Topical Diclofenac Sodium Decreases the Substance P Content of Tears

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Objective: To explore the mechanism by which diclofenac sodium eyedrops exert an adverse effect on the cornea.

Methods: In 10 healthy Japanese volunteers, 0.1% diclofenac sodium solution was instilled into one eye 3 times daily for 2 weeks. Only vehicle was applied to the other eye. Tear samples were taken before drug treatment, at 2 weeks (on the final day of treatment), and at 4 weeks. Prostaglandin E2 and substance P concentrations in tears were measured using enzyme immunoassays.

Results: After treatment for 2 weeks, concentrations of both prostaglandin E2 and substance P in tears from diclofenac sodium–treated eyes had decreased significantly, and both had returned to baseline levels by 4 weeks. No significant changes were seen in prostaglandin E2 and substance P levels in vehicle-treated eyes at any time points.

Conclusions: Diclofenac sodium eyedrops concurrently reduced concentrations of prostaglandin E2 and substance P in tears. Depletion of substance P (a pain-associated neurotransmitter) by diclofenac sodium may promote development of corneal complications.

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DICLOFENAC SODIUM is a well-established nonsteroidal anti-inflammatory drug (NSAID) that blocks the cyclooxygenase pathway of arachidonic acid metabolism.1 Diclofenac sodium eyedrops have seen frequent use in the practice of ophthalmology during the past decade. The anti-inflammatory effects of diclofenac sodium have been well evaluated in patients undergoing cataract surgery and laser trabeculoplasty.2,3 Another important use for diclofenac sodium eyedrops is to ameliorate discomfort and pain after refractive surgery.4,5 Indications for these topical agents have recently been expanded to include pain relief following traumatic corneal erosion.6,7 Although the anti-inflammatory and analgesic effects of diclofenac sodium eyedrops are well recognized, adverse effects have been a concern, especially involving the cornea. A previous clinical study in Japan reported that punctate epithelial keratopathy developed in association with the use of diclofenac sodium drops in approximately 10% of patients treated in the context of cataract surgery.8 We previously reported that topical diclofenac sodium and indomethacin, another well-known cyclooxygenase inhibitor, caused significant enlargement of corneal epithelial cells as seen by specular microscopy in patients who had undergone cataract surgery.9 Recently, the American Society of Cataract and Refractive Surgery issued the statement that corneal complications after cataract and refractive surgery may be associated with the use of topical NSAIDs.10 According to this statement, at least 200 occurrences of corneal complications (ranging from punctate superficial keratopathy to full corneal melt) have been reported in the United States. However, the mechanism by which diclofenac sodium exerts an adverse effect on the corneal epithelium is not well understood.

Several authors reported a transient decrease in corneal sensitivity when diclofenac sodium was applied topically to healthy human eyes.11,12 This effect seems to be desirable when diclofenac sodium eyedrops are used for lessening pain and discomfort in the eye. On the other hand, corneal hypesthesia from other causes such as trauma, brain surgery, or herpetic infection often results in neurotrophic keratopathy.13 Although the mechanisms of neurotrophic keratopathy are not fully understood, depletion of substance P and
SUBJECTS AND METHODS

SUBJECTS

Ten healthy Japanese volunteers (3 men and 7 women) aged 24 to 31 years, with no history of eye disease except for refractive errors, were examined. Each participant underwent a thorough initial eye examination, including a slit-lamp evaluation, Schirmer testing, and a cotton-thread test, yielding no abnormal findings in either eye of any subject. All subjects showed more than 10 mm of Schirmer strip wetting and more than 15 mm of cotton-thread wetting. Normal corneal sensation was confirmed using a Cochet-Bonnet esthesiometer (threshold, 55 mm or longer). Each subject received a full explanation of all procedures and gave informed consent for participation prior to the experiment. Approval for this investigation was granted by the Committee for the Protection of Human Subjects at Keio University School of Medicine (Tokyo, Japan).

