A Closed Eye Contact Lens Model of Corneal Inflammation

Part 2: Inhibition of Cytochrome P450 Arachidonic Acid Metabolism Alleviates Inflammatory Sequelae

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Purpose. The authors have previously shown a marked increase in corneal epithelial arachidonic acid metabolism to 12-hydroxyeicosatetraenoic acid (12-HETE) and 12-hydroxyeicosatrienoic acid (12-HETrE) in a model of closed eye–contact lens wear. Their formation was predominantly cytochrome P450-dependent and significantly correlated with inflammatory score and corneal thickness. In the current study, the authors used stannous chloride to inhibit the epithelial cytochrome P450-dependent synthesis of 12-HETE and 12-HETrE to assess the role of these eicosanoids as mediators of the inflammatory response to contact lens wear in the closed eye.

Methods. Hydrogel contact lenses were soaked in stannous chloride (100 μg/ml) or vehicle and fitted to the rabbit eye in stacked fashion (two lenses/eye), followed by a silk suture tarsorrhaphy of >90%. Eyes were analyzed over a 7-day period for inflammatory responses through slit lamp biomicroscopy, subjective inflammatory scoring, ultrasonic pachymetry, and corneal epithelial [l-14C]-arachidonic acid metabolism.

Results. Closed eye–hydrogel contact lens wear resulted in a progressive anterior surface inflammatory response. Coinciding with these events was a time-dependent increase in corneal thickness and 12-HETE and 12-HETrE production rates by corneal epithelial homogenates. Treatment of the lenses with stannous chloride (100 μg/ml) significantly attenuated by day 7 the inflammatory score (56% decrease), corneal thickness (17% decrease), and 12HETE and 12HETrE synthesis (77% and 71% decrease, respectively).

Conclusions. This study further substantiates the involvement of cytochrome P450, through the synthesis of 12-HETE and 12-HETrE, in the inflammatory response associated with hydrogel contact lens wear in the closed eye. Thus, inhibition of cytochrome P450, with subsequent decreases in 12-HETE and 12-HETrE, may attenuate the pathophysiologic response to contact lens wear in the closed eye. Invest Ophthalmol Vis Sci. 1995;36:841–850.

Contact lens wear combined with eye closure can promote a significant anterior surface inflammatory response.1 Eye closure alone, however, has become a major etiologic factor influencing extended lens wear complications. Recently, Schein et al determined that overnight wear of contact lenses was the overwhelming risk factor for the development of ulcerative keratitis, further indicating the importance of studying contact lens wear in the closed eye. Analysis of the array of inflammatory mediators strongly suggests that there must be several different mechanisms stimulating inflammation, making it difficult to incriminate a single substance as most consequential. However, relationships between the degree of in situ inflammation and corneal acidosis, protease activity (plasmin), and/or eicosanoids have been established.5–7 Although discontinuance of wear is the most rational treatment for inflammatory sequelae, pharmacologic regimens including corticosteroids and nonsteroidal antiinflammatory drugs may also be used. These drugs in-
hibit eicosanoid biosynthesis, making the eicosanoid response relevant to the pathophysiology of contact lens intolerance.

Previously, we have shown, in a closed eye–hydrogel contact lens model of anterior surface inflammation, that the corneal epithelial capacity to synthesize 12-HETE and 12-HETrE was greatly enhanced. This increase in metabolism was predominantly cytochrome P450-dependent and significantly correlated to both the inflammatory score and the corneal thickness, thereby indicating a potential cause–effect relationship between these events. Because 12-HETE and 12-HETrE synthesis were shown to be cytochrome P450-dependent in rabbit corneal epithelial microsomes, manipulation of this enzymatic pathway would provide an excellent means of determining the importance of both eicosanoids, as well as of the cytochrome P450 pathway, in the inflammatory response to closed eye–hydrogel contact lens wear. The current cytochrome P450 enzyme inhibitors are not useful for this experimental model of contact lens wear because of considerable toxicity associated with their in vivo use, instability over time, and lack of inhibitory selectivity, especially when higher doses are used. Therefore, the need for a nontoxic yet stable, efficacious, and selective intervention is required to modify the pathway effectively. One strategy to alter cytochrome P450 is to deplete the levels of microsomal heme, an important component for the activity of this enzyme system.

