Aqueous, Oil, and Ointment Formulations of Ketorolac: Efficacy Against Prostaglandin E2–Induced Ocular Inflammation and Safety: A Technical Note

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INTRODUCTION

In ocular tissue, the polyunsaturated fatty acid arachidonic acid is metabolized by cyclooxygenase to prostaglandins (PG), which are the most important lipid-derived mediators of inflammation. Topical administration of exogenous PG induces characteristic signs of ocular inflammation, including conjunctival vasodilation and edema, mucous discharge, lid closure, increased intraocular pressure, and increased aqueous humor protein.[1-3] Topical PG also induce the migration of polymorphonuclear leukocytes (PMNs) in tear fluid.[4] Elevation of tear proteins has been reported during corneal inflammation[5,6] and allergic conjunctivitis.[7] Presently, nonsteroidal anti-inflammatory drugs (NSAIDs), which are cyclooxygenase inhibitors, are being used in clinical ophthalmology for treatment of inflammatory disorders. The anti-inflammatory activity of ketorolac (an NSAID) is primarily attributed to its inhibitory effect on cyclooxygenase or PG formation.[8] Ketorolac is available as a tromethamine salt, ketorolac tromethamine (KT), which is water-soluble. Aqueous ocular drops of KT (0.5%) are an effective anti-inflammatory agent for topical use following cataract surgery and intraocular lens implantation.[9-11] KT is also a viable alternative to corticosteroids in treating ocular inflammation in the presence of pathogens.[12,13] A beneficial effect of KT (0.5%) topical solution in reducing postoperative pain after laser in situ keratomileusis has been reported.[14] Oral administration of NSAIDs has been associated with gastrointestinal ulceration, and topical application of these agents is irritating to the eye, but KT applied topically to the eye up to a concentration of 0.5% (wt/vol) is nonirritating.[15] Previously, this laboratory has reported in vitro transcorneal permeation of KT from 0.5% (wt/vol) aqueous drops; a formulation containing benzalkonium chloride (BAC, 0.01% wt/vol) and disodium edetate (EDTA, 0.01% wt/vol) provided maximum corneal permeation.[16] Similarly, sesame and soybean oil drops containing 0.2% (wt/vol) ketorolac free acid and benzyl alcohol (BA, 0.5% vol/vol), and ophthalmic ointment containing 0.5% (wt/wt) KT (in the dissolved state) showed higher in vitro transcorneal permeation with minimum corneal damage.[17] In vivo ocular availability of ketorolac has also been evaluated following ocular instillation of aqueous, oil, and ointment formulations in normal corneas of rabbits. Compared with aqueous drops, sesame and soybean oil drops of ketorolac provided higher ocular availability, followed by ophthalmic ointment. Ketorolac aqueous drops with BAC and EDTA improved the rate but not the extent of ocular absorption.[18] The in vivo pharmacokinetic data need validation by pharmacodynamic evaluation; that is, it is necessary to find out whether the quantity of drug absorbed topically is sufficient to protect the eye against inflammation.

Accordingly, the purpose of this research was to evaluate the efficacy of aqueous, oil, and ointment formulations of ketorolac on topical instillation against PG-induced ocular inflammation in rabbits. The protein and PMN migration in tear fluid and rate of blinking following topical instillation of prostaglandin E_2 (PGE_2) in the eye were used as study parameters. The effect of the formulations on rats' gastrointestinal ulceration during chronic dosing was also evaluated.

MATERIALS AND METHODS

Materials

KT (purity 99%) was a gift from Ranbaxy Laboratories Ltd (Gurgaon, India). The preservatives were a gift from Max India Ltd (New Delhi, India). Refined food-grade vegetable oils used in the study were soybean (Alpine Industries Ltd, Madhya Pradesh, India) and sesame (Ahmed Mills, Mumbai, India) oils. The eye ointment base used was of Indian Pharmacopoeial[19] grade, and all other chemicals were of analytical grade.

Albino rabbits (2.0-3.0 kg) and albino Wistar rats (160-210 g) were obtained from Lucky Zoological House (New Delhi, India). The animals were kept under standard laboratory conditions and were fed on a standard diet from Lipton India Ltd (New Delhi, India). Water was allowed ad libitum.

