

# Effect of Benzalkonium Chloride/EDTA on the Ocular Bioavailability of Ketorolac Tromethamine following Ocular Instillation to Normal and De-epithelialized Corneas of Rabbits

CHERUKURY MADHU, PETER J. RIX, MARTHA J. SHACKLETON, THAI G. NGUYEN, AND DIANE D.-S. TANG-LIU<sup>x</sup>

Received October 5, 1995, from the Department of Pharmacokinetics, Allergan, Inc., 2525 Dupont Drive, P.O. Box 19534, Irvine, CA 92713-9534. Final revised manuscript received December 8, 1995. Accepted for publication January 9, 1996<sup>®</sup>.

**Abstract** □ This study was designed to examine the effect of benzalkonium chloride/ethylenediaminetetraacetic acid (BAK/EDTA) on the ocular bioavailability ( $F_{\text{ocular}}$ ) of ketorolac tromethamine after ocular instillation to normal and de-epithelialized corneas of rabbits both *in vitro* and *in vivo*. The *in vitro*  $F_{\text{ocular}}$  of the formulations was measured in flow-through perfusion chambers. For *in vivo* studies, a 35  $\mu\text{L}$  dose of 0.5% ketorolac tromethamine with or without BAK/EDTA was instilled into rabbit eyes with intact or de-epithelialized corneas. At 0.5, 1, 2, 4, 6, and 8 h postdose, rabbits were euthanized, and the corneas and aqueous humor were collected from both eyes. The ketorolac concentrations from both *in vivo* and *in vitro* samples were quantified by reversed-phase high-performance liquid chromatography. The *in vitro* study results indicated that BAK/EDTA statistically significantly increased the  $F_{\text{ocular}}$  of ketorolac through de-epithelialized corneas but not through intact corneas. The *in vivo* study results showed that BAK/EDTA had no effect on the  $F_{\text{ocular}}$  of ketorolac in rabbits with intact corneas, based on the values of the area under the aqueous humor concentration versus time curves ( $\text{AUC}_{0-6\text{h}}$ ) of ketorolac. As expected, de-epithelialization of the corneas produced a faster and greater ocular absorption of ketorolac as evidenced by the smaller  $T_{\text{max}}$  and larger AUC values compared to those for the intact corneas *in vivo*. However, BAK/EDTA decreased the ocular absorption of ketorolac in rabbits with de-epithelialized corneas. The half-lives ( $t_{1/2}$ ) of ketorolac in corneal issue and aqueous humor were longer in rabbits with intact corneas than those in rabbits with de-epithelialized corneas. In conclusion, the *in vivo*  $F_{\text{ocular}}$  of ketorolac was not altered by BAK/EDTA in rabbits with intact corneas, but it was decreased by BAK/EDTA in rabbits with de-epithelialized corneas. Therefore, the formulation with ketorolac alone may be better as a postoperative ocular analgesic.

## Introduction

Steroids are used in the treatment of allergic ocular disorders, corneal burns, uveal tract inflammation, and other ocular inflammations, but their use is limited by their tendency to increase intraocular pressure and to cause cataracts upon chronic administration.<sup>1</sup> The advantage of nonsteroidal anti-inflammatory drugs (NSAIDs) is that they do not increase intraocular pressure.<sup>2</sup>

Ketorolac tromethamine is a potent NSAID, which is an effective treatment for postoperative inflammation in eyes. It is nonirritating when topically administered to eyes at concentrations of up to 0.5% and does not increase intraocular pressure.<sup>3</sup> The corneal epithelium is often damaged during ocular surgery, and alterations of the corneal epithelium have been shown to influence the corneal permeability of various compounds. Also, preservatives such as benzalkonium chloride (BAK) are known to enhance the corneal permeability of ketorolac *in vitro*.<sup>2</sup> Therefore, the objective of this study was

to evaluate the effect of BAK/ethylenediaminetetraacetic acid (EDTA) (both known corneal penetration enhancers) on the ocular bioavailability of ketorolac following ocular instillation to both intact and de-epithelialized corneas of rabbit eyes.

## Experimental Section

Ophthalmic solutions of 0.5% ketorolac tromethamine (pH 7.4) with or without 0.01% benzalkonium chloride/0.1% EDTA were provided by Allergan (Irvine, CA).