Studies by Maines and Kappas were among the first to show that certain heavy metals at relatively low, nontoxic doses could induce the synthesis and activity of the ubiquitous heme-metabolizing enzyme, heme oxygenase. This microsomal enzyme normally functions to convert cellular heme to biliverdin through an NADPH-dependent reaction that is subsequently converted to bilirubin in the presence of cytosolic biliverdin reductase. With induction, various heme-containing enzymes, particularly those with a more rapid turnover, are eventually depleted because of a lack of available heme within the cell. Further studies showed a selective depletion in cytochrome P450 hemoproteins, whereas other more stable hemoproteins, such as those of the respiratory chain, remained unaltered. The use of heavy metals to inhibit cytochrome P450-dependent arachidonic acid metabolism has been established in the kidney. However, their use in ocular tissues for manipulation of arachidonic acid metabolism is yet to be explored. Topical heavy metal therapy is preferred because systemic toxicity is avoided while the ocular surface is treated effectively. However, only a few metals are nontoxic with respect to the anterior surface. Stannous chloride (tin [II] chloride) is such a metal. It is nontoxic at effective doses, yet efficacious as a heme oxygenase inducer. Therefore, depletion of cytochrome P450 and subsequent inhibition of enzyme activity is achieved.

In the current study, we examined the effect of stannous chloride on the epithelial cytochrome P450-arachidonic acid metabolism (formation of 12-HETE and 12-HETrE) in corneas fitted with hydrogel contact lenses in the closed eye. We herein describe the effects of this treatment on the inflammatory score, corneal thickness, and corneal epithelial synthesis of 12-HETE and 12-HETrE in this model of anterior surface inflammation.

MATERIALS AND METHODS

Materials

[1-14C]-arachidonic acid (53 mCi/mmol) was obtained from NEN DuPont (Boston, MA). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) and phenylmethylsulfonylfluoride were obtained from Sigma (St. Louis, MO). Anhydrous stannous chloride (SnCl2) was purchased from Aldrich (Milwaukee, WI). All solvents were high-performance liquid chromatography (HPLC) grade purchased from JT Baker (Phillipsburg, NJ). The rabbits were obtained from Hazelton (Denver, PA).

Animal Treatment

Male New Zealand white rabbits (each weighing 2.0 to 2.5 kg) were anesthetized with ketamine HCL (50 mg/kg) and xylazine (20 mg/kg) intramuscularly. The right eye (OD) was used, and the left eye (OS) was the control. Seven days before contact lens placement, the right nictitating membrane was surgically removed in anesthetized rabbits after the administration of topical proparacaine–HCl 0.5% (Bausch & Lomb, Tampa, FL), and the remaining tissue was cauterized. All investigations conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Extended wear contact lenses (Vistakon; Acuvue: +4.0 D, 9.1 mm base curve, 14.0 mm diameter, 58% water) were dehydrated for 30 minutes and rehydrated in either phosphate buffer (0.1 M, pH 7.4) or stannous chloride in phosphate buffer (10, 100, or 1000 μg/μl; 0.05, 0.5, or 5 mM, respectively) for 30 minutes. In this fashion, the hydrogels functioned as a drug depot and an inflammatory stimulus. Lenses were then placed onto the proposed OD in stacked fashion (two lenses, one on top of the other), and the OD was gently reposited. To keep the lenses in place, five to seven interrupted 5-0 silk sutures (Ethicon, Somerville, NJ) were placed through the superficial tarsus of each eyelids, with care taken not to penetrate the conjunctival tissues. Suture tarsorrhaphy closed the eye 80% to 90%. At 1-, 3-, 5-, and 7-day intervals,
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animals were anesthetized, sutures were removed, test eye was exposed, and lenses were removed. After the administration of topical proparacaine, the eyes were subjected to ultrasonic pachymetry to assess central corneal thickness and then were examined with a slit lamp and photographed to document the degree of in situ inflammation. Animals were killed with an overdose of sodium pentobarbital (90 mg/kg) through the marginal ear vein.

