Effect of OPC-12759 on EGF receptor activation, p44/p42 MAPK activity, and secretion in conjunctival goblet cells

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Abstract

The purpose of the study was to determine if OPC-12759 stimulates secretion from conjunctival goblet cells in culture and if it activates the EGF receptor (EGFR) and p44/p42 mitogen-activated protein kinase (MAPK) to cause mucin secretion. Conjunctival goblet cells were cultured from pieces of male rat conjunctiva. OPC-12759 was added at increasing concentrations and for varying times to the cultured cells. The cholinergic agonist carbachol was used as a positive control. In selected experiments an inhibitor of the EGFR, AG1478, or an inhibitor of the kinase that activates MAPK, U0126, were added before OPC-12759. Goblet cell secretion of high molecular weight glycoconjugates was measured by an enzyme-linked lectin assay using the lectin UEA-1. Activation of the EGFR and MAPK were determined with Western blotting analysis using antibodies specific to the phosphorylated and the total amounts of these proteins. We found that OPC-12759 induced goblet cell secretion in a time- and concentration-dependent manner. Inhibition of the EGFR with AG1478 blocked secretion stimulated by OPC-12759. Inhibition of MAPK with U0126 also blocked secretion stimulated by OPC-12759. OPC-12759 increased the phosphorylation of the EGFR and MAPK in a time-dependent manner. We concluded that OPC-12759 stimulates secretion from cultured conjunctival goblet cells by activating the EGFR, which then induces MAPK activity.

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1. Introduction

Conjunctival goblet cells synthesize, store, and secrete a complex of high-molecular-weight glycoproteins that includes mucins. The large gel-forming mucin, MUC5AC is the only mucin so far identified in the conjunctival goblet cells (Gipson, 2004). Once mucin is secreted by the goblet cells, it contributes to the formation of the inner mucous layer of the tear film and provides a physical and chemical barrier to protect the ocular surface from dryness or other deleterious environmental and noxious agents (Dartt, 2002).

Instability of the tear film with resultant drying, and inflammation of the conjunctiva and cornea culminates in a spectrum of ocular surface diseases known as dry eye syndromes (DES) (Lemp, 1995; Tiffany, 2003). In disorders of aqueous tear deficiency, aqueous and mucin tear disturbances may occur simultaneously (Lemp, 1995). This leads to decreases in the number of goblet cells as determined in conjunctival biopsy specimens from human patients affected by mucin-deficient ocular disease and dry eye (Danjo et al., 1998; Argueso et al., 2002). In tears, dehydration of the ocular surface may cause qualitative changes in the secreted mucous glycoproteins. Thus changes in goblet cell mucin production appear to be an important cause of DES.

Treatments for managing DES present a formidable problem. Conventional approaches for treatment of DES are palliative and consist of the use of artificial tears or punctal...
occlusion. New treatment options for DES include the use of anti-inflammatory agents such as cyclosporine, or autologous serum (Tsubota et al., 1999; Kunert et al., 2002; Matsumoto et al., 2004). Autologous serum application has been shown to be effective in the treatment of severe dry eye states associated with ocular pemphigoid, Stevens–Johnson syndrome, and chronic graft-versus-host disease, and in persistent epithelial defects (Tsubota and Higuchi, 2000; Poon et al., 2001; Nakamura et al., 2006). However, there are no effective treatments for the majority of individuals with DES.

OPC-12759, 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolino-4-yl]-propionic acid known as Rebamipide (Mucosta®) is used as an antigastric ulcer drug (Arakawa et al., 2005). Its mechanisms of actions are different from anti-secretory drugs. OPC-12759 stimulates the synthesis of prostaglandin E2 to increase gastric mucus glycoprotein secretion (Arakawa et al., 2005), stimulates migration and proliferation of wounded epithelial cell monolayers (Watanabe et al., 1998, 2005), increases expression of epidermal growth factor (EGF) and its receptor, and scavenges active oxygen radicals (Yoshikawa et al., 1993; Naito et al., 1995, 1998; Kurokawa et al., 1998; Watanabe et al., 1998; Sakurai et al., 2005). All these actions contribute to healing of gastric ulcers.