Female New Zealand albino rabbits weighing between 2 and 3.5 kg were obtained from Myrtle's Rabbitry (Thomson Station, TN). The rabbits were quarantined for at least 1 week upon arrival and examined for clinically normal eyes. The rabbits were individually housed with food and water provided *ad libitum*.

**In Vitro Studies—Corneal Dissection**—The rabbits were euthanized with Eutha-6 (Western Medical Supply Co. Inc., Arcadia, CA). The corneal epithelium was removed by careful scraping of the cornea's surface with a scalpel blade until the stroma was exposed.

De-epithelialization was confirmed by microscopic examination of the corneas after scraping. The eyes were then enucleated, and the corneas were excised. The freshly-excised corneas were mounted in flow-through perfusion chambers as previously described.<sup>3</sup>

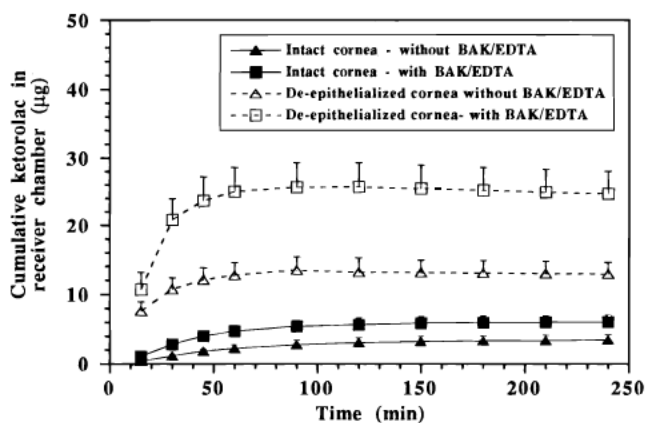
**Dosing and Sampling**—Glutathione-enriched bicarbonate Ringer's solution (GBR) was added to the receiver chamber<sup>4</sup> and bubbled with  $\text{O}_2/\text{CO}_2$  (95%/5%). One hundred fifty microliters of the 0.5% ketorolac tromethamine formulation was instilled into the donor chamber in flow line followed by 100  $\mu\text{L}$  of blank GBR. One minute after dosing, blank GBR buffer was infused into the donor chamber at a flow rate of  $\sim 28 \mu\text{L}/\text{min}$ . The donor effluent was collected over four 60 min intervals, and the volume and drug concentrations were measured. Samples of 100  $\mu\text{L}$  were collected with replacement from the receiver chamber at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min postdose. The ketorolac concentrations in all samples were quantified by HPLC. At the end of the experiment the corneas were weighed, soaked in 1 mL of methanol overnight, dried at 120 °C, and reweighed. The corneal hydration was calculated, and the methanol extracts were assayed for ketorolac content by HPLC.

**In Vivo Studies—De-epithelialization of Corneas**—Rabbits were anesthetized with ketamine (Ketaset; Fort Dodge Labs, Fort Dodge, IA) and xylazine (Xylazine; American Animal Health; Wisner, NE). One drop of proparacaine (Ophthetic; Allergan Inc., Irvine, CA) was topically applied to the cornea of the left eye. The corneal epithelium of the left eye was removed by scraping of the cornea's surface with a scalpel blade until the stroma was exposed. The right eye was used intact. For another set of animals, both eyes were scraped.

**Animal Dosing and Tissue Collection**—One 35  $\mu\text{L}$  drop of 0.5% ketorolac tromethamine ophthalmic formulation with or without BAK/EDTA was instilled into the lower cul-de-sac of each eye after the animals recovered from the general anesthesia. The upper and lower eyelids were gently held closed for  $\sim 10$  s to maximize drug–cornea contact. At 0.5, 1, 2, 4, 6, and 8 h postdose, six rabbits each were euthanized, after which the cornea and aqueous humor were collected and each stored in amber glass tubes containing 1 mL of methanol. All samples were stored at  $-20$  °C until analysis. Additional rabbits (two rabbits per formulation) were treated with placebo formulations with or without BAK/EDTA, and tissues were taken at 2 h postdose.