Assessment of Inflammatory Response

Corneal Edema. Central corneal thickness was measured by ultrasonic pachymetry (Villasenor Ultrasonic Pachymeter: CILCO, Huntington, WV), as the mean of five independent determinations at 1550 m/second. Measurements were taken at the same time for all animals to account for potential diurnal variations in corneal thickness. 17

Slit Lamp Analysis and Subjective Inflammatory Scoring. Animals were placed on a special platform built for the slit lamp, and eyes were assessed for in situ inflammation (Model SL-7E; Topcon, Paramus, NJ). Biomicroscopic photographs of the anterior surface tissues were taken with a photographic attachment to the slit lamp. Slit lamp photographs of the anterior surface were subjectively scored based on a modification of the methods described by McDonald et al. 18 Briefly, the conjunctiva was assessed for congestion and swelling; the iris was evaluated for hyperemia and swelling; and the cornea was examined for cloudiness, opacity, and neovascularization. Each ocular tissue analysis was graded separately; therefore, no preference was given to the scoring of one tissue over another. The scoring system ranges from 0 to 3+ or 0 to 5+, with 0 indicating normal or lack of response 3+ or 5+ indicating the most severe degree of inflammation in that tissue. The values from each subset were added, and severity of the inflammation was graded from 0 to 2.5 (essentially noninflamed), 2.5 to 15 (minimally inflamed), 15 to 25 (mildly inflamed), 25 to 50 (moderately inflamed), 50 to 80 (severely inflamed), and 80 to 110 (extremely inflamed).

Tissue Preparation and Arachidonic Acid Metabolism

Corneal epithelial homogenates were prepared by sonication in 0.1 M phosphate buffer (pH = 7.4) containing 0.1 mM phenylmethylsulfonyl fluoride. Protein content was measured by the Bradford method using bovine serum albumin as a standard (Bio-Rad, Richmond, CA). Homogenates (3 to 4 mg/ml) from corneas treated with SnCl2 or vehicle were incubated with [1-14C]-arachidonic acid (0.2 μCi, 3.6 nmol) and NADPH (1 mM) in a final volume of 150 μl. Boiled preparations were run as a control for enzymatic conversion. Incubations were carried out at 37°C for 60 minutes. The reaction was terminated by acidification, pH 3.5 to 4.0, with 0.2 M formic acid, and the metabolites were extracted three times with ethyl acetate.

Arachidonic acid metabolites (12-HETE and 12-HETrE) were separated by reverse-phase HPLC (RP-HPLC) on a C18 Microsorb column, 4.6 × 100 mm (Rainin Instruments, Woburn, MA) using a linear gradient running from 100% acetonitrile–water–acetic acid, 50:50:0.1 (vol/vol/vol) to 100% acetonitrile–acetic acid, 100:0.0 (vol/vol) at a flow rate of 1 ml/minute for 25 minutes. Radioactivity was monitored by an on-line flow detector (Radiomatic Instruments, Tampa, FL). Identification of metabolites was based on comigration with authentic standards. Specific activity for conversion to products was based on the specific activity of [1-14C]-arachidonic acid added and the extraction efficiency and was expressed as pg-ng of product formed per hour per milligram of homogenate.

Statistical Analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined by analysis of variance for multiple comparisons, followed by a modified t-test to determine specific differences among groups. Relationships between variables were analyzed by linear regression analysis. In all test cases, a critical significance level of 0.05 was assumed for statistical significance.

RESULTS

Initial experiments were conducted in the closed eye–hydrogel contact lens model to determine the optimal dose of SnCl2 to reduce corneal epithelial cytochrome P450 activity, i.e., production of 12-HETE and 12-HETrE. As seen in Figure 1A, pretreatment of the lenses with SnCl2 resulted in a dose-dependent decrease in the capacity of the corneal epithelium to produce 12-HETE + 12-HETrE at 6 days. At 100 μg/ml, there was a 54% ± 3% decrease (mean ± SEM, n = 4) in the formation of 12-HETE and 12-HETrE. Functionally, there was an 18% ± 5% decrease (mean ± SEM, n = 4) in the corneal thickness at this same dose (Fig. 1B). No significant differences were observed between the 100 μg/ml and 1000 μg/ml doses for inhibition of 12-HETE + 12-HETrE synthesis and the associated decrease in corneal thickness. Therefore, the lower dose of SnCl2 (100 μg/ml) was used to avoid the potential for tin-associated epithelial toxicity. A histogram transformation of a representative RP-HPLC trace depicting the effect of a 100 μg/ml dose on the formation of 12-HETE and 12-HETrE is shown in Figure 2. At 6 days, treatment with SnCl2 markedly inhibited the corneal epithelial production of 12-HETE and 12-HETrE.
A.