Recent studies in several experimental animal models have demonstrated that topical application of OPC-12759 improved the ocular surface by increasing the amount of mucin-like substances in the cornea and conjunctiva, and by attenuating the UVB-induced damage to the cornea (Fujisawa et al., 1999; Urashima et al., 1999, 2001, 2002, 2003, 2004; Tanito et al., 2003). OPC-12759 improved rose bengal staining scores in UVB-induced damage to the cornea (Fujisawa et al., 1999; Urashima et al., 2001). In addition, OPC-12759 increased the number of periodic Schiff’s reagent-positive goblet cells in the conjunctiva and reduced rose bengal staining scores in rabbits after ocular surface dryness induced by air blast (Fujisawa et al., 1999; Urashima et al., 1999). This drug also induced the proliferation of rat conjunctival goblet cells in culture (Rios et al., 2006).

As OPC-12759 has been shown to stimulate gastric mucus secretion, elevate the levels of mucin-like substances in the ocular surface in vivo, and increase the number conjunctival goblet cells in vitro, we investigated whether OPC-12759 stimulates conjunctival goblet cell mucin secretion in vitro. In addition, we determined whether OPC-12759 utilizes the epidermal growth factor receptor (EGFR) and p44/p42 mitogen-activated protein kinase (MAPK) to stimulate goblet cell secretion.

2. Materials

RPMI-1640 culture medium, L-glutamine, penicillin-streptomycin, Hanks’ balanced salt solution, and trypsin-EDTA solution were obtained from BioWhittaker (Walkersville, MD). Fetal bovine serum (FBS) was purchased from HyClone Laboratories (Logan, UT). Falcon tissue culture plastic wear was obtained from Becton Dickson Labware (BD, Franklin lakes, NJ). The MAPK inhibitor, U0126 and the epidermal growth factor receptor inhibitor, AG1478 were obtained from EMD Biosciences (San Diego, CA). Monoclonal antibodies that detect phosphorylated (active) p42/p44 MAPK, and total p42 MAPK were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The polyclonal antibodies against EGFR and its phosphorylated form (p-EGFR) were purchased from Biorad Laboratories (Camarillo, CA). Alkaline phosphatase-conjugated streptavidin, p-nitrophenyl phosphate, disodium salt (ImmunoPure® NPNN), and SuperSignal® Substrate Western Blotting, were purchased from Pierce Biotechnology (Rockford, IL). Protein assay dye reagent was from BioRad Laboratories (Hercules, CA). The antibody against the mucin MUC5AC was purchased from NeoMarkers (Fremont, CA) and the antibody against cytokeratin-7 was purchased from ICN (Aurora, OH). Biotinylated-conjugated lectin Ulex europaeus agglutinin I (UEA-I), carbachol, bovine submaxillary mucin, and other chemicals were obtained from Sigma (St. Louis, MO). OPC-12759 was provided by Otsuka Pharmaceutical (Tokyo, Japan).

2.1. Animals

Male Sprague–Dawley rats were purchased from Taconic (Germantown, NY). The rats were anesthetized for 1 min in CO2 and killed by decapitation. All experiments were approved by the Schepens Eye Research Institute Animal Care and Use Committee.