**HPLC Analysis**—The methanol extracts were centrifuged at 1500g for 15 min, and the supernatants were dried and reconstituted in mobile phase for HPLC analysis. A Beckman pump Model 126 (San Ramon, CA) was used to deliver the mobile phase at a flow rate of

<sup>®</sup> Abstract published in *Advance ACS Abstracts*, March 1, 1996.



**Figure 1**—Effect of BAK/EDTA on the penetration profiles of ketorolac through intact and de-epithelialized rabbit corneas *in vitro*. Corneas were perfused with 0.5% ketorolac tromethamine solutions with or without BAK. Values are mean  $\pm$  SEM,  $n = 8$ .

1.5 mL/min. A dry-packed precolumn was placed between the injector (Wisp 710B, Waters Assoc., Milford, MA) and the analytical column (Spherisorb ODS 5 $\mu$ , 4.6 mm  $\times$  25 cm, Alltech, Deerfield, IL). The effluent was monitored at 254 nm with a UV detector (Beckman Model 166, San Ramon, CA). The injection volume was 50  $\mu$ L. The retention time and lowest limit of quantitation of ketorolac were  $\sim$ 10.8 min and 15 ng/mL, respectively. Standards of ketorolac tromethamine ranging from 0.015 to 20.0  $\mu$ g/mL were analyzed with samples. Assay selectivity was verified by analysis of ocular tissues from animals treated with placebo formulations.

## Data Analysis

***In vitro* Studies**—The maximum cumulative total mass of drug in the receiver chamber ( $Q_{max}$ ) was directly obtained from the cumulative amount of ketorolac versus time curve, which was corrected for the mass of drug removed during sampling at each time point. The *in vitro* ocular bioavailability was calculated as  $F_{ocular} = Q_{max}/dose$ .

***In vivo* Studies**—the maximum concentration ( $C_{max}$ ) of drug in the aqueous humor and the time required to reach  $C_{max}$  ( $T_{max}$ ) were obtained from the aqueous humor concentration versus time curves. The area under the concentration versus time curve (AUC) was calculated as previously described.<sup>6</sup> The half-life ( $t_{1/2}$ ) of ketorolac was given by  $t_{1/2} = 0.693/k$ , where the rate constant ( $k$ ) for ketorolac was obtained by log linear regression of the last three points (terminal portion) of the aqueous humor or corneal concentration versus time curve. Student's  $t$ -test was used to compare values between groups. The level of statistical significance was set at  $\alpha = 0.05$ .

## Results

***In Vitro* Studies**—The effects of BAK/EDTA on the corneal absorption of ketorolac through intact and de-epithelialized corneas are shown in Figure 1 and Table 1. BAK/EDTA increased the corneal absorption of ketorolac in both intact and de-epithelialized corneas. As expected, de-epithelialization of the corneas markedly increased the corneal absorption of ketorolac from both formulations, as evidenced by shorter  $T_{max}$  and larger  $F_{ocular}$  values compared to those for the intact corneas. BAK/EDTA increased the  $F_{ocular}$  of ketorolac, but this increase was statistically significant only in the de-epithelialized corneas (Table 1). The ketorolac concentrations remaining in the corneal tissue 4 h after exposure to the ketorolac formulations with and without BAK/EDTA were  $113 \pm 17$  and  $142 \pm 15$   $\mu$ g/g, respectively, in the intact corneas.

In the de-epithelialized corneas, the concentrations were  $173 \pm 12$  and  $123 \pm 15$   $\mu$ g/g with and without BAK/EDTA, respectively.

***In Vivo* Studies**—The *in vivo* studies showed that the aqueous humor concentrations of ketorolac correlate well with the corneal tissue concentrations (Figure 2). BAK/EDTA had no effect on the corneal tissue or aqueous humor concentrations of ketorolac in rabbits with intact corneas (Figure 2, panels A and B, respectively). De-epithelialization of the corneas initially increased the corneal tissue and aqueous humor concentrations of ketorolac, but the concentrations decreased more rapidly than in rabbits with intact corneas (Figure 2, panels A and B). In rabbits with de-epithelialized corneas, BAK/EDTA decreased the corneal and aqueous humor concentrations of ketorolac compared to those of ketorolac alone.

