Intraocular Penetration of Periocular Ketorolac and Efficacy in Experimental Uveitis

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Purpose. To determine in rabbits whether periocular injection of ketorolac tromethamine effectively delivers the drug to the eye and, if so, whether this is efficacious in the treatment of experimental uveitis.

Methods. Ketorolac was administered by anterior subconjunctival injection, posterior periocular injection, intramuscular injection, or topical eye drops. The aqueous and vitreous were assayed for ketorolac. Anterior subconjunctival and topical ketorolac were compared to control as well as topical and anterior subconjunctival steroid treatments in uveitis induced by the intravitreal injection of tumor necrosis factor.

Results. Anterior subconjunctival injection led to high, though short-lived, levels of drug in the aqueous and vitreous. Posterior periocular injection led to much lower levels. Topical dosing led to relatively low aqueous and undetectable vitreous levels. No ocular levels were detected after intramuscular dosing. All tested antiinflammatory treatments were similarly effective in controlling uveitis.

Conclusions. Anterior subconjunctival injection of ketorolac produced high intraocular concentrations of drug and was beneficial in controlling the inflammation in this animal model of uveitis. Invest Ophthalmol Vis Sci. 1996;37:613-618.

Ketorolac tromethamine (ketorolac) is a nonsteroidal antiinflammatory agent available in parenteral form for use as an analgesic. The mechanism of action is thought to be principally inhibition of cyclooxygenase. Ketorolac has been used as a 0.5% topical ophthalmic preparation for ocular inflammatory conditions such as allergic conjunctivitis, chronic aphakic and pseudophakic cystoid macular edema, and postcataract surgery inflammation. As a nonsteroidal antiinflammatory agent, ketorolac may have fewer adverse effects than corticosteroids on ocular infections, corneal wound healing, and intraocular pressure.

Periocular injections of drugs such as corticosteroids have been used commonly for ocular inflammatory conditions and after ocular surgery. Possible advantages of this route of drug delivery include delivery of a high local concentration of drug using a relatively small dose compared to systemic (i.e., oral, intravenous, or intramuscular) dosing, reducing the possibility of systemic side effects; delivery of a higher concentration of a drug that poorly penetrates the corneal epithelium than is obtainable with topical administration; and more certain delivery of drug to the eye in patients who do not reliably administer topical medications. This study was undertaken in an animal model to assess whether ketorolac could be delivered effectively to the aqueous and vitreous compartments by periocular injection and, if so, whether this was efficacious in the treatment of experimental uveitis.

METHODS

The study design was approved by the Animal Care Committee of the University of Illinois at Chicago and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. New Zealand White female rabbits were used and were allowed food and water ad libitum. All injections and intracardiac puncture were carried out under intra-
Animals were killed at 30 minutes, 1 hour, 3 hours, was obtained by intracardiac puncture and centrifuged to separate the plasma. The animals were killed with intracardiac lidocaine.

**Intraocular Penetration**

Rabbits weighing 2 to 3 kg were used for the intraocular penetration studies. With the exception of control animals administered 0.5 ml unilateral anterior subconjunctival saline, all animals in this section of the study received ketorolac by one of four different routes: The first was anterior subconjunctival injection in which 15 mg of ketorolac (0.5 ml of a 30 mg/ml solution; Syntex Laboratories, Palo Alto, CA) was administered through a 30-gauge needle superotemporally near the limbus to the right eye only; the puncture site was small with this needle, and no drug solution was observed to leak from the site immediately after injection. These animals were killed at 15 minutes, 30 minutes, 1 hour, 3 hours, 8 hours, 24 hours, or 48 hours after dosing. The second route was posterior periocular injection of 15 mg to the right eye only. This was accomplished in the superotemporal quadrant with a 30-gauge needle injected as posteriorly as possible along the globe while hugging the external eye wall with the needle. We did not note dissection of drug solution anteriorly into the subconjunctival space, i.e., into the area in which solution normally accumulates after anterior subconjunctival injection. Animals were killed at 30 minutes, 1 hour, 3 hours, or 8 hours after dosing. The third route was intramuscular (gluteal) injection of 30 mg, and animals were killed at 30 minutes, 1 hour, 3 hours, or 8 hours after dosing. The fourth route was topical application of ketorolac 0.5% solution (Allergan, Irvine, CA), one drop to the right eye every 2 hours for four doses; the animals were killed at 1 hour after the last dose. Rabbits in the topical group received neither topical nor systemic anesthesia at the time of ketorolac dosing.