Methods

Preparation of Test Formulations

KT is soluble in water, so it was used for making aqueous drops. KT is insoluble in oil, but ketorolac free acid is

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oil-soluble, so ketorolac free acid was used for making oil drops. The concentration of ketorolac in oil drops was kept below the saturation solubility of the drug in the oils. The ophthalmic ointment also contained KT, as it provided enhanced permeation of the drug through isolated cornea.[17]

KT Aqueous Drops

The aqueous solution of KT, 0.5% (wt/vol), was formulated in glass-distilled water, and the resulting solution was adjusted to pH 6.5 using 0.1N NaOH and 0.1N HCL. The ionic strength (μ) of the solution was maintained at 0.2 with sodium chloride.

KT Aqueous Drops With BAC and EDTA

The KT 0.5% (wt/vol) solution was made by taking the aqueous solution of KT and adding BAC (0.01% wt/vol) and EDTA (0.01% wt/vol).

Ketorolac Oil Drops

Ketorolac free acid was made per a method published elsewhere.[17] Ketorolac 0.2% (wt/vol) solution was made in soybean oil and in sesame oil, and 0.5% (vol/vol) BA was added to each formulation.

KT Ointment

An ophthalmic ointment of KT, 0.5% (wt/wt), was prepared by dispersing the aqueous solution of the drug in simple eye ointment base using process 2 as specified in the Indian Pharmacopoeia.[19]

Anti-inflammatory Study

Twenty-eight albino rabbits of either sex were randomly divided into 7 groups of 4 animals each. Each rabbit received 50 μL of liquid formulation (or 25 mg of ophthalmic ointment) in the right eye and 50 μL (or 25 mg) of control vehicle (ie, vehicle without drug and preservative) in the contralateral eye. (This mode of drug dosing was used by Abelson et al for screening anti-inflammatory agents.[20]) Ten minutes later, 50 μL of PGE_2 (1 μg/mL normal saline, Dinoprostone, Astra IDL Ltd, Bangalore, India) was instilled in both eyes. All eyes were then evaluated for parameters of inflammation, that is, PMN and protein migration in tear fluid and blinking rate. The number of times the eye blinked in each rabbit was counted for 1 hour following PGE_2 instillation. Normal saline (100 μL) was instilled in the inferior cul-de-sac of the rabbit eye and after quick and gentle mixing, 50 μL of the tear fluid was withdrawn at 1, 2, 3, 4, and 5 hours following PGE_2 instillation. Similarly, tear fluids were also withdrawn before instillation of the drug formulation (ie, at 0 hour). The tear fluid was analyzed for PMN count and protein concentration.

PMN Count

Tear fluid PMN counts were performed on all eyes treated with either the control or the formulation. Tear fluid (0.02 mL) was gently mixed with 0.38 mL of white blood cell (WBC) diluting fluid (1.5 mL glacial acetic acid, 1 mL of 1% wt/vol aqueous solution of gentian violet, volume increased up to 100 mL with distilled water), and the PMNs in the mixture were counted in a Neubauer hemocytometer (Rohem Instruments, Nasik, India).[21]

Protein Estimation

The protein concentration was estimated in the tear fluid of all eyes treated with either the control or the formulation. The protein content was measured by the Lowry et al[22] method using bovine serum albumin as the standard. For solution A, sodium potassium tartarate (0.4 g) was dissolved in water, 0.2 g of copper sulfate was added to it, and the volume was increased up to 1 L. For solution B, 40 g of sodium carbonate was added to 1 L of 0.2N sodium hydroxide. Equal volumes of solution A and solution B were mixed just prior to use (alkaline copper sulfate solution). Tear fluid (20 μL) was mixed thoroughly with 5 mL of alkaline copper sulfate solution and was incubated at 37°C for 15 minutes. Then 0.5 mL of Folin phenol reagent (diluted to 1N) was added to it and mixed. After 30 minutes of incubation, the absorbance of the samples was measured at 660 nm in a colorimeter. The absorbance was compared with the absorbance values of standards (bovine serum albumin) in the range of 0 to 200 μg/mL treated similarly.