On the basis of the pharmacokinetic parameters, BAK/EDTA had no effect on the ocular absorption of ketorolac in rabbits with intact corneas (Table 2). De-epithelialization of the corneas produced a faster and greater ocular absorption of ketorolac as evidenced by shorter  $T_{max}$  and larger  $C_{max}$  and AUC values than for the intact corneas (Table 2). However, BAK/EDTA decreased the ocular absorption of ketorolac in the de-epithelialized corneas as evidenced by lower  $C_{max}$  ( $2.98 \pm 0.36$   $\mu$ g/mL) and AUC ( $403 \pm 47$   $\mu$ g $\cdot$ min/mL) values than those of ketorolac alone ( $7.39 \pm 1.15$   $\mu$ g/mL and  $854 \pm 96$   $\mu$ g $\cdot$ min/mL, respectively). BAK/EDTA had no effect on  $T_{max}$  values in either intact or de-epithelialized corneas.

In rabbits with intact corneas, the apparent half-lives ( $t_{1/2}$ ) of ketorolac in corneal tissue were longer (3.36 h without BAK/EDTA and 5.34 h with BAK/EDTA) than those in rabbits with de-epithelialized corneas (1.60 and 2.41 h, respectively) for both formulations. The half-lives of ketorolac in aqueous humor correlate well with corneal tissue half-lives. In aqueous humor, the half-life of ketorolac was shorter for rabbits with de-epithelialized corneas compared to that of rabbits with intact corneas (Table 2). BAK/EDTA had no effect on the half-lives of ketorolac (Table 2).

## Discussion

Our *in vitro* results are in agreement with previous reports in which BAK increased the *in vitro* corneal penetration of various compounds such as fluorescein,<sup>7</sup> horseradish peroxidase,<sup>8</sup> prednisolone phosphate,<sup>9</sup> dexamethasone and pilocarpine,<sup>10</sup> and ketorolac.<sup>3</sup> In the present study, BAK increased the corneal penetration of ketorolac *in vitro* (Table 1). Two mechanisms have been suggested to explain the BAK-enhanced corneal penetration of ketorolac: (a) BAK may disrupt the integrity of the epithelial membrane; and (b) BAK and ketorolac may form a more lipid-soluble ion pair, which may enhance corneal penetration.<sup>3</sup> However, the exact mechanism by which BAK enhances corneal penetration of ketorolac is not known.

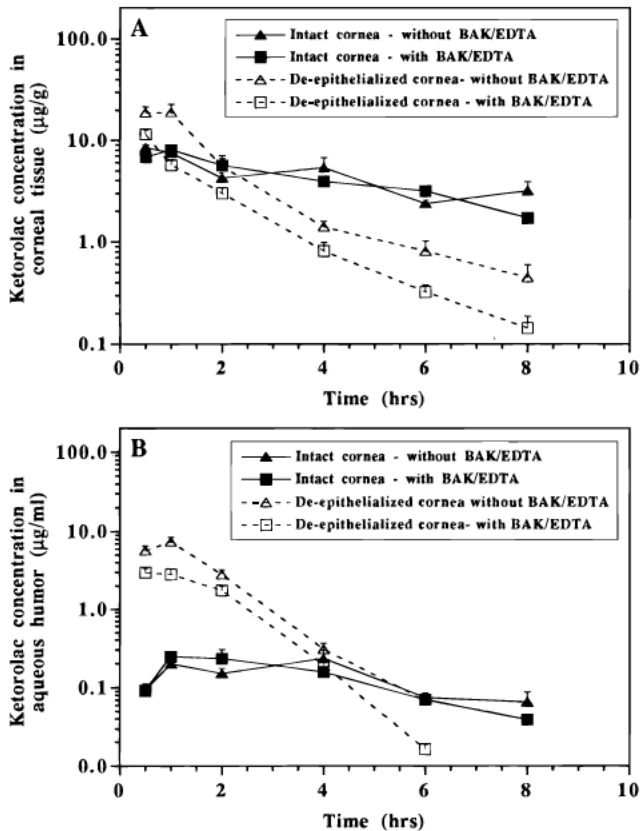
Additionally, the ophthalmic solution with BAK also contains a low concentration of EDTA (0.1%). EDTA, a known calcium-chelating agent, has been shown to act on cell junctions by interfering with calcium ions and altering intercellular integrity.<sup>11</sup> EDTA also disrupts the plasma membrane and consequently increases intercellular permeability.<sup>11</sup> EDTA has been shown to increase the absorption of various compounds through intact corneas.<sup>12,13</sup> Therefore, it is likely that EDTA in conjunction with BAK may jointly increase the extent of absorption through de-epithelialized corneas. Our results cannot exclude this possibility in that BAK/EDTA markedly enhanced the *in vitro* penetration of ketorolac through de-epithelialized corneas compared to that of ketorolac solution without BAK/EDTA (Figure 1).



