Suppression by an RAR-γ Agonist of Collagen Degradation Mediated by Corneal Fibroblasts

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PURPOSE. To examine the role of retinoic acid receptor (RAR) isoforms in interleukin-1β (IL-1β)–induced collagen degradation by corneal fibroblasts.

METHODS. Primary rabbit corneal fibroblasts embedded in a three-dimensional collagen gel were incubated with or without all-trans retinoic acid (ATRA), the RAR-α agonist Am580, the RAR-β agonist AC55649, or the RAR-γ agonist R667. Collagen degradation was determined by measurement of hydroxyproline produced in acid hydrolysates of culture supernatants. Matrix metalloproteinase (MMP) expression was evaluated by immunoblot analysis and gelatin zymography. The phosphorylation of mitogen-activated protein kinases (MAPKs) and the endogenous nuclear factor (NF)-κB inhibitor IκB-α was examined by immunoblot analysis. Cell proliferation was measured with a bromodeoxyuridine incorporation assay, and cell viability was determined by measurement of the release of lactate dehydrogenase.

RESULTS. Interleukin-1β–induced collagen degradation by corneal fibroblasts was inhibited by ATRA, Am580, and R667 in a concentration-dependent manner but was unaffected by AC55649, with the inhibitory effects of ATRA and R667 being markedly greater than that of Am580. The IL-1β–induced production of MMP-1, MMP-2, MMP-3, and MMP-9 by corneal fibroblasts was also inhibited by R667 in a concentration-dependent manner. R667 inhibited the IL-1β–induced phosphorylation of IκB-α but not that of MAPKs. R667 had no effect on the proliferation or viability of corneal fibroblasts.

CONCLUSIONS. The RAR-γ agonist R667 suppressed MMP production and thereby inhibited collagen degradation by corneal fibroblasts exposed to the proinflammatory cytokine IL-1β. These effects of R667 may be mediated by the NF-κB signaling pathway.

Keywords: cornea fibroblast, collagen, retinoid

CORNEAL transparency depends on the structure of the corneal stroma, which consists of keratocytes and an extracellular matrix composed mostly of type I collagen.1,2 The remodeling of collagen fibrils in the corneal stroma is mediated by corneal fibroblasts (activated keratocytes), and the destruction of these fibrils contributes to corneal disease. The production of matrix metalloproteinases (MMPs) by corneal fibroblasts is responsible in part for the remodeling of collagen fibrils in the corneal stroma.3,4 The production of tissue inhibitors of MMPs is also a determinant of collagen remodeling associated with corneal disease.5,6 Proinflammatory cytokines such as interleukin (IL)–1β and IL-6 also contribute to the pathogenesis of inflammatory corneal disease.7

Vitamin A (retinol) plays important roles in various biological processes including cell differentiation, inflammation, and development. Retinol is converted by the body to retinoic acids such as all-trans retinoic acid (ATRA), which exert their biological actions by binding to specific retinoic acid receptors (RARs) including RAR-α, RAR-β, and RAR-γ.8,9 Retinol also contributes to the maintenance of homeostasis at the ocular surface, with retinol deficiency giving rise to various ocular diseases including conjunctival and corneal xerosis, keratomalacia, retinopathy, and corneal ulceration.10,11 In addition, the expression of IL-1 is upregulated in the cornea of vitamin A-deficient rats.12 The various RAR isoforms and their corresponding selective agonists possess distinct biological activities, and RARs are expressed in the eye.13

Collagen degradation by corneal fibroblasts in three-dimensional culture has been studied as a means to characterize the remodeling of collagen fibrils in the corneal stroma.14-16 We have recently shown that ATRA inhibits IL-1β–induced collagen degradation by corneal fibroblasts in a manner likely dependent on attenuation of nuclear factor (NF)-κB signaling and MMP production.17 We now examined the effects RAR agonists including those selective for RAR-α, RAR-β, and RAR-γ on IL-1β–induced collagen degradation by rabbit corneal fibroblasts as well as on the release of MMPs by, and the activity of signaling pathways in, these cells.

