Effects of Topical Anti-inflammatory and Antiallergic Eyedrops on Prostaglandin E₂–Induced Aqueous Flare Elevation in Pigmented Rabbits

Yoriko Hayasaka, MD; Seiji Hayasaka, MD; Xue-Yun Zhang, PhD; Yasunori Nagaki, MD

Objective: To evaluate the role of topical instillation of anti-inflammatory or antiallergic agents on experimental elevation of aqueous flare induced by prostaglandin E₂ (PGE₂) in pigmented rabbits.

Methods: Transcorneal diffusion of PGE₂, 25 µg/mL (7.09 × 10⁻³ mmol/L), by means of a glass cylinder produced aqueous flare elevation. Anti-inflammatory or antiallergic agents were topically administered once or twice before PGE₂ application. Aqueous flare was measured with a laser flare-cell meter. Results are given as mean±SD.

Results: Double instillations of 0.1% betamethasone sodium phosphate and 0.1% fluorometholone acetate at 4 and 2 hours before PGE₂ application inhibited 61%±11% and 46%±14%, respectively, of flare elevation. Double instillations of 0.1% diclofenac sodium and 0.1% pranoprofen at 4 and 2 hours before PGE₂ application did not inhibit flare elevation. Double instillations of 0.1% betamethasone, 0.1% fluorometholone, 0.1% diclofenac, and 0.1% pranoprofen at 1 and 0.5 hour before PGE₂ application inhibited 16%±10%, 16%±6%, 24%±9%, and 23%±10%, respectively, of flare elevation. Double instillations of 2% cromolyn sodium, 0.5% tranilast, 0.025% levocabastine hydrochloride, 0.1% pemirolast potassium, and 0.01% ibudilast at 1 and 0.5 hour before PGE₂ application did not inhibit flare elevation. Single instillation of 0.1% betamethasone 6 hours before PGE₂ application inhibited 88% of PGE₂ induced aqueous flare elevation. Single instillation of 0.1% diclofenac 1 hour before PGE₂ application inhibited 23% of PGE₂ induced aqueous flare elevation.

Conclusions: Betamethasone needed several hours after topical instillation to inhibit flare elevation, but diclofenac needed 1 hour. Antiallergic agents did not affect disruption of the blood-aqueous barrier in rabbits.

Clinical Relevance: Corticosteroid eyedrops may need several hours from instillation to show action.

Arch Ophthalmol. 2002;120:950-953

Previous studies from our laboratory have shown that transcorneal diffusion of prostaglandin E₂ (PGE₂) by means of a glass cylinder induced aqueous flare elevation in pigmented rabbits, and that the elevation was reproducible when PGE₂ was applied at an interval of more than 1 week.¹² We also found that topical instillation of 0.25% clonidine hydrochloride, 0.5% betaxolol hydrochloride, 1.15% apraclonidine hydrochloride, 1.25% epinephrine bitartrate, or 0.04% dipivefrin hydrochloride inhibited flare elevation in rabbits.²³⁻⁵

Elevation of aqueous flare after cataract surgery was inhibited by anti-inflammatory agents. Othenin-Girard et al⁶ reported that diclofenac sodium seemed to be as effective as dexamethasone sodium phosphate. Miyake et al⁶ reported that the amount of flare after cataract surgery was lower in a diclofenac group than that in a fluorometholone group. Mast cells contain many small granules of substances that mediate inflammation. Histamine produces the itching and redness.⁸ In the present study, therefore, we evaluated the effects of anti-inflammatory or antiallergic agents on PGE₂–induced aqueous flare elevation in pigmented rabbits.

Corticosteroids bind to a specific receptor and enter into the cytoplasm and nucleus, which leads to the synthesis of specific proteins.⁹ Dexamethasone decreased a 4-kilobase messenger RNA encoding a cyclooxygenase-related protein.¹⁰ Glucocorticoids play a major role in the in vivo regulation of the cyclooxygenase 2 gene.¹¹ Nonsteroidal anti-inflammatory drugs block prostaglandin biosynthesis by inhibitory effects on cyclooxygenases 1 and 2.¹²⁻¹³ Thus, the mechanisms of actions of corticosteroids and nonsteroidal anti-inflammatory drugs are different. Therefore, several time points

From the Department of Ophthalmology, Toyama Medical and Pharmaceutical University, Toyama, Japan.
MATERIALS AND METHODS

ANIMALS

We used pigmented male rabbits (Japanese mongrel) that weighed 2.5 to 3.5 kg each. The animals were housed and treated according to the Association for Research in Vision and Ophthalmology Resolution on Use of Animals in Research. The study was approved by the Institutional Animal Care and Utilization Committee, Toyama Medical and Pharmaceutical University, Toyama, Japan. One eye of each animal was used for the experiment. Four to 8 eyes were used in each group.