DRUG TREATMENT AND REGIMEN

Diclofenac sodium was purchased from Cayman Chemical Co (Ann Arbor, Mich). Diclofenac sodium (0.1%) was dissolved in 0.067M phosphate-buffered saline with a pH of 7.4. Phosphate-buffered saline was used as a vehicle control. The subject was instructed to instill diclofenac sodium solution into one eye 3 times daily for 2 weeks, and to instill vehicle into the other eye. Tear samples were taken before treatment, at 2 weeks (on the final day of the treatment), and at 4 weeks. Twenty microliters of unstimulated tears were collected with a micropipette from the inferior tear meniscus in each eye of all subjects. The samples were placed in chilled test tubes containing 40 µL of an aprotinin-EDTA mixture (300 kallikrein inhibition units per milliliter of aprotinin and 1.2 mg/mL of EDTA), and they were immediately stored at −30°C until assay.

PGE₂ AND SUBSTANCE P ASSAY

Each tear sample was divided into 2 equal parts, one for PGE₂ assay and the other for substance P assay.

For the PGE₂ assay, samples were diluted 5-fold with phosphate-buffered saline, and acidified with formic acid to pH 4.0. Samples were loaded onto reversed-phase C-18 cartridges (Waters, Milford, Mass) and washed with water and hexane, followed by elution with an ethyl acetate–methanol mixture (100:1, v/v). The eluate was dried under nitrogen gas, and then reconstituted with 50 µL of phosphate-buffered saline. The PGE₂ concentrations in samples were measured using an enzyme immunoassay system (Cayman Chemical Co) and expressed per milliliter of tear fluid.

For the substance P assay, samples were diluted 5-fold with 4% acetic acid and loaded onto reversed-phase C-18 cartridges. After washing with the acetic acid, samples were eluted with an ethanol-water–acetic acid mixture (90:10:0.04, v/v/v). The eluate was dried by evaporation and then reconstituted with 50 µL of phosphate-buffered saline. Substance P concentrations in samples were measured using an enzyme immunoassay system (Cayman Chemical Co) and expressed per milliliter of tear fluid.

STATISTICAL ANALYSIS

Results are presented as mean±SD. Statistical significance was calculated by comparing results by t test or linear regression analysis, aided by Excel 98 software (Microsoft, Redmond, Wash). A P value less than .05 was considered statistically significant.

RESULTS

Before starting treatment, the mean concentration of PGE₂ in tears was 130.8±20.4 pg/mL in eyes subsequently treated with diclofenac sodium, and 125.6±19.6 pg/mL in eyes subsequently treated with vehicle (no significant difference; Figure 1). After drug treatment for 2 weeks, concentrations of PGE₂ in tears from diclofenac sodium–treated eyes decreased significantly to 69.1±15.8 pg/mL (P < .001, paired t test), returning to baseline concentrations at 2 weeks after discontinuation of treatment. No significant changes were seen in concentrations of PGE₂ in tears from vehicle-treated eyes throughout the experimental period.

Concentrations of substance P in tears from diclofenac sodium–treated eyes were also significantly decreased after 2 weeks of treatment (from 278.0±58.7 pg/mL to 171.7±52.2 pg/mL; P < .002, paired t test), returning to baseline concentrations at 2 weeks following discontinuation (Figure 2). Substance P in vehicle treated eyes showed no significant changes.

The ratio of PGE₂ to substance P concentration in tears before treatment to the concentration at conclusion of treatment was other neuropeptides may be involved.¹⁴⁻²⁰ The cornea is innervated by nerve fibers originating from the trigeminal ganglion that contain several neuropeptides, including substance P and calcitonin gene–related peptide.²¹⁻²³ Depletion of these neuropeptides induced by capsaicin delayed wound healing in the corneal epithelium.¹³ Substance P, both alone and in combination with other factors such as insulin and insulinlike growth factor 1, promotes migration¹⁶⁻²⁰ and proliferation¹⁰,¹⁷ of corneal epithelial cells. Nishida et al²⁴,²⁵ recently reported that topical application of substance P–derived peptide combined with insulinlike growth factor 1 may be effective in treating neurotrophic keratopathy.