Gastrointestinal Ulceration

Thirty-six adult male and female albino Wistar rats were randomly divided into 6 groups of 6 animals each. The first group of animals received oral KT aqueous solution at a dose of 0.14 mg/kg (0.1-0.3 mg/kg is the inhibitory dose 50 (ID_{50}) of ketorolac in an acute rodent model of inflammation[8]) 4 times a day. This was the control group. Four groups of animals received one of the liquid test formulations (50 μL) topically 4 times a day. The remaining group of animals was treated with 5 mg of ophthalmic ointment twice a day. Regular chronic dosing was continued for 5 or 10 days. On the 5th or 10th day, rats were fasted for 24 hours but water was provided ad libitum. On the 6th or 11th day, the rats were sacrificed by exposure to an atmosphere of chloroform in a chamber for 15 minutes. The stomach with part of the duodenum of the animals was removed, cut open along the

Table 1. Scoring of Ulcers*

Erosions	Score
1 mm or less	1
1 to 2 mm	2
More than 2 mm	3

*The sum of the scores was the ulcer index.

greater curvature, and observed for ulcers. Scoring of ulcers was done by the method described in Table 1.

Statistical Analysis

Statistical analysis was done by Student *t* test. A *P* value less than .05 was considered to be indicative of significance.

RESULTS AND DISCUSSION

The anti-inflammatory effects of ketorolac formulations against PGE_2-induced ocular inflammation in rabbits are shown in Table 2. Topical instillation of PGE_2 in the eye increased the blinking rate, tear fluid PMN count, and protein concentration. Both PMN count and protein concentration in the tear fluid increased up to the third hour and decreased afterward, which has been shown in Figures 1 to 4 for KT aqueous drops (0.5% wt/vol) with or without BAC and EDTA. The PMN count and protein concentration at the third hour, following PGE_2 instillation, was used to determine the statistical significance. KT aqueous drops (0.5%) with or without BAC and EDTA significantly inhibited the ocular inflammation, that is, PMN and protein migration in tear fluid and blinking rate. In an in vivo ocular availability study in rabbits, conducted earlier, KT aqueous drops with BAC and EDTA improved the rate but not the extent of ocular absorption,[18] but in the present experiment the quantity of drug absorbed from the formulation appeared to be sufficient to inhibit the inflammatory response. Similarly, ketorolac drops (0.2% wt/vol) in soybean or sesame oil and KT ointment also significantly inhibited the inflammatory response. The oil drops contained a lower quantity of drug (0.2% wt/vol ketorolac)

Table 2. Anti-inflammatory Effect of Ketorolac Formulations Against PGE_2-Induced Ocular Inflammation*

Formulation	Blinking (1 hour)	PMN/mm^3 (3rd hour)	Protein (%) (3rd hour)
Control†	75.58 ± 2.02	1150 ± 45.64	0.630 ± 0.02
KT aqueous drops	45.75 ± 2.78‡ (39.46%)	862.5 ± 42.70‡ (25%)	0.504 ± 0.01‡ (20%)
Control†	76.0 ± 1.47	1125.0 ± 52.04	0.642 ± 0.02
KT drops with BAC and EDTA	53.75 ± 2.29‡ (29.28%)	950.0 ± 45.64‡ (15.56%)	0.528 ± 0.01‡ (17.76%)
Control	76.25 ± 2.25	1112.5 ± 42.70	0.636 ± 0.02
KT ointment	48.25 ± 2.32‡ (36.72%)	825.0 ± 32.28‡ (25.84%)	0.534 ± 0.02‡ (16.03%)
Control	65.25 ± 1.25	937.5 ± 55.43	0.606 ± 0.02
Ketorolac drops in sesame oil with BA	40.0 ± 1.68‡ (38.69%)	750.0 ± 20.41‡ (20%)	0.492 ± 0.03‡ (18.81%)
Control†	68.75 ± 1.32	1012.5 ± 45.64	0.630 ± 0.02
Sesame oil	67.75 ± 1.65 (1.45%)	1000.0 ± 51.54 (1.23%)	0.615 ± 0.02 (2.38%)
Control	56.0 ± 1.47	812.5 ± 55.43	0.564 ± 0.01
Ketorolac drops in soybean oil with BA	30.75 ± 1.65‡ (45%)	675.0 ± 43.30‡ (16.92%)	0.468 ± 0.03‡ (17.02%)
Control†	68.0 ± 1.96	987.5 ± 65.75	0.642 ± 0.01
Soybean oil	58.5 ± 1.44‡ (13.97%)	812.5 ± 68.85‡ (17.72%)	0.564 ± 0.01‡ (12.15%)

*Values are mean ± SE (n = 4); figures in parentheses are percent inhibition. PGE_2 indicates prostaglandin E_2; PMN, polymorphonuclear leukocyte; KT, ketorolac tromethamine; BAC, benzalkonium chloride; EDTA, disodium edetate; BA, benzyl alcohol.
†Control vehicle contained water pH 6.5, ionic strength 0.2.
‡Statistically significantly different from control ($P < .05$) as per Student *t* test.