2.2. Culture of conjunctival goblet cell

Goblet cells were grown in organ culture as described previously (Shatos et al., 2001). In brief, the nictitating membranes and fornix were removed from rat conjunctiva, minced, and placed in culture with RPMI 1640 supplemented with 10% FBS and 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified 5% CO2 incubator. After nodules of cells were observed, the tissue plug was removed and goblet cells derived from the explants were permitted to grow. Goblet cells, identified by the presence of numerous secretory granules were further isolated from epithelial cells by scraping away contaminating nongoblet cells that grow with a rubber policeman (Shatos et al., 2001). The remaining cells were trypsinized and seeded at approximately 10,000 cells per well in a 24-well Primaria® cell culture plate (Becton Dickinson Labware). Cells were allowed to grow to confluence before use in experiments. The cells have been identified as goblet cells as they were positive for the following goblet cell markers: the lectin UEA-1, antibody to cytokeratin-7, or antibody to MUC5AC (Shatos et al., 2001; Rios et al., 2006).

2.3. Measurement of glycoconjugate secretion

Goblet cell secretion was measured by an enzyme-linked lectin assay (ELLA). Cells were serum-depleted in serum-free RPMI supplemented with 0.5% bovine serum albumin (BSA) as a protein source for 2 h, and then incubated with or without increasing concentrations of OPC-12759 in
serum-free RPMI supplemented with 0.5% BSA for 0–4 h. The cholinergic agonist carbachol at $10^{-4}$ M was used as the positive control for all experiments. In separate experiments, cells were preincubated with or without increasing concentrations ($10^{-8}$ to $10^{-6}$ M) of the inhibitors AG1478 or U0126 for 1 h respectively before OPC-12759 or carbachol addition.

After incubation, the media was removed and analyzed for amount of lectin-detectable glycoconjugate as previously described (Rios et al., 1999, 2007). These glycoconjugates include mucins and indicate goblet cell secretion. In brief, the collected conditioned cultured medium was centrifuged for 2 min at 14,000 rpm in 4°C. Aliquots of 230–250 μl of the samples and 100 μl of the mucin standard, including respective controls were loaded on Nunc microplates and dried overnight at 55°C. The ELLA was performed according a protocol described by Pierce, Inc. Biotinylated UEA-I lectin and alkaline phosphatase-labeled streptavidin were used. Bovine sub-maxillary mucin was used as a standard. The goblet cells remaining in the culture wells were scraped in Tris-buffer pH 7.5, collected, and sonicated. The amount of protein was determined by the Bradford assay. For each condition glycoconjugate secretion was normalized to total amount of protein in the cell homogenate. Glycoconjugate secretion was expressed as fold-increase over basal.

2.4. Western blot analysis to measure pEGFR and pMAPK

Cultured goblet cells were grown in 24-well plates. Agonists and antagonists were added for specified times as described for secretion and incubation terminated by addition of ice-cold PBS. Cells were solubilized in RIPA buffer (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 1 mM EDTA, 100 μg/ml phenylmethylsulfonyl fluoride, 30 μg/ml aprotinin, 1 mM Na3VO4). Proteins in homogenate were separated by SDS-PAGE and transferred to nitrocellulose membranes.

The membrane was analyzed for p44/p42 mitogen activated protein kinase (MAPK) activity using antibodies to phosphorylated (active) and total MAPK. The samples were also analyzed for EGFR using an antibody to phosphorylated (active) EGFR and total EGFR. Bands were detected by chemiluminescence and analyzed by NIH Image J (available at http://rsb.info.nih.gov/ij/). The amount of phosphorylated EGFR or MAPK was normalized to the total amount of EGFR or MAPK respectively. Basal values at zero time were set to 1 and results expressed as fold-increase over basal.

2.5. Statistical analysis

Data were expressed as mean ± SEM. Data were analyzed by Student’s t-test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of OPC-12759 on goblet cell secretion

Goblet cells in culture were periodically subjected to identification as goblet cells using UEA-I and antibodies against MUC5AC and cytokeratin 7 (Rios et al., 2006).