**Table 1—Pharmacokinetic Parameters after an Acute Administration of 0.5% Ketorolac Tromethamine Solutions with and without BAK/EDTA to Intact and De-epithelialized Rabbit Corneas *in Vitro*<sup>a</sup>**

Corneal Treatment	BAK/EDTA	$C_{\text{cornea}}^b$ ( $\mu\text{g/g}$ )	$Q_{\text{max}}^c$ ( $\mu\text{g}$ )	$T_{\text{max}}^d$ (min)	% $F_{\text{ocular}}^e$
Intact	–	113 ± 17	3.44 ± 0.77	236 ± 4	0.636 ± 0.122
	+	142 ± 15	6.07 ± 1.00	236 ± 4	1.07 ± 0.18
De-epithelialized	–	123 ± 15	13.3 ± 1.8	135 ± 21	2.48 ± 0.28
	+	173 ± 12* <sup>f</sup>	25.8 ± 3.6*	105 ± 10	4.64 ± 0.57*

<sup>a</sup> Values are mean ± SEM,  $n = 8$ . <sup>b</sup> Ketorolac concentration in corneal tissue, measured at the end of the 4 h perfusion. <sup>c</sup> Maximal cumulative mass of ketorolac in receiver chamber. <sup>d</sup> Time required to reach  $Q_{\text{max}}$ . <sup>e</sup> Percent ocular bioavailability. <sup>f</sup> Statistically significantly different from solution without BAK/EDTA in de-epithelialized cornea ( $p < 0.05$ ).



**Figure 2—Effect of BAK/EDTA on ketorolac concentrations in corneal tissue (panel A) and aqueous humor (panel B), after topical application of a 35  $\mu\text{L}$  eye drop of 0.5% ketorolac tromethamine with or without BAK/EDTA to intact and de-epithelialized rabbit corneas *in vivo*. Values are mean ± SEM,  $n = 6$  for intact cornea,  $n = 12$  for de-epithelialized cornea.**

**Table 2—Pharmacokinetic Parameters of Ketorolac in Aqueous Humor following Topical Application of a 35  $\mu\text{L}$  Eye Drop of 0.5% Ketorolac Tromethamine either with or without BAK/EDTA to Intact and De-epithelialized Rabbit Corneas *in vivo***

Corneal Treatment	BAK/EDTA	$C_{\text{max}}^a$ ( $\mu\text{g/mL}$ )	$T_{\text{max}}^b$ (h)	$t_{1/2}^c$ (h)	$\text{AUC}_{0-6h}^d$ ( $\mu\text{g}\cdot\text{min/mL}$ )
Intact <sup>e</sup>	–	0.229 ± 0.071	4	2.22	57.1 ± 8.9
	+	0.245 ± 0.028	1	2.00	57.2 ± 7.3
De-epithelialized <sup>f</sup>	–	7.39 ± 1.15	1	0.746	854 ± 96
	+	2.98 ± 0.36* <sup>g</sup>	0.5	0.594	403 ± 47*

<sup>a</sup> Maximum concentration of ketorolac in aqueous humor. <sup>b</sup> Time required to reach  $C_{\text{max}}$ . <sup>c</sup> Mean apparent half-life of ketorolac in aqueous humor. <sup>d</sup> Area under aqueous humor concentration vs time curve from 0 to 6 h. <sup>e</sup> Values are mean ± SEM,  $n = 6$ . <sup>f</sup> Values are mean ± SEM,  $n = 12$ . <sup>g</sup> (\*) Statistically significantly different from solution with BAK/EDTA in de-epithelialized cornea ( $p < 0.05$ ).