CHEMICAL SOLUTIONS

We used the following anti-inflammatory and antiallergic agents, which were purchased as ophthalmic solutions: betamethasone sodium phosphate (Shionogi Pharmaceutical Co Ltd, Osaka, Japan); fluorometholone acetate, levocabastine hydrochloride, and pemirolast potassium (Santen Inc, Napa, Calif); diclofenac sodium (Wakamoto Pharmaceutical Co, Ltd, Tokyo, Japan); pranoprofen (Senju Pharmaceutical Co, Ltd, Osaka); cromolyn sodium (cromoliglicate sodium) (Fujisawa Pharmaceutical Co, Ltd, Osaka); tranilast (Kissei Pharmaceutical Co, Ltd, Nagano, Japan); and ibudilast (Senju Pharmaceutical Co, Ltd) (Table 1). The PGE2 (Funakoshi Chemicals, Tokyo) was dissolved in 100% ethanol and stored at −70°C. Prostaglandin E2 solution was diluted in 5% ethanol with 0.9% sodium chloride (NaCl) just before use.

In 1 eye, 50 µL of eyedrops or placebo (0.9% NaCl) was topically instilled. Instillation took place once or twice just before use.

Table 1. Anti-inflammatory and Antiallergic Ophthalmic Solutions

<table>
<thead>
<tr>
<th>Generic Name</th>
<th>Concentration, %</th>
<th>Drug Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betamethasone sodium phosphate</td>
<td>0.1</td>
<td>Corticosteroid</td>
</tr>
<tr>
<td>Fluorometholone acetate</td>
<td>0.1</td>
<td>Corticosteroid</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>0.1</td>
<td>Nonsteroidal</td>
</tr>
<tr>
<td>Pranoprofen</td>
<td>0.1</td>
<td>anti-inflammatory drug</td>
</tr>
<tr>
<td>Cromolyn sodium</td>
<td>2.0</td>
<td>Mast-cell stabilizer</td>
</tr>
<tr>
<td>Tranilast</td>
<td>0.5</td>
<td>Mast-cell stabilizer</td>
</tr>
<tr>
<td>Levocabastine hydrochloride</td>
<td>0.025</td>
<td>Antihistamine</td>
</tr>
<tr>
<td>Pemirolast potassium</td>
<td>0.1</td>
<td>Mast-cell stabilizer</td>
</tr>
<tr>
<td>Ibudilast</td>
<td>0.01</td>
<td>Mast-cell stabilizer</td>
</tr>
</tbody>
</table>

Table 1: Anti-inflammatory and Antiallergic Ophthalmic Solutions

For application of eyedrops were chosen in the present study.

RESULTS

No remarkable changes in the systemic condition were noted after the transcorneal diffusion of PGE2 and the topical instillation of anti-inflammatory or antiallergic agents. Double instillation (1 and 0.5 hour before PGE2 application) of 0.1% betamethasone, 0.1% fluorometholone, 0.1% diclofenac, 0.1% pranoprofen, 2% cromolyn, 0.5% tranilast, 0.025% levocabastine, 0.1% pemirolast, and 0.01% ibudilast did not induce aqueous flare elevation.

After PGE2 was administered, aqueous flare increased, reached its maximum (450-470 photon counts/ms) at 60 to 90 minutes, and then gradually decreased and returned to baseline levels at 7 to 8 hours (Figure 2). When
0.1% betamethasone was topically instilled 4 and 2 hours before PGE2 application, aqueous flare elevated to 225 photon counts/ms at 60 minutes and then gradually decreased (Figure 2A). When 0.1% betamethasone was instilled 1 and 0.5 hour before PGE2 application, aqueous flare elevated to 365 photon counts/ms at 60 minutes and then gradually decreased (Figure 2B).

The effects of double instillation of anti-inflammatory and antiallergic eyedrops on aqueous flare elevation are shown in Table 2. Double instillations of 0.1% betamethasone and 0.1% fluorometholone at 4 and 2 hours before PGE2 application inhibited 61%±11% and 46%±14%, respectively, of the flare elevation.

Double instillations of 0.1% diclofenac and 0.1% pranoprofen at 4 and 2 hours before PGE2 application did not inhibit the flare elevation. Double instillations of 0.1% betamethasone, 0.1% fluorometholone, 0.1% diclofenac, and 0.1% pranoprofen at 1 and 0.5 hour before PGE2 application inhibited 16%±10%, 16%±6%, 24%±9%, and 23%±10%, respectively, of the flare elevation. Double instillations of 2% cromolyn, 0.5% tranilast, 0.025% levocabastine, 0.1% pemirolast, and 0.01% ibudilast at 1 and 0.5 hour before PGE2 application did not inhibit flare elevation.

Betamethasone needed several hours after topical instillation to exhibit inhibition of flare elevation (Table 3). When instilled 1 hour before PGE2 application, a single drop of 0.1% diclofenac inhibited flare elevation more strongly (23%±10%) than did 0.1% betamethasone (12%±6%). When instilled 6 hours before PGE2 application, a single drop of 0.1% diclofenac did not inhibit flare elevation, but 0.1% betamethasone did (88%±10%).