METHODS

Materials

Eagle’s minimum essential medium (MEM), Dulbecco’s phosphate-buffered saline (PBS), antibiotic–antimycotic mixture, dispase, and trypsin-EDTA were obtained from Invitrogen-Gibco (Grand Island, NY, USA); fetal bovine serum (FBS) was from JRH Biosciences (Lenexa, KS, USA); native porcine type I collagen from JRH Biosciences (Lenexa, KS, USA); type I collagen from JRH Biosciences (Lenexa, KS, USA).
collagen (acid solubilized), 5× Dulbecco’s modified Eagle’s medium, and collagen reconstitution buffer were from Nitta Gelatin (Osaka, Japan); and bovine plasminogen, collagenase, ATRA, and a protease inhibitor cocktail were from Sigma-Aldrich Corp. (St. Louis, MO, USA). Recombinant human IL-1β was obtained from R&D Systems (Minneapolis, MN, USA), R667 (palovarotene) was from NARD Institute (Hyogo, Japan), and Am580, AC55649, and MM11253 were from Tocris (Minneapolis, MN, USA). Mouse monoclonal antibodies to rabbit MMP-1 and MMP-3 were obtained from Daiichi Fine Chemicals (Toyama, Japan), and those to β-actin were from Sigma-Aldrich Corp. Antibodies to phosphorylated ERK1/2 (extracellular signal–regulated kinase 1/2) (Thr202, Tyr204), to ERK1/2, to phosphorylated p38 MAPK (mitogen-activated protein kinase) (Thr180, Tyr185), to p38 MAPK, to phosphorylated JNK (c-Jun NH2-terminal kinase) (Thr183, Tyr185), to JNK, to phosphorylated IκB-α, and to IκB-α were obtained from Cell Signaling (Beverly, MA, USA). Rabbit polyclonal antibodies to RAR-α, RAR-β, and RAR-γ were from Abcam (Cambridge, UK). An enhanced chemiluminescence (ECL) kit as well as horseradish peroxidase–conjugated goat secondary antibodies were from GE Healthcare (Piscataway, NJ, USA). Culture plates (24- and 96-well) and 60-mm cell culture dishes were obtained from Corning (Corning, NY, USA). Gelatin and Coomassie brilliant blue were from Bio-Rad (Hercules, CA, USA). A cell proliferation assay kit was from Roche (Basel, Switzerland), and a cytotoxicity assay kit (CytoTox 96 Non-Radioactive) was from Promega (Madison, WI, USA). All media and reagents used for cell culture were endotoxin minimized.

**Isolation of Rabbit Corneal Fibroblasts**

Corneal fibroblasts were isolated from male Japanese albino rabbits (body weight, 2.0–2.5 kg; Biotec, Saga, Japan) and were maintained as described previously. This study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and was approved by the Animal Experimental Committee of Yamaguchi University School of Medicine. In brief, eyes were enucleated and washed with Dulbecco’s PBS containing antibiotic–antimycotic mixture. The endothelial layer of the excised cornea was removed mechanically, the remaining corneal tissue was incubated with dispase (2 mg/mL, in MEM) for 1 hour at 37°C, the epithelial sheet was removed, and the remaining tissue was then digested with collagenase (2 mg/mL, in MEM) at 37°C until a single-cell suspension of corneal fibroblasts was obtained. The isolated fibroblasts were cultured under a humidified atmosphere of 5% CO2 at 37°C in 60-mm culture dishes containing MEM supplemented with 10% FBS. Proliferating cells at the subconfluent stage were used for experiments after four to six passages in monolayer culture.