When ketorolac solutions with and without BAK/EDTA were instilled into rabbit eyes *in vivo*, BAK/EDTA failed to increase the corneal penetration of ketorolac. This may be due to the fact that the corneal penetration enhancement of

BAK/EDTA is dependent on various conditions such as the concentration of BAK used in the dosing solution, number of ophthalmic doses administered, coadministration with other compounds, and the species tested. It has been reported that 0.01% BAK<sup>14</sup> and 0.02% BAK<sup>15</sup> increased the corneal permeability of fluorescein and inulin, respectively, in rabbits *in vivo*. In contrast, 0.01% BAK failed to increase the corneal permeability of inulin.<sup>15</sup> Similarly 0.01% BAK did not alter the corneal permeability of fluorescein in humans *in vivo*. Additionally, three repeated administrations (50  $\mu\text{L}$  each) of 0.01% BAK/0.1% EDTA with 2 min intervals failed to increase the corneal permeability, but five repeated administrations of this solution did increase the corneal permeability in humans *in vivo*.<sup>16</sup> Additionally, it has been reported that neither 0.34 nor 1.0% EDTA eye drops had any influence on the anterior chamber fluorescein concentration in humans.<sup>13</sup> Therefore, it is not surprising that, in the present study, a single ophthalmic dose of 0.01% BAK/0.1% EDTA had no effect on the corneal penetration of ketorolac in rabbits with intact corneas.

*In vivo* ocular bioavailability is known to be altered by changes in lacrimation;<sup>17</sup> that is, increased lacrimation causes increased washout of drug, thereby decreasing the ocular absorption of drug from the precorneal region. BAK is known to cause ocular irritation.<sup>18</sup> Therefore, one can speculate that BAK may enhance ocular absorption of ketorolac in rabbits with intact corneas as it does for other compounds<sup>3,7-9,14,19</sup> but, at the same time, BAK in combination with EDTA (another potential ocular irritant) might have produced increased irritation, and thus increased lacrimation, thereby reducing drug absorption. This effect could be expected to be exacerbated in the de-epithelialized cornea *in vivo*. Our results are consistent with this hypothesis. In the de-epithelialized corneas, BAK/EDTA decreased ocular absorption of ketorolac as evidenced by lower  $C_{\text{max}}$  and AUC values than those observed after topical administration of ophthalmic solution with ketorolac alone, which was shown to be nonirritating when applied to the corneal surfaces of rats, dogs, and rhesus monkeys at concentrations up to 0.5%.<sup>20</sup>

We need to explain why the corneal penetration enhancement of BAK/EDTA was observed *in vitro*, but not *in vivo*. This is likely due to the fact that the *in vitro* model is completely devoid of complication by variability in precorneal factors such as blinking, lacrimation, tear turnover, and drug washout. Therefore, the corneal penetration enhancement of BAK/EDTA was not diminished *in vitro*.

It has been reported that de-epithelialization of the cornea increased the penetration of pilocarpine, dexamethasone, and sorbitol.<sup>17,21</sup> In agreement with these reports, our *in vitro* and *in vivo* results showed that de-epithelialization of the cornea produced faster and greater ocular absorption of ketorolac as evidenced by shorter  $T_{\text{max}}$  and larger AUC values (Tables 1 and 2). These results suggest that the corneal epithelium is rate limiting in the ocular absorption of ketorolac.

The mean apparent half-life of ketorolac in aqueous humor was longer in rabbits with intact corneas than in rabbits with de-epithelialized corneas (Table 2). These results correlate

well with the corneal tissue half-lives of ketorolac, suggesting that the corneal epithelium may be acting as a reservoir for drug accumulation, similar to the situation reported for ketorolac<sup>22</sup> and pilocarpine.<sup>23</sup> Thus the longer half-lives observed in rabbits with intact corneas may be due to a continued flux of drug into the aqueous humor from the corneal reservoir as previously reported.<sup>22,23</sup>