FIGURE 1. Dose-dependent effect of SnCl₂ at 6 days of closed eye-hydrogel lens wear on (A) 12-HETE + 12-HETrE synthesis by the corneal epithelium and (B) the degree of corneal thickness. Data are expressed as a percentage of the control. Control specific activity for 12-HETE + 12-HETrE was 282 ± 50 ng/h • mg (n = 4). Control corneal thickness was 607 ± 48 μm (n = 4). Significant differences (*P < 0.05) were found at 100-μg/ml and 1000-μg/ml doses compared to control. However, there was no significant difference between the 100-μg/ml and 1000-μg/ml doses.

To confirm that the cytochrome P450 activity contributes to the formation of 12-HETE and 12-HETrE, epithelial homogenates were pooled from inflamed corneas (day 9) and incubated with arachidonic acid and NADPH in the presence and absence of selective metabolic inhibitors. The addition of SKF-525A, a cytochrome P450 enzyme inhibitor, and BW755C, a cyclooxygenase and lipooxygenase inhibitor, inhibited 12-HETE/12-HETrE synthesis by 70% ± 11% and 39% ± 9% (mean ± SE, n = 3), respectively, indicating the presence of both metabolic pathways in the corneal epithelial homogenates from inflamed eyes. Furthermore, 12-HETE synthesizing capacity in corneal epithelial homogenates from SnCl₂-treated eyes was reduced by 4.6-fold compared to inflamed, non-treated eyes. The remaining capacity was only inhibited by BW-755C (59% ± 10%, n = 3) with no effect by SKF-525A, suggesting that the cytochrome P450 activity was selectively affected by the SnCl₂ treatment whereas lipooxygenase activity was still present in these homogenates and was capable of being affected by BW-755C. SnCl₂ at micromolar concentrations had no direct effect on lipooxygenase or cyclooxygenase activities in corneal epithelial homogenates or calf conjunctival cytosol (a rich source of 12-lipoxygenase activity). For example, cytosolic 12-HETE production was 162 ± 30 and 265 ± 80 ng/h • mg in the presence and absence of SnCl₂, respectively (n = 3).

To address the effect of SnCl₂ treatment of the hydrogel lenses on the inflammatory response in the closed eye, a time-course study was performed. Figure 3 depicts representative slit lamp photographs of the ocular surface (n = 3 to 5 per time point) throughout the 7 days of closed eye–hydrogel contact lens wear. In the vehicle treatment, there was a progressive in situ inflammatory response that became prominent at day 3 and was more severe through days 5 and 7. The inflammatory response consisted of limbal vasodilation, conjunctival swelling, dilation of the iridial vessels, decreased corneal transparency (increased thickness, cloudiness, and opacity), and neovascularization. There was no evidence of ulcerative keratitis in any of the animals studied. One-time treatment of the hydrogel lenses with SnCl₂ (100 μg/ml) resulted in a marked attenuation of the inflammatory response. The pharmacologic effect was noticeable by day 3 and was maintained through day 7. SnCl₂ was effective in suppressing limbal and iridial vasodilation as well as the extent of epithelial defects and neovascularization of the cornea. Quantitative analysis by subjective inflammatory scoring demonstrated a significant correlation between the inflammatory response and the extent of closed eye–hydrogel contact lens wear over 7 days (r = 0.990, P = 0.001, n = 5) (Fig. 4). Treatment with SnCl₂ (100 μg/ml) attenuated the increase in inflammatory score beginning at day 3 and reached significance at days 5 and 7 (Fig. 4).

