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 been used 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 eyedrops 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-Bonnetesthesiometer (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 a 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 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.15 Substance P, both alone and in combination with other factors such as insulin and insulinlike growth factor 1, promotes migration16-20 and proliferation16,17 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.26-28 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 so-
dium, characterized as a cyclooxygenase inhibitor, re-
duced the PGE2 concentration in human tears. Prosta-
glandin synthesis in the cornea is up-regulated in response
to injury,29-31 and is blocked by topical NSAIDs.30,31 The
presence of PGE2 in human tears was first reported by
Gluud et al32 who noted that PGE2 became increased in
tears in response to cataract surgery. Half of their pa-
tients with chronic conjunctivitis exhibited high con-
centrations of PGE2 in tears. These observations suggest
that PGE2 in tears reflects prostaglandin content in ocu-
lar 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 di-
clofenac sodium concurrently reduced substance P con-
centrations in tears. Not only is the cornea innervated
by nerve fibers that contain substance P, but also the con-
junctiva and lacrimal gland.33 Therefore, the source of
substance P in tears remains unclear. We recently re-
ported that substance P concentrations in tears from pa-
tients with unilateral corneal hypesthesia were de-
creased compared with contralateral healthy eyes.34 Sub-
crstance 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, al-
though further studies should be done to determine the
source of substance P in tears.

Diclofenac sodium reportedly reduced substance P con-
centrations in synovial fluid from patients with rheu-
matoid arthritis,26 and also in the murine snout.37 Indom-
ethacin reduced substance P concentrations in the rat gas-
tric mucosa.38 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.35 Small primary sen-
sory nociceptive neurons contain substance P; capsaicin-
induced substance P depletion also has an analgesic effect.36
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 be-
come a major concern.39 Results of experimental studies
are conflicting. Most in vivo studies27-30 have reported that
diclofenac sodium and other NSAIDs did not have a sig-
nificant effect on the rate of wound healing in corneal
epithelium, while one study40 found that impairment of
corneal epithelial wound healing resulted from applica-

**Figures**

**Figure 1.** Concentrations of prostaglandin E2 (PGE2) in tears from diclofenac
sodium–treated eyes (squares) and contralateral vehicle–treated eyes (circles). A significant difference was noted between the 2 groups at week 2 (P < .001).

**Figure 2.** Concentrations of substance P in tears from diclofenac
sodium–treated eyes (squares) and contralateral vehicle–treated eyes (circles). A significant difference was noted between the 2 groups at week 2 (P = .002).

**Figure 3.** Ratios of prostaglandin E2 (PGE2) concentration in tears before
treatment to the concentration after treatment were correlated with the corre-
csponding ratios for substance P concentrations. Ratios for PGE2
concentration were significantly correlated with ratios for substance P
concentration (r = 0.75, P = .012).
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 studies2,8 have reported that punctate
epithelial keratopathy developed in association with post-
operative use of diclofenac sodium eyedrops. In con-
trast, Shimazaki et al43 failed to detect any significant
operative use of diclofenac sodium eyedrops. In con-
cept, epithelial keratopathy developed in association with post-
keratoplasty44 may also implicate
who have undergone keratoplasty44 may also implicate
prior insults. Our present study demonstrates that topi-
cal diclofenac sodium decreases the substance P con-
tent of tears. Depletion of substance P may be involved in
the development of neurotrophic keratopathy.13-23
Therefore, we speculate that additional depletion of sub-
stance P occurs when diclofenac sodium eyedrops are used in
patients with corneal hypesthesia, which may pro-
mote development of corneal complications.

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