Several studies have shown that diclofenac sodium or other NSAIDs decreased concentrations of substance P in synovial fluids from patients with arthritis and in gastric mucosa and snouts from experimental animals.²⁰⁻²⁴ Therefore, we reasoned that the analgesic actions of diclofenac sodium may involve similar depletion of substance P in ocular tissues, with a risk of development of corneal complications. In the present study, we evaluated the effect of topical diclofenac sodium on concentrations of prostaglandin E₂ (PGE₂) and substance P in human tears.
calculated in each subject, and then correlated with the ratio for substance P concentrations (Figure 3). Pre-treatment and posttreatment ratios for PGE2 correlated significantly with those for substance P ($r=0.75$, $P=.01$).

**COMMENT**

In the present study, we demonstrated that diclofenac sodium, characterized as a cyclooxygenase inhibitor, reduced the PGE2 concentration in human tears. Prostaglandin synthesis in the cornea is up-regulated in response to injury, and is blocked by topical NSAIDs. The presence of PGE2 in human tears was first reported by Gluud et al, who noted that PGE2 became increased in tears in response to cataract surgery. Half of their patients with chronic conjunctivitis exhibited high concentrations of PGE2 in tears. These observations suggest that PGE2 in tears reflects prostaglandin content in ocular tissues, especially in the anterior segment of the eye. Our finding of a diclofenac-sodium–related decrease in PGE2 in tears is consistent with these observations.

The most important result of our study is that diclofenac sodium concurrently reduced substance P concentrations in tears. Not only is the cornea innervated by nerve fibers that contain substance P, but also the conjunctiva and lacrimal gland. Therefore, the source of substance P in tears remains unclear. We recently reported that substance P concentrations in tears from patients with unilateral corneal hyposthesia were decreased compared with contralateral healthy eyes. Substance P concentrations in tears from patients with diabetic keratopathy are also lower than those of healthy controls (Masaro Ogata, MD, et al, unpublished data, 2000). It is likely that substance P concentrations in tears reflect the neuropeptides levels in ocular tissues, although further studies should be done to determine the source of substance P in tears.

Diclofenac sodium reportedly reduced substance P concentrations in synovial fluid from patients with rheumatoid arthritis, and also in the murine snout. Indomethacin reduced substance P concentrations in the rat gastric mucosa. Taken together with past observations, our present findings suggest that NSAIDs decrease amounts of prostaglandins and substance P in the ocular surface as in other tissues. Proposed analgesic mechanisms of NSAIDs are multiple, including central and peripheral nitric oxide synthase inhibition, central prostaglandin suppression, and down-regulation of pain receptors. Small primary sensory nociceptive neurons contain substance P; capsaicin-induced substance P depletion also has an analgesic effect. Therefore, our finding may partly explain the analgesic effect of diclofenac sodium on the cornea.

Although beneficial analgesic effects of diclofenac sodium eyedrops are marked, potential adverse effects, especially damage to the corneal epithelium, have become a major concern. Results of experimental studies are conflicting. Most in vivo studies have reported that diclofenac sodium and other NSAIDs did not have a significant effect on the rate of wound healing in corneal epithelium, while one study found that impairment of corneal epithelial wound healing resulted from applica-
tion of these agents. In vitro studies have failed to dem-
strate a delay of epithelial wound healing in the cor-
nea.41,42 Some clinical studies2,3,8 have reported that punctate epithelial keratopathy developed in association with postoperative use of diclofenac sodium eyedrops. In con-
trast, Shimazaki et al13 failed to detect any significant
operative use of diclofenac sodium eyedrops. In con-