Using this model, the corneal thickness increased steadily, correlating significantly with the extent of closed eye–hydrogel contact lens wear (r = 0.962, P = 0.009, n = 5) and the inflammatory score (r = 0.936, P = 0.019, n = 5) (Fig. 5). Treatment with SnCl₂ (100 μg/ml) progressively attenuated the increase, statistical significance was reached at 7 days. This corresponded to a 17% decrease in central corneal thickness (Fig. 5) and a marked difference in corneal clarity when compared to the vehicle control (Fig. 5).
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Effect of SnCl₂ on corneal thickness is potent. Although contact lens wear in the absence of SnCl₂ treatment increased corneal thickness by 273 ± 33 µm, the addition of SnCl₂ to the lens resulted in an increase of only 160 ± 61 µm, a 42% reduction in the contact lens-induced increase in corneal thickness.

Coinciding with inflammatory score and corneal thickness was the time-dependent changes in corneal epithelial synthesis of 12-HETE and 12-HETrE. In Figure 6, the specific activities for 12-HETE and 12-HETrE synthesis by the homogenates of the corneal epithelium are described. There was a time-dependent increase in the synthesis of 12-HETE that was significantly correlated with the extent of closed eye–hydrogel contact lens wear (r = 0.972, P = 0.006, n = 5) and the inflammatory score (r = 0.989, P = 0.001, n = 5) (Fig. 6A). One-time treatment of the hydrogel contact lenses with SnCl₂ (100 µg/ml) inhibited the conversion of [1-¹⁴C]-arachidonic acid to 12-HETE beginning at day 3 (84% decrease), with a marked significant inhibition at days 5 (96% decrease) and 7 (77% decrease), compared to the vehicle control. Figure 6B describes a time-dependent increase in the corneal epithelial synthesis of 12-HETrE that was significantly correlated with the extent of closed eye–hydrogel contact lens wear (r = 0.908, P = 0.035, n = 5) and the inflammatory score (r = 0.954, P = 0.012, n = 5). One-time treatment of the hydrogel lenses with SnCl₂ (100 µg/ml) markedly inhibited the synthesis of corneal epithelial 12-HETrE at days 5 (93% decrease) and 7 (71% decrease). Overall, SnCl₂ treatment significantly suppressed the in situ inflammatory response to closed eye–hydrogel contact lens wear with concomitant inhibition of corneal epithelial cytochrome P450-dependent formation of 12-HETE and 12-HETrE, indicating a strong relationship between these events.

DISCUSSION

The correlation between the known functional properties of both 12-HETE and 12-HETrE, their time-
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Figure 5. Time-dependent changes in central corneal thickness with vehicle and SnCl₂ (100 μg/ml) treatment. Corneal thickness was assessed by ultrasonic pachymetry as described in Methods. Data are expressed as mean ± SEM (n = 3 to 5). A trend to decrease was noted by day 3 and reached statistical significance by day 7 (*P < 0.05). D = day of closed eye–hydrogel contact lens wear; DO = control, no eye closure, or hydrogel contact lens.

One-time treatment of the hydrogel contact lens with SnCl₂ (100 μg/ml) for 30 minutes, resulted in the significant inhibition of cytochrome P450-dependent arachidonic acid metabolism. For example, Sacerdoti et al.²⁰ used SnCl₂ (100 mg/kg) in the spontaneously hypertensive rat to inhibit renal cytochrome P450 activity. From these studies, an association between the inhibition of cytochrome P450-dependent arachidonic acid metabolism and the development of hypertension in these animals was established. With chronic treatment, Escalante et al.²¹ showed that continuous dosing of the spontaneously hypertensive rat with SnCl₂ not only maintained the inhibition of cytochrome P450-dependent arachidonic acid activity and normalization of blood pressure but also showed no evidence of systemic toxicity even at the microscopic level.