**Three-Dimensional Culture**

Collagen gels were prepared as described. In brief, corneal fibroblasts were harvested by exposure to trypsin-EDTA followed by centrifugation at 150g for 5 minutes and resuspension in serum-free MEM. Acid-solubilized type I collagen (3 mg/mL, 5× Dulbecco’s modified Eagle’s medium, collagen reconstitution buffer (0.05 M NaOH, 0.26 M Na2CO3, 0.2 M HEPES [pH 7.3]), and corneal fibroblast suspension (2.2 × 10^5 cells/mL, in MEM) were mixed on ice in a volume ratio of 7:2:1:1. Portions (0.5 mL) of the resultant mixture were added to culture wells of a 24-well culture plate and allowed to solidify in an incubator at 37°C, after which 0.5 mL serum-free MEM containing test reagents and plasminogen (60 μg/mL) was overlaid and the cultures were returned to the incubator for 36 hours.

**Assay of Collagenolytic Activity**

Collagen degradation was measured as previously described. In brief, the supernatants from collagen gel incubations were collected, and native collagen fibrils with a molecular size of >100 kDa were removed by ultrafiltration. The filtrate was subjected to hydrolysis with 6 M HCl for 24 hours at 110°C, and the amount of hydroxyproline in the hydrolysate was determined by measurement of absorbance at 558 nm with a spectrophotometer.

**Immunoblot Analysis**

Immunoblot analysis of MMP-1 and MMP-3 was performed as described previously. In brief, culture supernatants from collagen gel incubations were subjected to SDS-polyacrylamide gel electrophoresis on a 10% gel, and the separated proteins were transferred electrophotographically to a nitrocellulose membrane. Nonspecific sites of the membrane were blocked, and it was then incubated with primary antibodies. Immune complexes were detected with the use of horseradish peroxidase–conjugated secondary antibodies and ECL reagents. Immunoblot analysis of total or phosphorylated forms of ERK, p38 MAPK, JNK, and IκB-α, as well as of RAR-α, RAR-β, and RAR-γ, was also performed as described previously. In brief, cells were lysed in a solution containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 5 mM NaF, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM Na2VO4, and 1% protease inhibitor cocktail, and the cell lysates (10 μg protein) were subjected to immunoblot analysis.

**Gelatin Zymography**

Gelatin zymography was performed as described previously. In brief, culture supernatants (8 μL) from collagen gel incubations were mixed with 4 μL nonreducing SDS sample buffer (125 mM Tris-HCl [pH 6.8], 20% glycerol, 2% SDS, 0.002% bromophenol blue), and 5 μL of the resulting mixture was subjected to SDS-polyacrylamide gel electrophoresis in the dark at 4°C on a 10% gel containing 0.1% gelatin. The gel was then washed with 2.5% Triton X-100 for 1 hour before incubation for 18 hours at 37°C in a reaction mixture containing 50 mM Tris-HCl (pH 7.5), 5 mM CaCl2, and 1% Triton X-100. The gel was finally stained with Coomassie brilliant blue.

**Cell Proliferation Assay**

Cells (2 × 10^4 per well) seeded in a 96-well plate were incubated in MEM with or without 10% FBS or R667 for 24 hours, with bromodeoxyuridine (BrdU) being added to the culture medium for the final 2 hours. The incorporation of BrdU into the cells was then assessed with a colorimetric cell proliferation assay kit and measurement of absorbance assessed at 570 nm with a microplate reader.

**Cytotoxicity Assay**

Cells (2 × 10^4 per well) seeded in a 96-well plate were incubated in serum-free MEM with or without R667 for 36 hours, after which the activity of lactate dehydrogenase (LDH) released into the culture medium was determined with a colorimetric assay kit and measurement of absorbance at 490 nm with a microplate reader.
Role of RAR-γ Agonist on Collagen Degradation

Effects of RAR Agonists on IL-1β–Induced Collagen Degradation by Corneal Fibroblasts