At the appropriate time, the eyes were examined with a hand light and then were irrigated with saline solution. Aqueous humor sampling was performed with a 27-gauge needle through clear cornea. For the anterior subconjunctival injection group only, blood was obtained by intracardiac puncture and centrifuged to separate the plasma. The animals were killed, and the eyes were enucleated and irrigated with saline. All specimens were placed promptly in a −70°C freezer. At a later time, a surgical blade was used to incise the wall of the eye to expose the frozen vitreous body, which was delivered intact from the globe. Care was taken to exclude any adherent material from the vitreous body, such as retina, iris, lens, or aqueous humor; this was accomplished easily as long as the dissection was performed quickly before any thawing of the tissues occurred.

All specimens, including bilateral aqueous, bilateral vitreous, and plasma samples from each animal, were assayed for ketorolac by a previously described high-performance liquid chromatography method. The lower limit of the assay was 0.040 μg/ml for aqueous and vitreous and 0.010 μg/ml for plasma.

**Treatment of Experimental Uveitis**

Rabbits weighing 1.4 to 1.8 kg were used in the uveitis experiments. Uveitis was induced by the intravitreal injection of recombinant human tumor necrosis factor (1 × 10^7 U/μg; Cellular Products, Buffalo, NY), which was diluted in Dulbecco’s phosphate buffered saline. A 30-gauge needle was used to enter the eye approximately 2 to 3 mm posterior to the limbus in the superonasal quadrant, and the needle tip was visualized through the pupil. When the needle tip was assessed to be in the center of the vitreous body, injection of 100,000 U in 0.1 ml was completed. The needle was removed, and no extravasation was noted from the puncture site. The intravitreal injection was made to both eyes of each animal in this section of the study. On day 3, the eyes were examined with a hand light and generally showed a mild injection of the conjunctiva, iris, or both. Animals with severe injection of the conjunctiva and/or iris were excluded from the study; such animals were few, and the appearance of their highly inflamed eyes was clearly atypical of the other animals. Treatments were bilateral, were initiated on day 3, and consisted of one of the following: a single 0.5 ml injection (per eye) of superotemporal anterior subconjunctival saline (control), 15 mg ketorolac in 0.5 ml, or 2 mg dexamethasone in 0.5 ml; or prednisolone acetate 1% or ketorolac 0.5% administered four times daily (8 AM, 12 PM, 4 PM, and 8 PM) as eye drops for the duration of the study. Rabbits in the topical treatment groups received topical and systemic anesthesia at the initiation of the treatment, as did rabbits in the injection treatment groups; anesthesia was not repeated for the subsequent topical doses in the topical treatment groups. On day 7, aqueous humor was sampled from each eye using a ZBI Coulter counter (Coulter Electronics, Hialeah, FL).

To study whether periocular ketorolac affected the intraocular inflammation by a local or systemic effect, a second group of animals with similarly induced bilateral uveitis was treated with 15 mg anterior subconjunctival ketorolac to one eye and anterior subconjunctival saline to the other. Statistical analysis on the aqueous cell counts was performed using the unpaired Student’s t-test.

**RESULTS**

**Intraocular Penetration**

Hand light examination of all eyes of animals that received ketorolac by any route showed no evidence
of conjunctival injection, corneal irregularity, or other signs of ocular irritation at all time points. Ketorolac was undetectable in the aqueous and vitreous bilaterally, as well as in the plasma from control animals that did not receive ketorolac (n = 3 to 4).

Ipsilateral aqueous and vitreous and plasma concentrations of ketorolac after uniocular anterior subconjunctival injection of 15 mg for up to 8 hours after dosing are shown in Figure 1. The 24-hour aqueous level was 0.09 ± 0.06 µg/ml, the vitreous level was undetectable, and the plasma level was 0.02 ± 0.02 µg/ml. All 48-hour aqueous, vitreous, and plasma levels were undetectable. Contralateral aqueous and vitreous concentrations were undetectable at each time point except for the aqueous at 8 hours (0.10 ± 0.10 µg/ml) and 24 hours (0.11 ± 0.10 µg/ml).

