In Vivo Ocular Availability of Ketorolac Following Ocular Instillations of Aqueous, Oil, and Ointment Formulations to Normal Corneas of Rabbits: A Technical Note

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INTRODUCTION

Topical therapy with corticosteroids is quite common in the treatment of ocular inflammatory disorders but their use is often associated with increase in intraocular pressure, cataract formation, and the risk of infection.[1] Many nonsteroidal anti-inflammatory drugs (NSAIDs) have been tested as ocular antiinflammatory agents[2-4] so as to diminish the well-documented ocular side effects caused by corticosteroids. Ketorolac tromethamine (KT), an aryl-acetic acid NSAID is nonirritating to the eye at 0.5% wt/vol concentration.[5] Aqueous ocular drop of KT is an effective and safe anti-inflammatory agent for topical use following cataract surgery and intraocular lens implantation.[6-8] KT is also known to be a viable alternative to corticosteroids in treating ocular inflammation in presence of pathogens.[9,10] Ophthalmic solution of KT (0.5%) has been shown to be effective in treatment of chronic aphakic and pseudoaphakic macular edema.[11] Beneficial effect of KT (0.5%) topical solution in reducing postoperative pain after laser in situ keratomileusis has been reported.[12] Previously this laboratory has reported in vitro transcorneal permeation of KT from 0.5% (wt/vol) aqueous drops, where formulation containing benzalkonium chloride (0.01% wt/vol) and disodium edetate (0.01% wt/vol) provided maximum corneal permeation.[13] Similarly sesame and soybean oil drops containing 0.2% (wt/vol) ketorolac free acid and benzyl alcohol (0.5% vol/vol) and ophthalmic ointment containing 0.5% (wt/wt) KT (in dissolved state) showed higher in vitro transcorneal permeation with minimum corneal damage.[14]

The in vitro corneal diffusion model, used in the said studies, was completely devoid of complication by variability in precorneal factors such as blinking, lacrimation, tear turnover, and drug washout. The in vitro studies provided relative permeation characteristics of ketorolac from different formulations, but the same could not simulate real in vivo conditions. It is therefore necessary to study the in vivo ocular absorption of drug from the said formulations. Ling and Combs studied the ocular bioavailability and tissue distribution of [^{14}C] KT in rabbits.[15] Madhu et al[16] evaluated the effect of benzalkonium chloride/ethylene diamine tetraacetic acid (EDTA) on the ocular bioavailability of KT following ocular instillation to normal and de-epithelialized corneas of rabbits.

Accordingly, the purpose of the present research was to determine the ocular availability of ketorolac following ocular instillation of aqueous, oil, and ointment formulations of the drug to normal corneas of rabbits.

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MATERIALS

KT (purity 99%) was a gift from Ranbaxy Laboratories Limited (Gurgaon, India). Similarly, preservatives were received as gift from Max India Limited (New Delhi, India). Refined food grade vegetable oils used in the study were soybean (Alpine Industries Limited, Madhya Pradesh, India) and sesame (Ahmed Mills, Mumbai, India) oils. Eye ointment base used was of Indian Pharmacopoeial (IP)[17] grade and all other chemicals were of analytical grade. Methanol, acetic acid, and water used in high performance liquid chromatography (HPLC) were of HPLC grade.

Albino rabbits weighing between 2.0 and 3.0 kg were obtained from Lucky Zoological House (New Delhi, India). The rabbits were quarantined for at least 2 weeks upon arrival and animals having clinically normal eyes (ie, free from signs of ocular inflammation) were used in the study. The rabbits were individually housed with food and water provided ad libitum.

METHODS

Preparation of Test Formulations

KT is soluble in water, hence for making aqueous drops KT was used. However, KT is insoluble in oil, whereas ketorolac-free acid is 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 drug in the oils. The ophthalmic ointment also contained KT, as it provided enhanced permeation of drug through isolated cornea.[14]

Ketorolac Tromethamine Aqueous Drop

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 0.1 N NaOH and 0.1 N HCL. The ionic strength (μ) of the solution was maintained at 0.2 with sodium chloride.

Ketorolac Tromethamine Aqueous Drop With Benzalkonium Chloride and EDTA

KT, 0.5% (wt/vol) solution was prepared as the aqueous solution of KT above and benzalkonium chloride (BAC, 0.01% wt/vol) and disodium edetate (EDTA, 0.01% wt/vol) was added to the solution.

Ketorolac Oil Drops

Ketorolac-free acid was made as per method published elsewhere.[14] Ketorolac 0.2% (wt/vol) solution was made in each of soybean and sesame oil, and 0.5% (vol/vol) benzyl alcohol was added to each formulation.