We first examined the effect of ATRA on IL-1β–induced collagen degradation in three-dimensional cultures of rabbit corneal fibroblasts. The cells were incubated with various concentrations of ATRA (10 nM–1 μM) in the presence of IL-1β (0.1 ng/mL) for 36 hours, after which the culture supernatants were collected for determination of the extent of collagen degradation. Consistent with our previous findings,17 ATRA inhibited IL-1β–induced collagen degradation in a concentration-dependent manner, with the inhibition being significant at ATRA concentrations of ≥10 nM (Fig. 1A). We next examined the effects of RAR agonists on IL-1β–induced collagen degradation by rabbit corneal fibroblasts. The RAR-α agonist Am580 inhibited IL-1β–induced collagen degradation in a concentration-dependent manner, with this effect being statistically significant at Am580 concentrations of ≥0.1 μM and maximal at 1.0 μM (Fig. 1B). In contrast, the RAR-β agonist AC55649 had no effect on IL-1β–induced collagen degradation by corneal fibroblasts (Fig. 1C). The RAR-γ agonist R667 also inhibited IL-1β–induced collagen degradation in a concentration-dependent manner, with this effect being significant at R667 concentrations of ≥0.1 nM and approaching maximal at 10 nM (Fig. 1D). The inhibitory effect of R667 on IL-1β–induced collagen degradation was markedly greater than that of Am580, suggesting that RAR-γ is most active among the RAR isoforms in this regard.

Effects of R667 on MMP Expression

We examined the effects of the RAR-γ agonist R667 on the abundance and activation state of MMPs released from rabbit corneal fibroblasts cultured in collagen gels for 36 hours in the presence of IL-1β (0.1 ng/mL). We have previously shown that the amounts of pro or active forms of MMP-1, MMP-2, MMP-3, and MMP-9 in such culture supernatants are increased by exposure of the cells to IL-1β14 and that these effects are inhibited by ATRA.17 Immunoblot analysis and gelatin zymography revealed that the amount of ProMMP-1, active MMP-1, ProMMP-2, active MMP-2, ProMMP-3, active MMP-3, ProMMP-9, and active MMP-9 in the supernatants from cultured cells were increased by IL-1β. Immunoblot analysis also revealed that R667 inhibited the IL-1β–induced increase in the amounts of both the pro and active forms of MMP-1 and MMP-3 in a concentration-dependent manner (Figs. 2A, 2B). Gelatin zymography also revealed that the IL-1β–induced increase in the amounts of both the pro and active forms of MMP-9 and the active form of MMP-2 was inhibited by the RAR-γ agonist in a concentration-dependent manner (Figs. 2A, 2B).

Lack of Effect of R667 on Cell Proliferation and Viability

We examined whether the RAR-γ agonist R667 might affect the proliferation of rabbit corneal fibroblasts by measuring incorporation of BrdU. R667 had no effect on cell proliferation.
FIGURE 2. Inhibitory effects of an RAR-γ agonist on IL-1β-induced MMP-1, MMP-2, MMP-3, and MMP-9 release by rabbit corneal fibroblasts. (A) Cells were cultured in collagen gels for 36 hours in the absence or presence of IL-1β (0.1 ng/mL) and the indicated concentrations of the RAR-γ agonist R667, after which the culture supernatants were subjected to immunoblot analysis with antibodies to MMP-1, to MMP-3, and to β-actin (loading control) as well as to gelatin zymography for analysis of MMP-2 and MMP-9. Bands corresponding to the pro and active forms of the MMPs are indicated. (B) Quantification of data for cells incubated as in (A). The intensity of each band is expressed relative to the corresponding value for cells incubated with IL-1β alone, and data are means ± SD from three independent experiments. *P < 0.05 (Dunnett’s test). Similar results were obtained in three separate experiments.