REFERENCES

1. Ku EC, Lee W, Kothari HV, Scholer DW. Effect of diclofenac sodium on the arachidi
3. Herbst CP, Mermaid A, Schmider C, Rittet N. Anti-inflammatory effect of di-
clofenac drops after argon laser trabeculoplasty. Arch Ophthalmol. 1993;111:
481-483.
5. Epstein RL, Laurence EP. Effect of topical diclofenac solution on discomfort af-
6. Jayamannne DGR, Fitt AWD, Dayan M, Andrews RM, Mitchell KW, Griffiths PG. The effectiveness of topical diclofenac in relieving discomfort following tra-
7. Szucs PA, Nashed AH, Allegra JR, Eskin B. Safety and efficacy of diclofenac oph-
35:131-137.
8. Kitaooji H, Kitaooji S, Naka Y. Diclofenac sodium ophthalmic solution before and af-
9:1583-1587.
118:312-314.
thalmol. 1995;113:262.
16. Reid TW, Murphy CJ, Iwahashi CK, Foster BA, Mannis MJ. Stimulation of epi-
thelial cell growth by the neuropeptide substance P. J Cell Biochem. 1993;52:
476-485.
18. Nishida T, Nakamura M, Ujii F, Reid TW, Mannis MJ, Murphy CJ. Synergistic ef-
20. Nakamura M, Chikama T, Nishida T. Up-regulation of integrin alpha S expres-
21. Tervo T, Tervo K, Franko L, Vannas A, Erano K, Cuello AC. Substance P immu-
noreaction and acetylcholinesterase activity in the cornea and Gasserian gan-
22. Lehtosalo JI. Substance P-like immunoreactive trigeminal ganglion cells sup-
24. Brown SM, Lamberts DW, Reid TW, Nishida T, Murphy CJ. Neurotrophic and an-
25. Chikama T, Fukuda K, Morishige N, Nishida T. Treatment of neurotrophic ker-
topathy with substance-P-derivative (FGLM) and insulin-like growth fac-
29. Bazan HEF, Birke DL, Beuerwan RM, Bazan NG. Inflammation-induced simul-
30. Phillips AF, Szerenyi K, Campos M, Krueger RR, McDonnell PJ. Arachidonic acid mem-
eteors after eximer laser corneal surgery. Arch Ophthalmol. 1993;111:
1273-1278.
31. Yamada M, Kawai M, Kawai Y, Mithma Y. The effect of selective cyclooxygen-
ase-2 inhibitor on corneal angiogenesis in the rat. Curr Eye Res. 1999;19:300-
304.
63(suppl):28-29.
33. Nikkenen A, Lehtosalo JI, Uusitalo H, Palkama A, Panula P. The lacrimal glands of the rat and the guinea pig are innervated by nerve fibers containing immuno-
reactivities for substance P and vasoactive intestinal polypeptide. Histochimis-
34. Yamada M, Ogata M, Kawai M, Mithma Y. Decreased substance P concentra-
129:671-672.
39(suppl)103:1-44.
37. Srinivasan BD, Kulkarni PS. The effect of steroidal and nonsteroidal anti-
38. Waterbury L, Kunysz EA, Beuerman R. Effects of steroidal and non-steroidal anti-
inflammatory agents on corneal wound healing. J Ocular Pharmacol. 1987;3:
43-54.
39. Srinivasan BD, Srinivasan BA. Anti-inflammatory effects of ketoprofen in rabbit cor-
41. Gupta AG, Hirakata A, Proia AD. Effect of inhibitors of arachidonic acid metabo-
43. Shimazaki J, Fujishima H, Yagi Y, Tsutoba K. Effects of diclofenac eye drops on corneal epithelial structure and function after small-incision cataract surgery. Oph-
thalmology. 1996;03:50-57.
44. Shimazaki J, Saito H, Yang H-Y, Toda I, Fujishima H, Tsutoba K. Persistent epi-