To determine whether OPC-12759 stimulates goblet cell mucin secretion, cultured goblet cells were incubated for 0–4 h in the presence of OPC-12759 ($10^{-7}$ M) or the cholinergic agonist carbachol ($10^{-4}$ M, the positive control). Basal secretion values were 0.6 ± 0.4, 0.4 ± 0.2, 0.5 ± 0.3, and 1.1 ± 0.3 at 0.25, 0.5, 1, and 4 h and did not change over the 4-h period (Fig. 1). OPC-12759 significantly stimulated goblet cell secretion at 1 h to 2.4 ± 0.4 fold above basal. OPC-12759-stimulated secretion continued to increase in a time-dependent manner to 4.0 ± 0.9 fold above basal at 4 h (Fig. 1) which was significantly increased above basal.

In the same experiments carbachol-stimulated secretion was significantly increased to 6.0 ± 2.2 fold above basal at 1 h before declining to 3.2 ± 1.0 fold at 4 h above basal. Thus OPC-12759 stimulated goblet cell secretion.

To determine the effect of increasing concentrations of OPC-12759 on goblet cell secretion, cultured goblet cells were incubated for 4 h in the presence of OPC-12759 from $10^{-12}$ to $10^{-5}$ M. As a positive control carbachol at $10^{-4}$ M was incubated for 1 h, as this time point gave a significant increase as shown in Fig. 1. OPC-12759 stimulated secretion in a concentration-dependent manner with a maximum secretion at $10^{-8}$ M. (Fig. 2). Goblet cell secretion was significantly increased by OPC-12759 at $10^{-8}$, $10^{-7}$ and $10^{-6}$ M of 3.6 ± 0.9, 3.6 ± 0.8, and 3.9 ± 1.1 fold over basal, respectively. In the same experiments carbachol stimulated secretion 4.6 ± 1.0 fold above basal (Fig. 2).

Fig. 1. Effect of time on OPC-12759-dependent goblet cell glycoconjugate secretion. Conjunctival goblet cells were treated with (closed triangles) or without (closed circles) $10^{-7}$ M OPC-12759 for 0–240 min at 37°C. The cholinergic agonist carbachol (open circles) at $10^{-4}$ M was the positive control. Data are mean ± SEM of 4 independent experiments. * indicates significant difference from basal at each time point.
3.2. Effect of OPC-12759 on EGFR activation

A previous study reported that OPC-12759 increased the expression of EGF and EGFR in normal and ulcerated gastric mucosa in rats (Tarnawski et al., 1998). To determine whether OPC-12759 activates the EGFR in rat conjunctival goblet cells, cultured goblet cells were stimulated by OPC-12759 (10^−7 M) or carbachol (10^−4 M) for 0–15 min and the amount of activated (phosphorylated) EGFR was determined by Western blot analysis. Basal activity of MAPK was 1.2 ± 0.3, 1.7 ± 0.2, 1.3 ± 0.9, and 2.3 ± 0.9 fold above time zero at 1, 5, 10, and 15 min, respectively and did not differ significantly from time zero (data not shown). OPC-12759 significantly activated EGFR in a time dependent manner with an increase of 1.6 ± 0.3 and 3.4 ± 1.0 fold at 5 and 10 min before declining to 1.7 ± 0.3 fold at 15 min above zero time (Fig. 3). The positive control carbachol also stimulated EGFR activity in a time dependent manner with a maximum 2.1 ± 0.5 fold at 5 min above zero time.

3.3. Effect of inhibition of the EGFR on OPC-12759-stimulated secretion

To determine whether OPC-12759 induced glycoconjugate secretion via activation of the EGFR, cultured goblet cells were pre-incubated with the EGFR inhibitor AG1478 (10^−8 and 10^−7 M). We previously showed that 10^−7 M AG1478 inhibited activation of the EGFR stimulated by EGF (Horikawa et al., 2003). Cells were then stimulated with OPC-12759 (10^−7 M) for 4 h. Secretion in the presence of AG1478 10^−8 and 10^−7 M alone was 1.1 ± 0.3 and 1.35 ± 0.2 fold above basal (data not shown). Neither of these values was significantly increased above basal. OPC-12759 significantly stimulated secretion 2.4 ± 0.5 fold compared to the basal level. This secretion was significantly inhibited by AG1478 by 54 ± 17 and 59 ± 16% at 10^−8 and 10^−7, respectively (Fig. 4).