FIGURE 3. Lack of effect of an RAR-γ agonist on the proliferation or viability of rabbit corneal fibroblasts. (A) Cells were cultured in MEM without addition (negative control) as well as with or without 10% FBS in the absence or presence of 0.01 or 0.1 μM R667 for 24 hours, after which cell proliferation was evaluated by measurement of BrdU incorporation with a colorimetric assay. (B) Cells were cultured in MEM in the absence (negative control) or presence of 0.01 or 0.1 μM R667 for 36 hours, after which cell viability was assessed by measurement of LDH activity in the culture supernatants with a colorimetric assay. The amount of LDH released from cells by a cell lysis solution was determined as a positive control. All data are means ± SD of quadruplicates from experiments that were repeated three times with similar results. *P < 0.05 versus the negative control (Dunnett’s test).
at concentrations of 0.01 or 0.1 μM in the absence or presence of serum (Fig. 3A). Moreover, it did not exhibit cytotoxicity for corneal fibroblasts at these same concentrations, as revealed by measurement of LDH release from the cells (Fig. 3B).

Effects of R667 on MAPK and Nuclear Factor (NF)-κB Signaling Pathways
We examined the effects of the RAR-c agonist R667 on MAPK and NF-κB signaling pathways activated by IL-1β in corneal fibroblasts, having previously shown that ATRA inhibits the IL-1β-induced phosphorylation of the endogenous NF-κB inhibitor IκB-α in these cells.17 Corneal fibroblasts were incubated with R667 (0.1 μM) for 12 hours before stimulation with IL-1β (0.1 ng/mL) for 30 minutes. Immunoblot analysis showed that R667 inhibited the IL-1β–induced phosphorylation of IκB-α without affecting that of the MAPKs ERK, p38, or JNK in corneal fibroblasts (Fig. 4).

Effect of an RAR-γ Antagonist on the Inhibition of IL-1β–Induced Collagen Degradation by R667
Finally, we examined the expression of RAR isoforms in rabbit corneal fibroblasts as well as the role of RAR-γ in the inhibitory effect of R667 on IL-1β–induced collagen degradation by these cells. Immunoblot analysis showed that RAR-α, RAR-β, and RAR-γ are each expressed in rabbit corneal fibroblasts (Fig. 5A). In addition, the RAR-γ antagonist MM11253 (0.0–0.03 nM) attenuated the inhibitory effect of R667 (0.1 nM) on IL-1β–induced collagen degradation by corneal fibroblasts in a concentration-dependent manner, with this action being significant at MM11253 concentrations of ≥0.03 nM (Fig. 5B).

DISCUSSION
We showed here that ATRA as well as RAR-α and RAR-γ agonists inhibited IL-1β–induced collagen degradation by rabbit corneal fibroblasts in a concentration-dependent manner, with the effects of ATRA and the RAR-γ agonist being markedly greater than that of the RAR-α agonist. The RAR-γ agonist R667 also suppressed the IL-1β–induced release of pro and active forms of MMPs from corneal fibroblasts in a concentration-dependent manner. The proliferation and viability of these cells were not affected by R667. Finally, R667 inhibited activation of the NF-κB signaling pathway by IL-1β. Our observations thus suggest that the RAR-γ agonist R667 inhibited collagen degradation by corneal fibroblasts through suppression of MMP production, and that RAR-γ signaling might therefore attenuate collagen remodeling in corneal disease.

The onset and progression of corneal pathology can result in the disruption of collagen arrangement in the stroma.22,23 Collagenase has thus been implicated in corneal destruction after alkali-induced burn or other injury.24–26 The expression of collagenase (MMP-1) has been found to be increased in association with destructive corneal disease.26 Collagenase inhibitors have therefore been considered as potential drugs for the treatment of corneal diseases.27,28 We now showed that an RAR-γ agonist inhibited IL-1β–induced collagen degradation by rabbit corneal fibroblasts as well as the expression of MMP-1 in these cells, suggesting that this inhibition of collagen degradation

![Figure 4](http://arvojournals.org/)