Ipsilateral aqueous and vitreous concentrations after uniocular posterior periocular injection of 15 mg ketorolac are shown in Figure 2. Contralateral aqueous and vitreous concentrations were undetectable at all time points.

In the topical ketorolac dosing study, the ipsilateral aqueous concentration was 0.89 ± 0.04 µg/ml, whereas the ipsilateral vitreous and contralateral aqueous and vitreous levels were undetectable (n = 3). Aqueous and vitreous concentrations of ketorolac after intramuscular injection were undetectable at all time points (n = 2).

**Treatment of Experimental Uveitis**

Aqueous cell counts in control rabbits not given tumor necrosis factor (n = 6 eyes) were 23 ± 18 cells/µl. Aqueous cell counts on day 7 after bilateral treatment of bilateral tumor necrosis factor-induced uveitis using the different treatment regimens are shown in Table 1. All treatment groups differed significantly from the untreated group at P < 0.0005, but there were no significant differences among the different types of treatment. In this section of the study, 5 of 46 animals were excluded on day 3 before treatment because of severe conjunctival and/or iris injection. Two outlier animals were excluded from the data analysis, one from the subconjunctival ketorolac treatment group (aqueous cell counts of 3470 and 2640 cells/µl) and one from the prednisolone acetate 1% treatment group (3099 and 2611 cells/µl); these animals had aqueous cell counts that were extremely atypical for their respective groups.

Aqueous cell counts after uniocular treatment with anterior subconjunctival ketorolac in five rabbits with bilateral uveitis were 154 ± 96 cell/µl in the treated eyes and 749 ± 343 cells/µl in the contralateral saline-treated eyes. The ketorolac-treated eyes differed significantly from the saline-treated eyes at P < 0.005. These saline-treated eyes differed significantly from the bilaterally saline treated group above at P < 0.05.

**DISCUSSION**

Little is known regarding the intraocular penetration of ketorolac after local dosing. One study in unanes-
FIGURE 2. Ipsilateral aqueous and vitreous concentrations of ketorolac after uniocular posterior periocular injection of 15 mg ketorolac. Data are presented as mean ± standard deviation. n = 3 at each time point.

Unanesthetized rabbits found a peak aqueous concentration of 0.217 μg/ml at 1 hour after a single drop of 0.5% ketorolac solution to the eye; the peak vitreous concentration was 0.036 μg/ml at 30 minutes. In our topical dosing regimen of one drop every 2 hours for four doses in unanesthetized rabbits, we obtained aqueous concentrations though no detectable vitreous concentration and no detectable contralateral aqueous or vitreous concentrations at the arbitrarily selected 1 hour interval after dosing. For this particular dosing regimen, the time to maximal intraocular ketorolac levels was not studied. Also, because systemic anesthesia may increase the ocular availability of topically applied drops from altered eye drop and/or tear fluid dynamics, it is emphasized that these animals were unanesthetized.

Relative to these levels after topical dosing, very high concentrations were obtained in the aqueous and vitreous after anterior subconjunctival injection. This statement must, of course, be qualified by the fact that the injection dose (15 mg) was 15 times the topical dose (0.25 mg times four doses). Nonetheless, anterior subconjunctival injection appeared to be an effective method of achieving high intraocular ketorolac concentrations. The levels were short lived; they were undetectable within 48 hours in the aqueous and within 8 hours in the vitreous. Posterior periocular injection gave much lower levels in the aqueous and vitreous. Despite using an intramuscular dose in the rabbit typical of that used for analgesia in adult humans, ketorolac was undetectable in the eyes after intramuscular injection.

Ketorolac has been shown to penetrate the cornea, and it seems that this is the most important route of entry for this drug after local dosing. Presumably, this is why anterior subconjunctival injection led to higher intraocular levels than posterior periocular injection; it is possible that aside from simply being nearer the cornea, the anteriorly injected ketorolac may have leaked into the tear film, thereby gaining access to the cornea. This concept has been suggested before for other drugs injected subconjunctivally. The posteriorly injected drug would not have such easy access to the tear film and, indeed, might be taken up more readily in the systemic circulation by the rich orbital vasculature. In accordance with this, higher intraocular levels of gentamicin after subconjunctival versus retrobulbar injection in the rabbit have been shown.