Our results indicate that the corneal epithelium is important in the elimination/loss of drug from the anterior chamber. It has been reported that the mean half-life of [<sup>14</sup>C]ketorolac in the anterior chamber after intracameral injection to rabbits with intact corneas was 2.1 h.<sup>22</sup> In this study, the mean half-lives of ketorolac in aqueous humor after ophthalmic administration to rabbits with de-epithelialized corneas were much shorter (0.594–0.746 h). It is likely that, once the drug reaches  $C_{max}$  in the aqueous humor, it may diffuse back through the cornea. This would lead to a more rapid elimination of ketorolac from the aqueous humor of rabbits with de-epithelialized corneas than that observed after intracameral injection where the corneal epithelium of rabbits was still intact.<sup>22</sup>

In conclusion, BAK had no effect on the ocular absorption of ketorolac in intact corneas *in vivo*. The ocular absorption of ketorolac was increased by de-epithelialization of the corneas *in vivo*, but it was decreased by BAK. Therefore, the formulation with ketorolac alone may be better as a postoperative ocular analgesic. This result is unexpected and should be of interest to ophthalmic formulators.

## References and Notes

- Haynes, R. C., Jr. In *The pharmacological Basis of Therapeutics*; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 1456–1458.
- Flach, A. J.; Jaffe, N. S.; Akers, W. A. *Ann. Ophthalmol.* **1989**, *21*, 407–411.
- Fu, R. C.-C.; Lidgate, D. M. *Drug Dev. Ind. Pharm.* **1986**, *12*, 2403–2430.
- Richman, J. B.; Tang-Liu, D. D.-S. *J. Pharm. Sci.* **1990**, *79*, 153–157.
- O'Brien, W. J.; Edelhofer, H. F. *Invest. Ophthalmol.* **1977**, *16*, 1093–1103.
- Tang-Liu, D.; Burke, P. J. *Pharm. Res.* **1988**, *5*, 238–241.
- Green, K.; Tonjum, A. *Am. J. Ophthalmol.* **1971**, *72*, 897–905.
- Tonjum, A. M. *Acta Ophthalmol.* **1975**, *53*, 335–347.
- Green, K.; Downs, S. J. *Invest. Ophthalmol.* **1974**, *13*, 316–319.
- Camber, O.; Edman, P. *Int. J. Pharm.* **1987**, *39*, 229–234.
- Grass, G. M.; Wood, R. W.; Robinson, J. R. *Invest. Ophthalmol. Visual Sci.* **1985**, *26*, 110–113.
- Ashton, P.; Diepold, R.; Platzer, A.; Lee, V. H. L. *J. Ocular Pharmacol.* **1990**, *6*, 37–42.
- Rojanasakul, Y.; Liaw, J.; Robinson, J. R. *Int. J. Pharm.* **1990**, *66*, 131–142.
- Burstein, N. L. *Invest. Ophthalmol.* **1984**, *25*, 1453–1457.
- Keller, N.; Moore, D.; Carper, D.; Longwell, A. *Exp. Eye Res.* **1980**, *30*, 203–210.
- Ramselaar, J. A. M.; Boot, J. P.; van Haeringen, N. J.; van Best, J. A.; Oosterhuis, J. A. *Curr. Eye Res.* **1988**, *9*, 947–950.
- Conrad, J. M.; Reay, W. A.; Polcyn, R. E.; Robinson, J. R. *J. Parenter. Drug Assoc.* **1978**, *32*, 149–161.
- Kennah, H. E.; Higney, S.; Laux, P. E.; Dorko, J. D.; Barrow, C. S. *Fundam. Appl. Toxicol.* **1989**, *12*, 258–268.
- Smolen, V. F.; Clevenger, J. M.; Williams, E. J.; Bergdolt, M. W. *J. Pharm. Sci.* **1973**, *62*, 958–961.
- Mohoney, J. M.; Waterbury, L. D. *Invest. Ophthalmol. Visual Sci. (Suppl.)* **1983**, *24*, 151.
- Ashton, P.; Diepold, R.; Platzer, A.; Lee, V. H. L. *J. Ocular Pharmacol.* **1990**, *6*, 37–42.
- Ling, T. L.; Combs, D. L. *J. Pharm. Sci.* **1987**, *76*, 289–294.
- Sieg, J. W.; Robinson, J. R. *J. Pharm. Sci.* **1976**, *65*, 1816–1822.

JS9504189