**Figure 4.** Effects of an RAR-γ agonist on IL-1β–induced MAPK and IκB-α phosphorylation in rabbit corneal fibroblasts. Cells were incubated in MEM with or without the RAR-γ agonist R667 (0.1 μM) for 12 hours and then in the additional absence or presence of IL-1β (0.1 ng/mL) for 30 minutes. Cell lysates were then subjected to immunoblot analysis with antibodies to phosphorylated (p-) or total forms of ERK (A), JNK (B), p38 MAPK (C), or IκB-α (D). The relative ratios of phosphorylated to total forms of MAPKs as well as of phosphorylated IκB-α to β-actin (loading control) were determined by densitometric analysis and are presented as means ± SD for three independent experiments. *P < 0.05 (Dunnett’s test) versus the corresponding value for cells cultured without addition; †P < 0.05 (Dunnett’s test) versus the corresponding value for cells cultured with IL-1β alone.
 Role of RAR-γ Agonist on Collagen Degradation

A

RAR-α RAR-β RAR-γ Actin

B

![Graph showing collagen degradation](Image)

**FIGURE 5.** Expression of RAR isomers in rabbit corneal fibroblasts as well as the effect of an RAR-γ antagonist on the inhibition by R667 of IL-1β-induced collagen degradation mediated by these cells. (A) Cell lysates were subjected to immunoblot analysis with antibodies to RAR-α, RAR-β, RAR-γ, and to β-actin (loading control). Similar results were obtained in three separate experiments. (B) Cells in collagen gels were incubated in the absence or presence of IL-1β (0.1 ng/mL), R667 (0.1 nM), and the RAR-γ antagonist MM11253 (0.01 or 0.03 nM) for 36 hours, after which culture supernatants were assayed for collagen degradation. Data are expressed as micrograms of HIP per well and are means ± SD of quadruplicates from an experiment that was repeated three times with similar results. *P < 0.05 (Dunnett’s test).

Degradation is mediated at least in part by attenuation of MMP-1 production. In addition to MMP-1, MMP-8, MMP-13, and MMP-18 also possess collagenase activity. Increased production of both MMP-1 and MMP-8 has been detected during chronic skin ulceration. The possible effects of RAR-γ agonists on the production of collagenases other than MMP-1 by corneal fibroblasts during corneal disease thus warrants further study.

Retinoids including ATRA bind to RAR-α, RAR-β, and RAR-γ as well as to the retinoid X receptor (RXR) and thereby modulate cellular functions. RAR-γ has been found to contribute to maintenance of hematopoietic stem cells by ATRA in an ex vivo progenitor cell culture system. On the other hand, RAR-β contributes to neural differentiation of mesenchymal stem cells induced by ATRA. In the present study, we confirmed the expression of RAR-α, RAR-β, and RAR-γ in rabbit corneal fibroblasts as well as compared the effects of RAR-α, RAR-β, and RAR-γ agonists on IL-1β-induced collagen degradation by these cells, with the RAR-γ agonist R667 showing the greatest inhibitory effect and therefore likely being largely responsible for the inhibitory action of ATRA in this system. Moreover, an RAR-γ antagonist suppressed the inhibitory effect of R667 on IL-1β-induced collagen degradation by corneal fibroblasts. Retinoids also regulate the expression of RAR isomers and RXR. Thus, ATRA thus induces the expression of RAR-α and RAR-γ in adipocytes as well as promotes that of RAR-β in conjunctival fibroblasts. It remains to be determined whether ATRA and RAR-γ agonists inhibit collagen degradation by corneal fibroblasts in part by regulating the expression of RARs and RXR in these cells.

The remodeling of collagen fibrils in the corneal stroma is mediated by the production or degradation of extracellular matrix and contributes not only to physiological homeostasis but also to pathologic changes. Expression of MMPs is upregulated in association with injury or infection in the cornea. Matrix MMPs have also been detected at the wound margin in the cornea during wound healing after LASIK (laser-assisted in situ keratomileusis) surgery. We now showed that the RAR-γ agonist R667 suppressed the expression or activation of MMP-1, MMP-2, MMP-3, and MMP-9 in collagen gel cultures of corneal fibroblasts stimulated with IL-1β. We have previously shown that dexamethasone and female sex hormones as well as ATRA also inhibit collagen degradation by corneal fibroblasts likely through suppression of MMP expression. These data thus suggest that RAR-γ signaling modulates the expression of MMPs in corneal fibroblasts and thereby contributes to the regulation of collagen remodeling in the corneal stroma both under physiological conditions and during the development of pathologic conditions such as corneal infection, scar formation, and inflammation. Indeed, we have recently found that topical application of ATRA ameliorates corneal ulceration induced by injection of bacterial lipopolysaccharide into the corneal stroma of rabbits.