Drug delivery involving the local vasculature could also be significant; however, systemic uptake of the drug with subsequent delivery to the eye is unlikely given the nearly total absence of detectable drug in the contralateral eyes of the three locally dosed groups and the absence of any ocular levels in the intramuscularly dosed group. Another study found detectable ketorolac levels in the aqueous and vitreous of rabbits after intravenous injection of only 1 mg, but these

TABLE 1. Aqueous Cell Counts After Bilateral Treatment of Bilateral Tumor Necrosis Factor-Induced Uveitis*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Number of Eyes</th>
<th>Aqueous Cell Counts/μl</th>
</tr>
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<tbody>
<tr>
<td>Subconjunctival saline</td>
<td>22</td>
<td>1583 ± 995</td>
</tr>
<tr>
<td>Subconjunctival ketorolac</td>
<td>17</td>
<td>215 ± 273</td>
</tr>
<tr>
<td>Subconjunctival dexamethasone</td>
<td>10</td>
<td>67 ± 97</td>
</tr>
<tr>
<td>Topical prednisolone acetate</td>
<td>10</td>
<td>184 ± 266</td>
</tr>
<tr>
<td>Topical ketorolac</td>
<td>18</td>
<td>238 ± 257</td>
</tr>
</tbody>
</table>

* Data are mean ± standard deviation. All treatment groups were significantly different from the saline-treated group at P < 0.0005.
were extremely low levels and were well below the limits of assay of the current study. Other drugs have been shown to be much more effectively delivered to the eye by periocular injection than by systemic administration. 19-21

Given the earlier peaking of levels in the vitreous versus the aqueous in the anterior injection group and the higher vitreous levels versus aqueous levels in the posterior injection group, a small role for the transcleral route 22 could not be excluded. These findings, however, could be explained by a possibly higher likelihood of contamination of the vitreous by periocular ketorolac during dissection of the globe. For example, soon after ketorolac dosing, a large amount of drug would be in the periocular space and could potentially contaminate the vitreous during globe dissection; similarly, a more posterior location of the ketorolac dose would be more likely to lead to contamination because the globe incisions were made posteriorly.

In the current model of rabbit uveitis induced by the intravitreal injection of tumor necrosis factor, all treatment modalities appeared to be similarly effective in controlling the inflammation as assessed by aqueous humor cell counts. Subconjunctival ketorolac, when given unilaterally, significantly controlled inflammation in the contralateral eye though not as much as in the ipsilateral eye. Given the apparent lack of ocular uptake after systemic administration, there are several potential explanations for this contralateral effect. First, ketorolac may have a peripheral action in controlling the inflammation as well as a more local effect on the eye itself. Second, the reduction in inflammation in the contralateral eye may have been caused by levels of ketorolac in the eye that were below the assay limit of the current study. Third, ketorolac may have entered the inflamed, uveitic eyes to a greater extent than the uninflamed eyes of the intraocular penetration study, as has been shown for other drugs. 23,24 In addition, aside from any alterations in the blood–ocular barriers caused by inflammation, the pharmacokinetics of ketorolac in the uveitic eyes may have been altered by increased protein concentrations in the ocular humors because ketorolac is known to be highly protein bound in plasma. 25 Fourth, although the aqueous humor often is considered the "target area" of ocular drug delivery, it may be that, from a therapeutic standpoint, drug concentrations in other ocular tissues are more important than aqueous concentrations. 26 After topical application of ketorolac, higher concentrations were achieved in several ocular tissues, including the iris–ciliary body, than in the aqueous humor. 15

We have shown that ketorolac can be delivered to the eye in high concentrations by anterior subconjunctival injection. The adverse effects on the eye, if any, from these high intraocular levels is unknown. This form of therapy was effective in controlling uveitis but was not shown to be more effective than other forms of therapy, such as eye drops. For this therapy to become clinically useful, it should be shown that high intraocular levels are not harmful to the eye and that, given the potential complications of injections, there is some advantage to using the injection route over eye drops. 19 The development of a depot form of ketorolac, such as is available for steroid medications, could be useful clinically.

Key Words
animal model, ketorolac tromethamine, nonsteroidal antiinflammatory agent, tumor necrosis factor, uveitis

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References