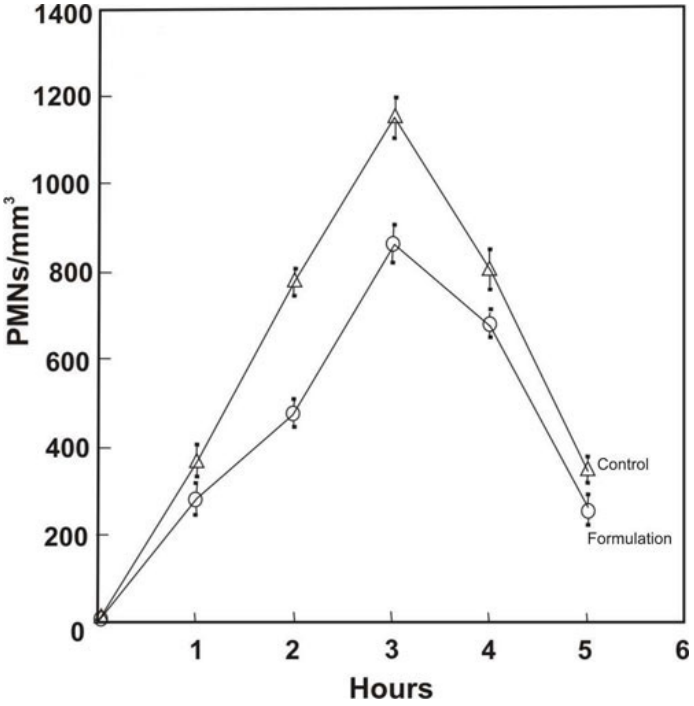

Figure 1. Effect of aqueous drops of ketorolac tromethamine against prostaglandin E_2–induced PMN migration in tears of rabbits. PMNs indicates polymorphonuclear leukocytes.

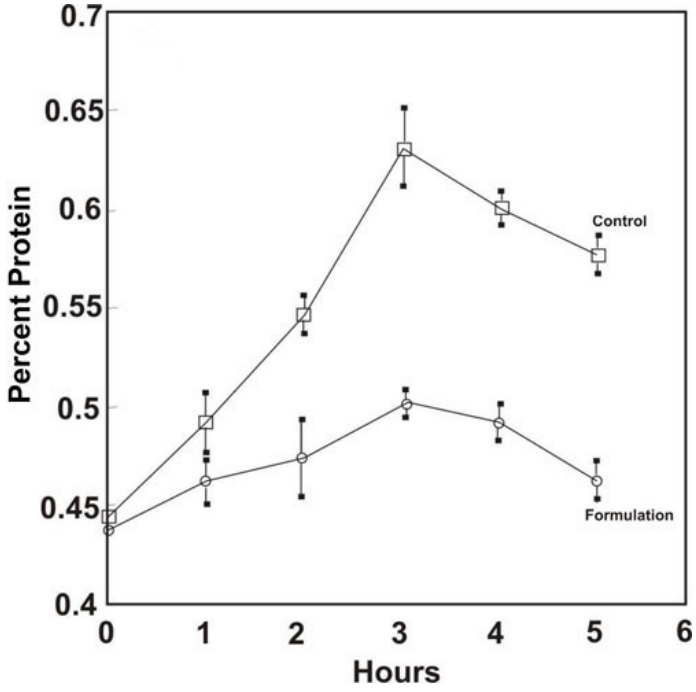

Figure 3. Effect of aqueous drops of ketorolac tromethamine against prostaglandin E_2–induced protein migration in tears of rabbits.

than did the aqueous drops, which contained 0.5% KT (equivalent to 0.35% wt/vol ketorolac). Thus, although the oil drops contained less of the drug, they still inhibited the inflammatory response. The ointment also inhibited the inflammatory response, though the drug contained in a dose of ointment was 50% of that contained in a dose of aqueous drops. To