Retinoic acids play essential roles in biological processes including cell proliferation, differentiation, and apoptosis as well as development. Moreover, they exhibit an antiinflammatory action mediated through regulation of collagen turnover. Corneal melting or scarring results from abnormal collagen remodeling in the corneal stroma. We now showed that an RAR-γ agonist inhibited IL-1β-induced collagen degradation by corneal fibroblasts in vitro. These results suggest that activation of retinoic acid signaling mediated by RAR-γ is a potential new approach to the treatment of corneal inflammatory diseases. ATRA has also been found to inhibit the proliferation of corneal fibroblasts but not that of corneal epithelial cells, although in the present study we found that R667 had no effect on the proliferation of rabbit corneal fibroblasts. On the other hand, ATRA induces the expression of cytochrome P450 (CYP) 4B1 in corneal epithelial cells and thereby promotes the production of proinflammatory 12-hydroxyeicosanoids. These various observations suggest that the effects of retinoid signaling in the cornea are complex. RAR-γ signaling has also been found to promote the conversion of fibroblasts to neurons as well as the reprogramming of somatic cells into induced pluripotent stem cells. The expression of α, β, and γ isomers of RAR has been found to change during eye development, and their individual inactivation in mice results in hypoplasia of the anterior segment of the eye. These various observations suggest that treatment with an RAR-γ agonist may not only modulate the remodeling of collagen fibrils but also regulate the regeneration or differentiation of corneal tissue after injury.

Steroid hormones as well as vitamins A and D act as ligands of nuclear receptors. We have previously shown that female sex hormones including 17β-estradiol and progesterone inhibit IL-1β-induced collagen degradation by corneal fibroblasts as well as the IL-1β-induced phosphorylation of p38 MAPK, but not that of ERK or JNK, in these cells. In addition, the glucocorticoid receptor agonist dexamethasone inhibits IL-1β-induced collagen degradation by corneal fibroblasts as well as the IL-1β-induced phosphorylation of ERK and JNK, but not that of p38 MAPK, in these cells. We now showed that the phosphorylation of MAPKs including ERK, p38, and JNK induced by IL-1β was not inhibited by the RAR-γ agonist R667 in rabbit corneal fibroblasts. In contrast, the RAR-γ agonist suppressed the IL-1β-induced phosphorylation of IkB-α in these cells. These various observations suggest that different nuclear receptor agonists may target MAPK and NF-kB signaling pathways differentially to achieve inhibition of IL-1β-induced collagen degradation by corneal fibroblasts.
We showed that RAR-α and RAR-γ agonists inhibited IL-1β-induced collagen degradation by corneal fibroblasts, with the effect of the RAR-γ agonist being markedly greater than that of the RAR-α agonist and accompanied by the inhibition of MMP-1, MMP-2, MMP-3, and MMP-9 production. An RXR ligand has been found to inhibit MMP-1 and MMP-3 expression in human chondrosarcoma. Doxycycline inhibits collagen gel remodeling through suppression of MMP-1, MMP-9, and MMP-12 expression in conjunctival fibroblasts. These observations suggest that drugs other than RAR-γ agonists that target collagenase function warrant further study for evaluation of a potential therapeutic action in the cornea.

In conclusion, we showed that an RAR-γ agonist inhibited IL-1β-induced collagen degradation by corneal fibroblasts as well as the IL-1β-induced production and activation of MMPs in three-dimensional cultures of these cells. These effects of the RAR-γ agonist may be mediated by inhibition of the NF-κB signaling pathway. RAR-γ agonists thus warrant further investigation as potential new drugs for the treatment of corneal inflammatory diseases.

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