Ketorolac Tromethamine Ointment

Ophthalmic ointment of KT, 0.5% (wt/wt), was prepared by dispersing aqueous solution of drug in simple eye ointment base using process 2, specified in the IP.[17]

In Vivo Study

A 50-μL drop or 25 mg ointment formulation of ketorolac was instilled in the lower cul-de-sac of each eye, and the upper and lower eyelids were gently held closed for 10 seconds to maximize drug cornea contact. At 0.5, 1, 2, 4, 6, and 8 hours postdose, eyes were anesthetized using 4% xylocaine solution topically and aqueous humor was sampled from 4 eyes using a 28-gauge needle. Aqueous humor samples (100 μL) were mixed with 100 μL of methanol and kept in a refrigerator for 1 hour. The mixture was then centrifuged at 3000 rpm for 15 minutes and 20 μL of the supernatant, thus obtained, was analyzed for ketorolac content by HPLC. The remaining supernatant was again mixed with 100 μL of methanol, kept in the refrigerator for 1 hour, and centrifuged at 3000 rpm. With all samples, this repeat exercise showed no protein precipitation.

HPLC Analysis

Quantitative estimation of ketorolac was done by HPLC. Filtered and degassed mixture of methanol, water, and acetic acid (60:39.9:0.1) was used as the mobile phase. The equipment included the following: Waters 486 tunable absorbance detector; Waters 746 data module integrator; Waters 501 HPLC pump (Milford, MA); μ Bondapak C-18, 10 μ column (30 cm × 4.6 mm) and injector (Rheodyne Inc, Cotati, CA) fitted with 20-μL loop). The mobile phase was delivered at a flow rate of 2.0 mL/min, single injection volume was 20 μL and the effluent was monitored at 254 nm. Three solutions of known ketorolac concentration were used as external standards and the 3 standards were run after every 10 to 15 samples in order to ensure reliable and accurate quantification.[18]

Data Analysis

The maximum concentration of drug in the aqueous humor (Cmax) and the time required to reach the maximum concentration (Tmax) were obtained from the aqueous humor drug concentration versus time curves. The area under the aqueous humor concentration versus time curve (AUC) was calculated by trapezoidal rule. The rate constant (k) of ketorolac was calculated by log linear regression of the last data points (terminal portion) of the aqueous humor concentration versus time curve. The half-life of ketorolac was calculated from the following equation:

$$t_{1/2} = \frac{0.693}{K} \qquad (1)$$

(Statistical analysis was done by 1-way analysis of variance (ANOVA) followed by Dunnett test. A P value less than .05 was considered as indicative of significance .

Results and Discussion

The results of the in vivo studies are shown in Table 1 and Figure 1. On topical instillation of KT aqueous drop into rabbit's eye, maximum concentration of ketorolac in aqueous humor (Cmax) was achieved slowly and the time to reach maximum aqueous humor concentration of ketorolac (Tmax) was 4 hours. Formulation with BAC and EDTA reduced Tmax to 1 hour and increased Cmax (statistically not significant). Thus BAC and EDTA increased the rate of absorption of the drug into the eye, and the results are consistent with the findings of Madhu et al.[16] AUC obtained with formulation with BAC and EDTA was however smaller (statistically significant) than that obtained with aqueous drop without the additives. Madhu et al[16] used 0.5% KT drops (pH 7.4) with or without 0.01% BAC/0.1% EDTA for their studies. AUC obtained with formulation with BAC & EDTA was similar to that obtained without BAC and EDTA and $t_{1/2}$ obtained were 2.0 and 2.22 hours, respectively. We have used 0.5% wt/vol KT drops (pH 6.5, ionic strength 0.2) with or without 0.01% BAC and 0.01% EDTA (ie, EDTA concentration was one

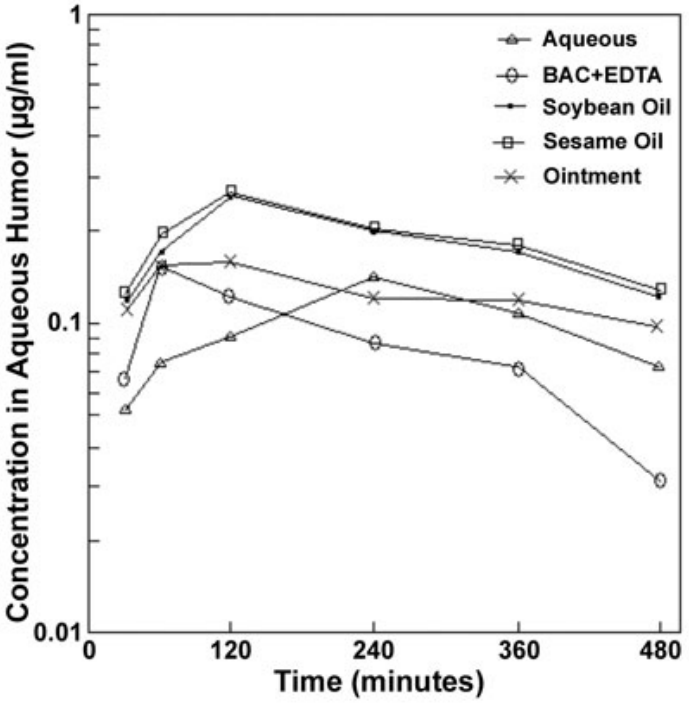

Figure 1. Ketorolac concentrations in aqueous humor after topical administration of ketorolac formulations to normal corneas of rabbits. Values are Mean ± SE, n=4.