3.4. Effect of OPC-12759 on MAPK activity

As shown in Fig. 3, OPC-12759 activated the EGFR that led to mucin secretion. The EGFR can be phosphorylated at different tyrosine sites, leading to subsequent activation of different pathways, including the MAPK pathway. To determine if activation of the EGFR by OPC-12759 stimulates MAPK, cultured goblet cells were stimulated by OPC-12759 (10^−7 M) for 0–15 min or carbachol (10^−7 M) for 5 min and MAPK activity was measured. Basal activity of MAPK was 1.3 ± 0.2, 1.1 ± 0.2, 1.1 ± 0.5, and 1.3 ± 0.2 fold above time zero, at 1, 5, 10 and 15 min respectively (data not shown). Only the 1 min basal value differed significantly from time zero. OPC-12759 increased MAPK activity 1.7 ± 0.3 fold at 1 min, 1.5 ± 0.3 fold at 5 min, 2.4 ± 0.6 fold at 10 min, and 1.6 ± 0.2 fold at 15 min (Fig. 5). The effect of OPC-12759 was statistically significant over basal at 1 min. The positive control carbachol stimulated MAPK activity 2.5 ± 0.4 fold at 5 min.

Fig. 2. Effect of concentration on OPC-12759-dependent goblet cell glycoconjugate secretion. Cultured conjunctival goblet cells were incubated for 4 h at 37 °C, with increasing concentrations of OPC-12759 (closed circles). The cholinergic agonist carbachol (Cch) at 10^−5 M and incubated for 1 h was the positive control (open circles). Data are mean ± SEM of 5–8 independent experiments. * indicates significant difference from no addition.

Fig. 3. Effect of OPC-12759 on EGFR activation. Conjunctival goblet cells were treated with no addition (basal) and OPC-12759 (10^−7 M) for 0–15 min or carbachol (10^−7 M) (the positive control) for 5 min. The cultured goblet cells were then homogenized in RIPA buffer. The proteins were analyzed by Western blotting with an anti-phosphoEGFR antibody. To normalize the amount of phosphorylation in each sample, Western blot analysis was also performed with the anti-EGFR antibody. A representative Western blot is shown in (A). The experiments were analyzed and the amount of phosphorylated EGFR/total EGFR was determined (B). Open circles, 10^−4 M Cch; closed circles, 10^−7 M OPC-12759. Data are mean ± SEM of 5–6 independent experiments. * indicates significant difference from zero time.
3.5. Effect of inhibition of MAPK on OPC-12759-stimulated secretion

As OPC-12759 stimulated MAPK activity, we determined if MAPK played a role in OPC-12759-induced glycoconjugate secretion. Cultured goblet cells were pre-incubated with the MAPK inhibitor U0126 (10⁻⁷ and 10⁻⁶ M). We previously found that these concentrations of U0126 inhibited carbachol-induced mucin secretion from conjunctival goblet cells (Kanno et al., 2003). Cells were then stimulated with OPC-12759 (10⁻⁷ M) for 4 h. Secretion in the presence of U0126 10⁻⁷ and 10⁻⁶ M was 1.3 ± 0.2 and 1.1 ± 0.3, respectively (data not shown). Neither of these values were significantly different from the basal level of secretion. OPC-12759 significantly stimulated secretion by 2.2 ± 0.2 fold. U0126 at 10⁻⁷ and 10⁻⁶ M significantly inhibited OPC-12759 induced secretion by 80 ± 17 and 95 ± 5%, respectively (Fig. 6).