One-time treatment of the hydrogel contact lens with SnCl₂ (100 μg/ml) for 30 minutes, resulted in the significant inhibition of cytochrome P450-dependent arachidonic acid metabolism to 12-HETE and 12-HETrE between 3 and 7 days. This was associated with an attenuation of the inflammatory response, determined by subjective scoring, in the same time frame. There was no difference in the reduction of inflammatory response if the lenses were pretreated for 15 minutes or 5 hours, indicative of equilibrated uptake into the lens by 15 minutes (data not shown). There was also no evidence of heavy metal-induced toxicity to the ocular surface at all doses tested. The lag period in inhibitory effect may reflect the time needed to initiate transcription, translation, and activation of heme oxygenase with subsequent expression of its biologic activity, i.e., degradation of micromolar heme required for the de novo synthesis of new cytochrome P450 protein. To this end, da Silva et al.²² have shown that within 4 hours of a one-time systemic administration of SnCl₂ (150 mg/kg), increased mRNA for heme oxygenase could be detected with maximal effects between 8 and 16 hours. However, at least 24 hours were required to find substantial increases in heme oxygenase activity, which was sustained up to 72 hours. Decreased levels of cytochrome P450 content and inhibition of cytochrome P450-dependent arachidonate metabolism followed the increase in heme oxygenase activity. Kikuchi and Yoshida²³ have provided similar results with CoCl₂ showing elevated heme oxygenase activity up to 4 days after a single dose, with concomi-

Figure 6. Time-dependent changes in the synthesis of (A) 12-HETE and (B) 12-HETrE by corneal epithelial homogenates with vehicle or SnCl₂ treatment. Data are presented as mean ± SEM (n = 3 to 5). Significant differences (*P < 0.05) were noted at days 5 and 7 for both (A) 12-HETE and (B) 12-HETrE. D = day of closed eye–hydrogel contact lens wear; DO = control, no eye closure, or hydrogel contact lens.
tant decreases in cytochrome P450 content and activity. A similar scenario could potentially operate in our model. To this end, we obtained preliminary results that demonstrate a marked increase in HO-1 mRNA levels in corneal epithelium from SnCl₂-treated contact lens eyes within 8 to 24 hours compared to the untreated eyes. The increased HO-1 mRNA remained elevated as long as 5 to 6 days, after which a gradual decrease occurred. Within this time frame of heme oxygenase induction, a significant and marked inhibition of 12-HETE and 12-HETrE was obtained (Fig. 6). At this time, the identity of the cytochrome P450 isozyme that converts arachidonic acid to these metabolites in the corneal epithelium is uncertain. However, we have obtained preliminary evidence to suggest that a cytochrome P450 isozyme, namely CYP1A1, could be the corneal epithelial arachidonate metabolizing enzyme. The expression of this isoform seems to correlate with heme oxygenase induction.

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The expression of this isoform seems to correlate with heme oxygenase induction, a significant and marked inhibition of 12-HETE and 12-HETrE synthesis in this model of contact lens wear. This result provides strong pharmacologic evidence for the role of cytochrome P450 and lipoxygenase pathways. The dynamic process of inflammation encompassing the release of mediators from damaged tissues, with the development of an inflammatory infiltrate (and subsequent release of other proinflammatory mediators), creates an amplifying environment for the inflammatory response making targeted antiinflammatory therapy difficult to achieve. Nevertheless, a 60% inhibition in the inflammatory response is significant and further implies that manipulation of cytochrome P450 activity should be included in the pharmacologic regimen in the potential prevention and/or treatment of contact lens intolerance.