tenth of that used by Madhu et al[16]) and $t_{1/2}$ were 3.30 and 3.56 hours, respectively, but AUC obtained with the former was smaller. In in vitro studies conducted earlier we have observed maximum transcorneal permeation of KT from aqueous drop containing BAC (0.01%) and EDTA (0.01%).[13] BAC, a cationic surfactant, has been reported to increase in vitro permeation of ketorolac (an anionic drug) through rabbit cornea,[18] and the mechanism suggested for the same are (1) formation of a more lipid soluble ion pair and (2) disruption of corneal epithelium. EDTA, a known calcium-chelating agent, has been shown to act on cell junctions by interfering with calcium ions and altering intercellular integrity. EDTA also disrupts plasma membrane and consequently increases intercellular permeability.[19] Thus, it seems reasonable to expect that BAC and EDTA combination would also increase in vivo penetration of ketorolac. But formulation with BAC and EDTA increased the rate of absorption of ketorolac not the extent. One explanation could be that BAC being a surfactant reduces the interfacial tension between the formulation and corneal epithelium resulting in spreading of the formulation over the cornea. Furthermore, BAC may emulsify corneal epithelium resulting in quicker saturation of epithelium with the drug . Since only the drug present in the epithelium can partition through the stroma and endothelium to aqueous humor, quicker saturation of epithelium could possibly explain faster absorption. But BAC (being a surfactant) is known to cause ocular irritation[20] as does EDTA. Thus formulation with BAC and EDTA could cause ocular irritation, resulting in increased lacrimation and loss of drug from conjunctival sac leading to reduced extent of absorption of ketorolac. However further studies are needed to ascertain the fact.

In in vitro studies with excised cornea, conducted earlier, observations were made of enhanced permeation of ketorolac from ophthalmic ointment containing 0.5% (wt/wt) KT (in dissolved state) and sesame or soybean oil drop containing 0.2% (wt/vol) ketorolac-free acid and benzyl alcohol (0.5% vol/vol), with minimum corneal damage.[14] Accordingly, these formulations were chosen for in vivo studies. Compared with aqueous drop, ointment formulation provided higher Cmax and AUC (statistically significant) values, though the drug contained in the dose of ointment was 50% of that contained in a dose of aqueous drop. Tmax observed with ointment was shorter and $t_{1/2}$ was longer, indicating faster absorption and sustained effect. It was observed with fluorometholone ointment that on topical dosing into rabbit eye the peak aqueous humor concentration of drug was not reached until 3 hours after dosing, but drug concentrations persisted far longer than observed after instillation of suspension or solution of the drug.[21] But in this study, peak concentration (Cmax) was reached relatively quickly with ointment compared with aqueous drop. Presence of dissolved drug in ointment and reduced drainage of the dose could possibly account for this result. Formulations in sesame and soybean oil also provided shorter Tmax (similar to ointment) but the Cmax and AUC values were significantly much higher ($P < .05$) than that obtained with aqueous drop. The $t_{1/2}$ values were

Table 1. Pharmacokinetic Parameters of Ketorolac (as free acid) in Aqueous Humor After Topical Administration of Ketorolac Formulations to Normal Corneas of Rabbits*

Formulation	C_{max} (µg/mL)	T_{max} (hours)	$t_{1/2}$ (hours)	$AUC_{(0\text{-}8h)}$(µg.min/mL)
Aqueous	0.142 ± 0.008	4	3.56	47.14 ± 2.616
BAC + EDTA	0.153 ± 0.005	1	3.30	40.88 ± 1.285†
Soybean oil	0.259 ± 0.004†	2	6.51	85.03 ± 0.790†
Sesame oil	0.244 ± 0.021†	2	6.71	87.86 ± 3.259†
Ointment	0.164 ± 0.005	2	10.20	58.73 ± 1.307†

*BAC indicates benzalkonium chloride; and EDTA, disodium edetate. Values are mean ± SE, n = 4.

†Statistically significant ($P < .05$) from aqueous formulation, as determined by 1-way ANOVA followed by Dunnett test.

in between the corresponding $t_{1/2}$ obtained with aqueous drops and the ointment. It would be worthwhile to mention here that oil drops contained lesser quantity of drug (0.2% wt/vol ketorolac) compared with aqueous drop containing 0.5% KT (ie, equivalent to 0.35% wt/vol ketorolac). Thus, considering both rate (Tmax) and extent of absorption (AUC), the ocular availability of the formulations could be ranked as sesame oil ≈ soybean oil > ointment > aqueous drop ≈ aqueous drop with BAC and EDTA. The ointment formulation will have maximum sustained effect.

CONCLUSIONS

In vivo ocular availability of ketorolac was evaluated following ocular instillation of aqueous, oil, and ointment formulations to normal corneas of rabbits and monitoring ketorolac concentration in aqueous humor by HPLC. Compared with aqueous drop, sesame and soybean oil drops of ketorolac provided higher ocular availability followed by ophthalmic ointment. The ointment formulation provided maximum sustained effect. Ketorolac aqueous drop with BAC and EDTA improved the rate of ocular absorption though not the extent.

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