 50, 150 and 300 mg/kg/day for 26 weeks. The incidence was dose related. Four dogs, (1 and 3 in the 150 and 300 mg/kg/day dosage groups, respectively), were found dead or were sacrificed due to urinary obstruction from drug crystals. It was postulated that crystalluria in dogs and rats is the result of low solubility of norfloxacin in alkaline urine. This was supported by the observation of a significant decrease in the incidence of crystalluria and the absence of urinary obstruction in dogs in a separate study at 50, 150 and 300 mg/kg/day orally to 3 dogs/sex/group for three months where the urine was acidified by the administration of ammonium chloride. The pH after urine acidification was generally 6.0 or below whereas the urinary pH of dogs where drug crystals were formed was between 6.86 and 8.28.

Other frequently observed changes in the 150 and 300 mg/kg/day groups consisted of a dose-related incidence of emesis, grey or clay-coloured feces (due to unabsorbed drug) and retardation of body weight gain. There were also changes in the haematologic and serum biochemical parameters (leukocytosis, elevated serum urea nitrogen and creatinine) that are compatible with uremia and pyelonephritis secondary to intrarenal or urethral obstruction.

Reproduction Studies

Fertility

The effect of norfloxacin on fertility was assessed in male and female mice. Norfloxacin was administered orally at dosage levels of 0, 125, 250, 500 mg/kg/day (20 per sex per group) for 61 days before mating and during mating to male mice and for 15 days before mating until the sixth day of pregnancy to female mice. There were no treatment-related effects on the reproductive performances of the adults or differences in growth and characteristics of the fetuses.

Teratology

Mice

In mice oral doses of 0, 125, 250 or 500 mg/kg/day (31 mice/group) given on Days 6 to 15 of gestation did not have any adverse effect on the pregnant mother or F_1 or F_2 fetuses.

Rats

In rats, oral doses of 0, 50, 200 or 800 mg/kg/day (administered b.i.d. to 20 rats/group) given on days 6 to 17 of gestation did not adversely affect the progress or outcome of pregnancy.

Rabbits

In an oral teratology study, rabbits were given 0, 25, 50 or 100 mg/kg/day of norfloxacin orally (12 or 13 rabbits per group) from day 6 to 18 of gestation. In this study, rabbits in the 100 mg/kg/day group showed decreased activity, reduced appetite, diarrhea, and reduced body weight gain. Embryotoxicity (i.e., abortion and fetal resorption) was also observed at this dosage level. An additional study comparing the maternal and fetal toxicity of norfloxacin administered orally at doses of 100 mg/kg/day with that administered subcutaneously at doses of 20 mg/kg/day (which resulted in approximately 5-fold higher maximum plasma drug levels) was carried out. There was no evidence of embryotoxicity in the group that received norfloxacin at a dose of 20 mg/kg/day by the subcutaneous route. This suggested that the

embryotoxicity of norfloxacin in rabbits did not correlate with drug levels in blood.

Monkeys

In an oral teratology study in cynomolgus monkeys, norfloxacin was given at doses of 0, 50, 100, 150 or 300/200 mg/kg/day (the dose was reduced because of emesis) in single or divided doses from Days 20 to 50 of gestation. There were at least 10 female monkeys in each group. Doses of 200 mg/kg/day and greater were maternotoxic and produced vomiting, reduced appetite, and body weight loss. In the 150 and 300/200 mg/kg/day groups, 3 of 10 and 11 of 16 monkeys lost their embryos. There was no evidence of a teratogenic effect in surviving fetuses. Doses of 100 mg/kg/day did not result in any increase in embryonic losses relative to controls.

Gestation and Postnatal Development

The effect of norfloxacin during gestation and postnatal period was studied in mice at doses of 0, 125, 250 and 500 mg/kg/day (21 mice per group). Female mice were dosed orally from Day 15 of gestation to Day 21 postpartum. No significant difference was observed with newborn of the treated groups as compared to controls.

Mutagenicity

Norfloxacin was negative in a dominant lethal test in mice (300 or 800 mg/kg administered as a single dose), an *in vivo* chromosome aberration test in rats (1000 mg/kg/day for 38 days) and hamsters (250 or 500 mg/kg, one dose), and various *in vitro* genotoxicity studies including an Ames test, chromosomal aberration test, sister chromatid exchange assay, unscheduled DNA synthesis assay and V-79 mammalian cell mutagenesis assay. Norfloxacin was weakly positive in a test for DNA repair (rec assay), however, this was considered to be of questionable biological significance since a more sensitive test for DNA repair (V-79 mammalian cell mutagenesis assay) was negative.

Carcinogenicity

A 19-month chronic oral toxicity study was done in rats (50 per sex per group). The rats were autopsied after a 12 to 14 week withdrawal period. Norfloxacin was given in the diet at 0.05 or 0.2% in the feed. The estimated drug intake was 18-35 mg/kg/day for the low dose group and 70-140 mg/kg/day for the high-dose group. No drug related neoplastic changes were reported as compared to control animals. The highest dose was below the maximum tolerated dose (approximately 600 mg/kg/day).

Special Toxicity Studies

Dog

In an ocular irritation study in dogs (3 dogs/sex/group) norfloxacin administered topically at doses of 0, 0.3% and 1% three times a day, for 30 days, produced no treatment-related ocular changes including conjunctival mucous depletion.

Rabbit

In an ocular irritation study in rabbits, the animals received an instillation of 50 µL of saline, vehicle, 0.3, or 1% of norfloxacin solutions in the left eye, three times a day approximately three hours apart for 13 weeks (the right eye being untreated). Very slight signs of ocular irritation were occasionally seen after instillation throughout the study in the treated eye of animals receiving the vehicle, the 0.3% or 1% solutions of MK-366. They consisted of very slight transient blinking in all rabbits and very slight transient redness of the bulbar conjunctiva in most rabbits. These signs were considered due to the vehicle.

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