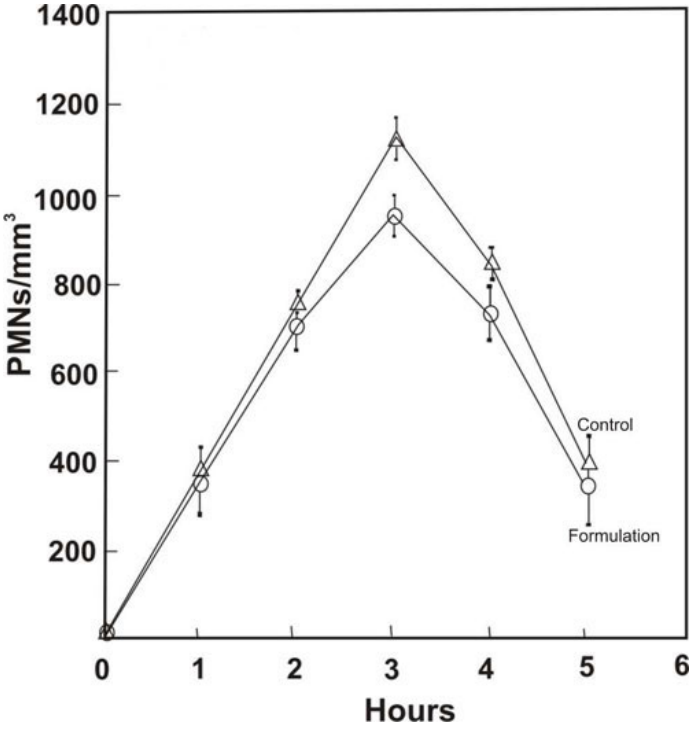

Figure 2. Effect of aqueous drops of ketorolac tromethamine containing benzalkonium chloride and disodium edetate against prostaglandin E_2–induced PMN migration in tears of rabbits. PMN indicates polymorphonuclear leukocytes.

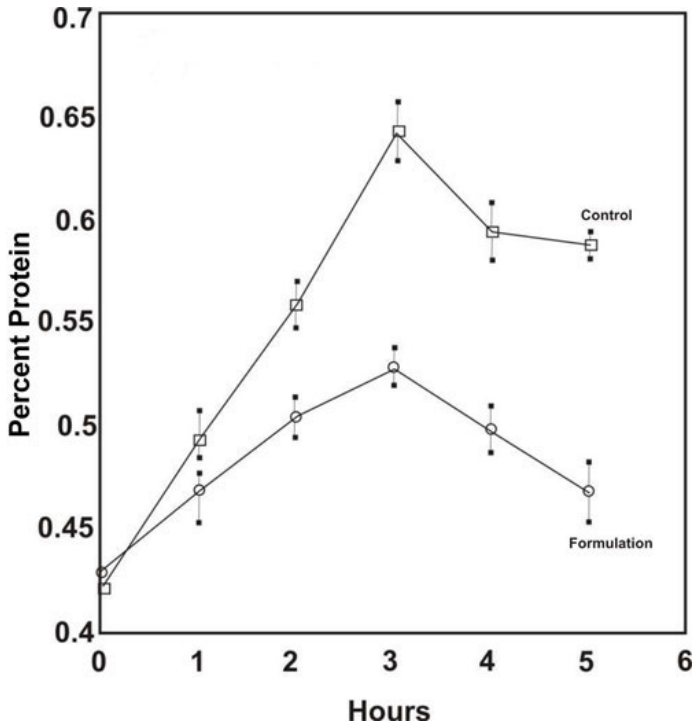

Figure 4. Effect of aqueous drops of ketorolac tromethamine containing benzalkonium chloride and disodium edetate against prostaglandin E_2–induced protein migration in tears of rabbits.

Table 3. Gastrointestinal Ulceration in Rats Following Chronic Dosing of Ketorolac Formulations*

Formulation	Day 6		Day 11	
	Ulcer Index	Mean	Ulcer Index	Mean
Control	4.0	0.66	4.0	0.66
KT aqueous drops	0.0	0.00	1.0	0.16
KT drops with BAC and EDTA	2.0	0.33	0.0	0.00
Ketorolac drops in sesame oil with BA	4.0	0.66	1.0	0.16
Ketorolac drops in soybean oil with BA	1.0	0.16	1.0	0.16
KT ointment	3.0	0.50	4.0	0.66

*Mean of 6 animals in each group. KT indicates ketorolac tromethamine; BAC, benzalkonium chloride; EDTA, disodium edetate; BA, benzyl alcohol.