We have described a significant correlation between the development of corneal edema with the increased corneal epithelial production of 12-HETE + 12-HETrE. Conversely, we show that a 95% decrease in metabolite production results in only a 17% decrease in central corneal thickness (edema) when compared to the vehicle control. However, this translates into a 42% reduction in the increase of corneal thickness brought about by contact lens wear alone. This degree of inhibition was sufficient to attenuate the in situ appearance of corneal edema (Fig. 3). However, there remains a weak correlation between the extent of inhibition of 12-HETE and 12-HETrE synthesis and the maximum inhibition in corneal thickness through 7 days. Because the remaining 58% of the edema response was not affected by SnCl₂, it may be assumed that the synthesis of 12-HETE and 12-HETrE are not strongly associated with the edematous response. This, however, does not preclude the possibility that they are involved in the development of the response, whereas other mediators also may play a role in the maintenance of corneal edema. For example, under the conditions of eye closure with a hydrogel contact lens in place, significant stromal lactate accumulation, with subsequent stromal acidosis, has been implicated in the edema response. Furthermore, because 12(R)-HETE is a substrate for the synthesis of...
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12(R)-HETE, its effectiveness as an NaK-ATPase inhibitor may be limited because of a potentially enhanced conversion, particularly at the later time points of closed eye—hydrogel contact lens wear. It is, therefore, possible that a combination of lactate accumulation, acidosis, cytochrome P450-dependent synthesis of 12-HETE and 12-HETE, and other potential mediators most likely work in conjunction in the development and maintenance of the edema response in the cornea under the conditions of closed eye—hydrogel contact lens wear. Nevertheless, as described for the inflammatory response, a 42% alleviation in the development of corneal edema is significant and further implies that manipulation of cytochrome P450 activity can markedly influence corneal deturgescence particularly under conditions of contact lens wear.

Correlations between one particular pathway of arachidonate metabolism and the inflammatory response have been shown through the use of enzyme inhibitors. Although this is a correct pharmacologic approach, particularly when studying semipurified enzyme preparations, one must be aware of the lack of selectivity between the inhibitors when higher doses are used. This issue may become relevant, particularly when topical applications are studied. It is not uncommon for a nonsteroidal antiinflammatory drug, such as indomethacin or flurbiprofen, to inhibit arachidonic acid metabolism through the lipoxygenase or cyclooxygenase P450 pathway, as well as the cyclooxygenase pathway. These results, therefore, make conclusions concerning the proinflammatory effects of prostanoids questionable as to whether they are the true mediators of the inflammatory response studied. Similar limitations also exist for inhibitors of the lipoxygenase pathway of arachidonate metabolism. Therefore, the use of more selective interventions to inhibit pathway-specific arachidonate metabolism are much more appropriate in this regard. The results of the current study demonstrate that SnCl₂ is exemplary of such an intervention; it inhibits cytochrome P450-dependent arachidonate metabolism in vivo, presumably through induction of heme oxygenase, as shown previously for other tissues. Furthermore, it does not have an effect on in vitro metabolism of arachidonic acid through lipoxygenase activity. However, tin has been shown to manipulate other heme-dependent enzyme systems that produce proinflammatory eicosanoids, such as thromboxane synthase and the subsequent production of thromboxane A₂ (TXA₂). Through effects on this cytochrome P450-like enzyme, SnCl₂ treatment could theoretically attenuate the production of this eicosanoid in the eye. However, TXA₂ production by the anterior surface plays a questionable role in the corneal inflammatory response. Another class of enzymes that also are heme dependent are the two isoforms of cyclooxygenase, COX 1 and COX 2. These enzymes, particularly COX 2, have been implicated in inflammatory responses in many tissues. However, in a previous study, we demonstrated that administration of SnCl₂ at doses that have significant inhibitory effects on cytochrome P450 activity showed no effect on cyclooxygenase activity, i.e., production of PGE₂ and PGF₂α in the rat kidney. Similarly, analysis of cyclooxygenase-like products from rabbit corneal epithelial homogenates and homogenates of the inflamed corneal epithelium indicates a relatively minor contribution of ≈3.5% (of the total radioactivity) as evidenced by an indomethacin-sensitive polar peak by HPLC analysis (data not shown). This peak is not significantly affected by SnCl₂ treatment. Based on this finding and on the evidence in the literature, the potential masking of 12-HETE’s and 12-HETE’s effects, through prostanoid and/or thromboxane synthesis, is unlikely.

These findings are novel in providing selective pharmacologic evidence for a role of the cytochrome P450 enzyme system, specifically 12-HETE and 12-HETE, production in the corneal inflammatory response to closed eye—hydrogel contact lens wear. The inhibition of their synthesis by SnCl₂ was selective without evidence of corneal toxicity. This could provide a basis for the treatment of the adverse effects of contact lens wear, especially in the closed eye.

Key Words: closed eye, contact lens, inflammation, arachidonic acid, cyclooxygenase P450

References


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