4. Discussion

The present study demonstrated that OPC-12759 induced a time- and concentration-dependent secretion of high molecular weight glycoproteins (including mucin) from cultured rat conjunctival goblet cells. Similar to the cholinergic agonist carbachol, OPC-12759 transactivated the EGFR, and activated p44/p42 MAPK to lead to mucin secretion (Kanno et al., 2003). Compared to carbachol (10⁻⁴ M), OPC-12759 caused about the same magnitude of secretion, but this maximum was reached at a lower concentration of OPC-12759 and extended for a longer incubation time than with carbachol. Thus OPC-12759 activates the same signaling pathway as cholinergic agonists to stimulate goblet cell secretion. These results suggest that p44/p42 MAPK activation is a major pathway for stimulating conjunctival goblet cell secretion.

The concentration of OPC-12759 that stimulated goblet cell secretion in the present study was several orders of magnitude higher than that which was effective to induce proliferation of cultured conjunctival goblet cells. We previously reported that OPC-12759 at 10⁻¹¹ M induced the proliferation of cultured goblet cells were pre-incubated with the MAPK inhibitor U0126 (10⁻⁷ and 10⁻⁶ M). We previously found that these concentrations of U0126 inhibited carbachol-induced mucin secretion from conjunctival goblet cells (Kanno et al., 2003). Cells were then stimulated with OPC-12759 (10⁻⁷ M) for 4 h. Secretion in the presence of U0126 10⁻⁷ and 10⁻⁶ M was 1.3 ± 0.2 and 1.1 ± 0.3, respectively (data not shown). Neither of these values were significantly different from the basal level of secretion. OPC-12759 significantly stimulated secretion by 2.2 ± 0.2 fold. U0126 at 10⁻⁷ and 10⁻⁶ M significantly inhibited OPC-12759 induced secretion by 80 ± 17 and 95 ± 5%, respectively (Fig. 6).

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conjunctival goblet cells when used for 24 h or at $10^{-10}$ M when renewed every day for 3 days (Rios et al., 2006). These differences in concentrations of OPC-12759 between proliferation and secretion are intriguing. In both studies, cells were grown from the tissue explants. For proliferation studies, cells were plated at low cell density. In contrast, for secretion studies, cells were grown to confluence. It is possible that differences in cell density, exposure time (2 versus 24 h), or the presence of tight junctions and a resultant establishment of polarity in confluent cells account for this difference.

In N-acetylcysteine-treated rabbit eyes, OPC-12759 increased the level of mucin-like substances on the cornea and conjunctiva that correlated with an improvement in rose bengal scores of N-acetylcysteine-treated eyes (Urashima et al., 2001). As whole conjunctiva were used and the type of mucin not identified, the effector cell type (stratified squamous or goblet) could not be identified. Thus, OPC-12759 could be increasing the synthesis of mucin, increasing mucin secretion, or stimulating epithelial cell proliferation in the conjunctiva. Our results imply that OPC-12759 affects goblet cell mucin production and plays at least two roles in the conjunctiva by stimulating goblet cell mucin secretion and by inducing goblet cell proliferation in vitro. We have not determined if OPC-12759 increases goblet cell mucin synthesis.

Studies have shown that epidermal growth factor (EGF) and the cholinergic agonist carbachol both phosphorylate the EGFR and activate p44/p42 MAPK in conjunctival pieces and in cultured goblet cells (Horikawa et al., 2003; Kanno et al., 2003). Activation of the EGFR then serves as a scaffold for the Shc-Grb2 complex, leading to the activation of Ras and Raf and the downstream activation of p44/p42 MAPK (Luttrell et al., 1999). Increases in extracellular Ca$^{2+}$, as well as activation of PKC, were also shown to activate p44/p42 MAPK in conjunctival goblet cells (Horikawa et al., 2003; Kanno et al., 2003). p44/p42 MAPK has been implicated in a variety of cellular processes including “long term” events such as cell proliferation and differentiation and gene expression (Sugden and Clerk, 1997) and “short term” events such as secretion (Horikawa et al., 2003; Kanno et al., 2003). In rat conjunctival goblet cells, OPC-12759 activated p44/p42 MAPK is responsible for both a “long term” event (goblet cell proliferation) and a “short term” event (mucin secretion).