In the present study, 0.1% betamethasone and 0.1% fluorometholone instilled 4 and 2 hours before PGE2 application showed stronger inhibition of flare elevation than those instilled 1 and 0.5 hour before PGE2 application. Single instillations of 0.1% betamethasone 6 hours before PGE2 application inhibited 88% of aqueous flare elevation. The peak of prednisolone acetate concentration occurred 30 to 45 minutes after topical instillation in humans and rabbits. Corticosteroids might need several hours after administration to show action by inhibitory effects on expression of the messenger RNA–encoding cyclooxygenase-related protein. Diclofenac and pranoprofen instilled 1 and 0.5 hour before PGE2 application inhibited flare elevation. A single instil-
lation of 0.1% diclofenac 1 hour before PGE\(_2\) application inhibited 23% of aqueous flare elevation. Diclofenac directly inhibits cyclooxygenase.\(^2\) The different times from administration to inhibition between betamethasone and diclofenac may be due to the dissimilar mechanisms of action of these agents. Several authors have reported that nonsteroidal anti-inflammatory drugs were more effective than corticosteroids in inhibiting blood-aqueous barrier breakdown after cataract surgery.\(^7,17,19\) However, the action of diclofenac was weaker than that of betamethasone in the present study. A quantitative study of inhibitory effects on postsurgical inflammation by these agents is needed. Some authors compared topical instillations of corticosteroid and nonsteroidal anti-inflammatory drugs 1 hour before the production of experimental uveits and then at hourly intervals for 6 hours.\(^20\) We believe that further studies looking at different dosing schedules could prove beneficial.

Antiallergic agents did not inhibit flare elevation. It is unlikely that these agents affected disruption of the blood-aqueous barrier in rabbits.

Another study found PGE\(_2\)-like activity in the aqueous humor after paracentesis in rabbits.\(^2\) The PGE\(_2\) may be involved in traumatic iridocyclitis. The blood-aqueous barrier in rabbits has unique sensitivity to prostaglandins.\(^2\) Therefore, the findings in the present study are not always identical to those seen in humans. The exact mechanisms of inhibition by corticosteroids and nonsteroidal anti-inflammatory drugs in rabbits and humans should be studied further.

Submitted for publication June 29, 2001; final revision received January 14, 2002; accepted March 20, 2002.

Corresponding author and reprints: Yoriko Hayasaka, MD, Department of Ophthalmology, Toyama Medical and Pharmaceutical University, 2630 Sugiitani, Toyama 930-0194, Japan (e-mail: ophthal@ms.toyama-mpu.ac.jp).

---

Table 3. Effects of Single Instillation of Eyedrops on PGE\(_2\)-Induced Aqueous Flare Elevation in Pigmented Rabbits*<sup>†</sup><sup>‡</sup>

<table>
<thead>
<tr>
<th>Single Instillation Before PGE(_2) Application, h</th>
<th>0.9% Sodium Chloride</th>
<th>0.1% Betamethasone Phosphate</th>
<th>0.1% Diclofenac Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2 ± 10 (5)</td>
<td>6 ± 3 (4)‡</td>
<td>2 ± 9 (8)</td>
</tr>
<tr>
<td>1</td>
<td>2 ± 8 (5)</td>
<td>12 ± 6 (4)§</td>
<td>23 ± 10 (8)‡</td>
</tr>
<tr>
<td>2</td>
<td>3 ± 9 (5)</td>
<td>20 ± 6 (9)§</td>
<td>8 ± 8 (6)</td>
</tr>
<tr>
<td>3</td>
<td>5 ± 11 (4)</td>
<td>35 ± 10 (4)§</td>
<td>4 ± 10 (4)</td>
</tr>
<tr>
<td>4</td>
<td>0 ± 12 (6)</td>
<td>48 ± 8 (6)§</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>1 ± 8 (6)</td>
<td>69 ± 14 (6)§</td>
<td>...</td>
</tr>
<tr>
<td>6</td>
<td>0 ± 9 (6)</td>
<td>88 ± 10 (8)§</td>
<td>2 ± 9 (4)</td>
</tr>
<tr>
<td>7</td>
<td>2 ± 10 (6)</td>
<td>57 ± 15 (6)§</td>
<td>...</td>
</tr>
<tr>
<td>8</td>
<td>0 ± 12 (6)</td>
<td>40 ± 14 (7)§</td>
<td>...</td>
</tr>
<tr>
<td>10</td>
<td>2 ± 10 (4)</td>
<td>14 ± 6 (4)§</td>
<td>...</td>
</tr>
</tbody>
</table>

*PGE\(_2\) indicates prostaglandin E\(_2\); ellipses, not examined.† Data are expressed as mean ± SD. Numbers in parentheses indicate number of eyes examined.‡P<.05, compared with eyes treated with 0.9% sodium chloride.§P<.001, compared with eyes treated with 0.9% sodium chloride.

---

REFERENCES


©2002 American Medical Association. All rights reserved.