ascertain whether oily vehicles had any anti-inflammatory effect, both sesame and soybean oils were tested against PGE_2-induced ocular inflammation. Soybean oil significantly inhibited the inflammatory response, but sesame oil did not. This laboratory has earlier reported anti-inflammatory activity of soybean oil against carrageenan-induced rat paw edema. The oil contains linolenic acid (2%-10%), which has been found to inhibit PGE_2-, Leukotriene B_4 (LTB_4-), and arachidonic acid–induced rat paw edema, indicating inhibition of both the cyclooxygenase and the lipoxygenase pathways of arachidonate metabolism.[23] Thus, it seems reasonable that soybean oil would inhibit PGE_2-induced ocular inflammation. Addition of ketorolac to the oil would potentiate the anti-inflammatory effect of the formulation.

Rabbits blink 4 times per hour.[24] Topical administration of exogenous PG induces characteristic signs of ocular inflammation, including conjunctival vasodilation and edema.[1-3] Topical PGE_2 also induces PMN migration in the tear fluid of rabbits.[4] The present study indicates that topical PGE_2 increases blinking rate and PMN and protein migration in tear fluid. PGE_2 is a powerful vasodilator and synergizes with other inflammatory vasodilators such as histamine and bradykinin. It is this combined dilator action on precapillary arterioles that contributes to the redness and increased blood flow in areas of acute inflammation. PGE_2 potentiates the effect of histamine and bradykinin in increasing the permeability of postcapillary venules. PGE_2 also potentiates the effect of bradykinin by sensitizing afferent C fibers to cause pain. The anti-inflammatory effects of the NSAIDs result largely from prevention of these PG actions.[25] It has been suggested that the COX-independent (or PG-independent) effect may contribute to the efficacy of NSAIDs. Salicylates and certain traditional NSAIDs can directly inhibit the activation and function of neutrophils, perhaps by blockade of integrin-mediated neutrophil adhesion by inhibiting downstream Erk signaling.[26] From the above discussion, it appears that topical PGE_2 would cause ocular inflammation, resulting in increased blinking and permeability of conjunctival blood vessels to PMNs and proteins that migrate to tear fluid. Ketorolac formulations significantly inhibited the inflammatory response, as NSAIDs are known to decrease the sensitivity of blood vessels to bradykinin and histamine and reverse vasodilation. NSAIDs can also inhibit chemotaxis.[27] The results indicate that the amount of drug absorbed, on topical administration of ketorolac formulations, is quite sufficient to protect the eye against inflammatory insult.

Chronic dosing of ketorolac aqueous and soybean oil drops topically for 5 to 10 days into rats' eyes yielded ulcer indexes less than those of controls, whereas sesame oil drops and ointment formulations yielded ulcer indexes similar to controls' on the 6th day and 11th day, respectively (Table 3). Since the mean ulcer index was less than 1, the formulations could be considered nonulcerogenic. In addition, the formulations did not produce any abnormalities in the treated eyes. On instillation to the eye, drops are drained via the nasolachrymal duct and gain entry into the gastrointestinal tract, from which they are absorbed into the systemic circulation. Systemic absorption may lead to adverse effects. Topical administration of an ophthalmic solution of timolol, a beta-adrenergic antagonist, has been reported to cause respiratory embarrassment and death in asthmatic patients.[28] Ketorolac is a PG inhibitor. The common side effect observed with PG inhibitors is gastrointestinal ulceration. Thus, questions may be raised about the safety of ketorolac topical formulations for long-term use. To explore this, the formulations were applied chronically into rat's eyes for 5 or 10 days. The formulations were not associated with any appreciable gastrointestinal ulceration. Thus, the results indicate that ketorolac formulations have anti-inflammatory activity and do not lead to gastrointestinal toxicity on chronic dosing.

Summary and Conclusions

The efficacy of aqueous, oil, and ointment formulations of ketorolac against PGE_2-induced ocular inflammation in rabbits was evaluated, with monitoring of blinking rate and PMN and protein migration in tear fluid, following topical PGE_2 instillation. Ketorolac ophthalmic formulations protected the eye against inflammatory insult. Chronic topical administration of formulations for 10 days into rats' eyes did

not lead to any appreciable gastrointestinal ulceration, which indicates that the formulations are safe for long-term use.

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