In gastric mucosa, OPC-12759 significantly accelerated ulcer healing, caused a significant increase in EGF and EGFR expression in normal gastric mucosa, and increased expression of EGF and EGFR in regenerating glands of the ulcer scar (Tarnawska et al., 1998). In addition, OPC-12759 has been shown to activate cyclooxygenase-2 (COX-2) (Sun et al., 2000; Buchanan et al., 2003; Murata et al., 2005). Buchanan et al. have shown that COX-2-derived prostanoids (E2 (PGE2) signaling occurs by transactivation of the EGFR by the PGE2 receptor, EP4 (Sun et al., 2000; Buchanan et al., 2003; Murata et al., 2005). The biological effects of PGE2 are mediated via four different G protein-coupled receptor subtypes (EP1–4). EP1 activation results in Ca$^{2+}$ mobilization. Activation of both EP2 and EP4 results in increased cAMP levels while EP3 receptor leads to decreased cAMP levels (Ohnishi et al., 2001). It has been shown that OPC-12759 promotes gastric PGE2 production and mucus secretion and these effects seen to be mediated by EP4 (Suetsugu et al., 2000; Arakawa et al., 2005). So far, all four different PGE2 receptor subtypes have been identified in the cornea and conjunctiva (Schlotzer-Schrehardt et al., 2002). Diverse inflammatory mediators include IL-1β and IL-6 through EP3 and 4 receptors (Yoon et al., 1999; Gray et al., 2004; Kook Kim et al., 2006) and prostaglandins (Kook Kim et al., 2006) using EP4 receptors have been shown to increase mucin secretion in human airway and nasal epithelial cells. Thus, it is tempting to speculate that OPC-12759 induces conjunctival goblet cell secretion through similar signaling pathways. Whether or not OPC-12759 mediates mucin secretion by the synthesis of prostaglandins in the conjunctiva needs to be addressed.

However, other possible mechanisms may exist by which OPC-12759 directly modulates mucin production in cultured goblet cells independent of prostaglandins. In rat pancreatic acini, OPC-12759 was shown to bind to the cholecystokinin receptor. There is evidence that the PGE2 inhibitor indomethacin does not alter OPC-12759-induced increase in soluble mucus in gastric mucosa (Ishihara et al., 1992; Murakami et al., 1997). Instead OPC-12759 appears to directly inhibit the production of superoxide (O2$^-$) and inhibits proinflammatory cytokines in gastric mucosa (such as TNFα and IL-8) (Kim et al., 2000; Choi et al., 2002). In addition, a recent study showed that OPC-12759 inhibited smoke-induced TNFα release, TNFα production, neutrophil recruitment, and activation of the EGFR in airway epithelium. In that study, activation of the EGFR caused MUC5AC mucin synthesis which was inhibited by OPC-12759 (Lee et al., 2006). OPC-12759 also attenuated the activity of neutrophils and the production of inflammatory cytokines stimulated by NSAIDs and/or H. pylori in gastric mucosa (Fujioka et al., 2003). This evidence suggests that OPC-12759 has multiple effects that might occur in the conjunctiva to stimulate secretion by the cultured goblet cells.

In summary, the present results indicate that OPC-12759 induces mucin-like secretion from rat conjunctival goblet cells in culture by activation of the EGFR and the MAPK pathway. OPC-12759 may thus be a useful therapy in DES by stimulating the secretion of mucin or by increasing the amount of goblet cells and thereby healing or protecting the ocular surface. Further investigation focusing on possible mechanisms by which OPC-12759 regulates conjunctival mucin production are needed in animal models of dry eye syndromes to further define the effects that this drug may have in the conjunctival mucus system in normal and dry eye states.

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References

to the new mechanisms and new applications. Dig. Dis. Sci. 50 (Suppl. 1), S3–S11.


