Effects of Antiglaucoma Drops on MMP and TIMP Balance in Conjunctival and Subconjunctival Tissue

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PURPOSE. To study the effects of antiglaucoma drugs on metabolism within the extracellular matrix (ECM) of the ocular surface, including corneal, conjunctival, and subconjunctival tissue.

METHOD. Several antiglaucoma drugs—including β-blockers, α/β-blockers, α-blocker, α2-agonist, and prostaglandin derivative—were topically administered to rat eyes daily for 2 weeks or were incubated with human corneal cells or human fibroblasts for 72 hours. Thereafter, expression and enzymatic activity of the matrix metalloproteinases (MMPs), a group of enzymes proteolyzing ECM and their inhibitors, called tissue inhibitors of metalloproteinase (TIMPs), were evaluated.

RESULTS. Quantitative RT-PCR revealed significantly upregulated and downregulated expression of MMPs and TIMPs, respectively, in rat conjunctival and subconjunctival tissue on the administration of α/β-blockers, α-blocker, α2-agonist, and prostaglandin derivative, suggesting that these drugs may enhance ECM degradation. However, in contrast, β-blocker administration caused reverse effects—that is, upregulation and downregulation of TIMPs and MMPs, respectively. Enzymatic activity of MMPs in rat conjunctival and subconjunctival tissue analyzed by biochemical assay and zymography was markedly enhanced on the administration of α/β-blockers, α-blocker, α2-agonist, and prostaglandin derivative, but not of β-blockers. Similar effects of these antiglaucoma drugs were observed in cultured human corneal cells and human fibroblast cells.

CONCLUSIONS. The present experimental observations suggest that some α/β-blockers, α-blocker, α2-agonist, and prostaglandin derivative stimulate ECM degradation of ocular surface tissue by modulating the balance between MMPs and TIMPs. (Invest Ophthalmol Vis Sci. 2006;47:823–830) DOI:10.1167/iovs.05-0902

Glaucoma is known to be a major cause of optic neuropathy that eventually leads to loss of vision. This is characterized by loss of retinal ganglion cells and their axons, excavated appearance of optic nerve head, and progressive loss of visual field sensitivities. Clinically open-angle glaucoma (OAG), the most common type of glaucomatous optic neuropathy, is further divided into primary open-angle glaucoma (POAG) and normal-tension glaucoma (NTG), which are associated with elevated (greater than 21 mm Hg) and normal (not exceeding 21 mm Hg) intraocular pressure (IOP), respectively. Clinical aspects of the optic neuropathy in POAG and NTG are identical with the exception of their IOP levels. In terms of possible etiology of glaucomatous optic neuropathy, retinal ganglion cell death by apoptosis has been identified in postmortem studies of human eyes with POAG and experimental glaucoma models with elevated IOP. Although the molecular mechanism causing apoptosis has been uncertain, deprivation of neurotrophic factors, ischemia, disorganized nitric oxide (NO) metabolism, and chronic elevation of glutamate have been suggested as possible mechanisms. In addition, it has been suggested that autoimmune reactions, such as rhodopsin, 60-kDa heat shock protein (hsp 60), 27-kDa heat shock protein (hsp 27), α-crystallin, and neuron-specific enolase directed toward retinal antigens may be involved in the apoptotic mechanism in some patients with glaucoma, particularly those with NTG. However, several clinical studies have shown that lowering IOP levels in patients with NTG caused significant decreases in the progression of glaucoma. Therefore, controlling IOP through antiglaucoma drug medication and performing surgery if necessary are critically important in the management of patients with POAG and NTP.

In extracellular matrix (ECM) metabolism within the trabecular meshwork, the juxtacanalicular tissue is critically involved in aqueous humor outflow resistance, which is the key factor for maintaining IOP levels. A pair of enzyme families called the matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs) are involved in the regulation and maintenance of the ECM. MMPs and TIMPs are involved in physiological mechanisms, including embryogenesis, development, and wound healing. Dysregulated expression of MMPs and TIMPs is implicated in many diseases accompanied by abnormal matrix production, such as fibrotic disorders, and many other disease states. Such abnormal mechanisms have been recognized in many ocular diseases, including proliferative vitreoretinopathy, secondary cataract, and pterygium.

In addition, ECM accumulation caused by changes of MMP and TIMP expression is significantly involved in the increased outflow resistance in glaucomatous eyes. In fact, it was recently reported that significantly reduced levels of MMPs in aqueous humor were present in pseudoexfoliation syndrome and POAG. Alternatively, the regulation of ECM metabolism is known as an important factor for success of glaucoma filter surgery. The filtering bleb after trabeculectomy, for instance, often fails because of scarring and ECM accumulation within the subconjunctival space, suggesting that the stimulation of ECM degradation may suppress subconjunctival scar formation and promote longer survival of filtering blebs. Interestingly, Li et al. reported that MMP-1 and MMP-3 were significantly overexpressed in conjunctivochalasis fibroblasts and suggested that this may cause redundant, loose, and nonedematous conjunctiva. This interesting observation allowed us to speculate that the overexpression of MMPs enhances the degradation of ECM in conjunctiva and may thereby cause long-term survival of postoperative filtering blebs. To test this hypothesis, we studied postoperative IOP lowering and survival of filtering blebs after the transfection of MMP-3 cDNA into...
rabbit subconjunctival tissue by electroporation (EP) and found that trabeculectomy performed after MMP-3 transfection caused significantly longer survival of filtering blebs and decreased IOP levels in comparison with controls (trabeculectomy alone or trabeculectomy after vector transfection) and that these levels were almost identical with those of trabeculectomy with 0.04% mitomycin C.26

Several kinds of antiglaucoma drugs, including β-blockers, α/β-blockers, α1-blocker, α2-agonist, prostaglandin deriva-
tives, and others, are frequently used for the treatment of glaucoma in our clinic. Therefore, we have many medication options. When trying to determine the most suitable antiglau-
coma treatment for each patient, we consider the effects of the drug on retinal neuronal cells and on the ocular surface, including corneal, conjunctival, and subconjunctival tissue, in addition to the therapeutic efficacy for managing IOP. In terms of drug effects on retinal neuronal cells, several recent in vivo and in vitro studies27–29 using animal models and including NMDA-induced retinal dysfunction and other mechanisms have suggested that some of these antiglaucoma drugs may provide retinal ganglion cells with neuroprotective effects against apoptosis. However, few data are available that specifically focus on the effects of antiglaucoma drugs toward ocular surface tissue.

Therefore, in the present study, to evaluate effects of anti-
glaucoma drugs toward ocular surface tissue, several major antiglaucoma drugs, among them β-blockers, α/β-blockers, α1-
blocker, α2-agonist, and prostaglandin derivative, were topo-
ically administered to rat eyes daily for 2 weeks or were in-
culated with human corneal cells or human fibroblast for 72 hours. Thereafter, expression and enzymatic activities of MMPs and TIMPs were systematically evaluated.

Materials and Methods

Studies were performed in accordance with our institutional review board guidelines, and all experimental procedures were designed to conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and to our own institution’s guidelines. Human keratocytes and fibroblasts and their cell culture media were purchased from Kurabo Industries Ltd. (Osaka, Japan) and were maintained according to the manufacturer’s protocol.

Antiglaucoma Drug Administration

Six-week-old Sprague-Dawley (SD) rats (each weighing approximately 150 g) reared in cyclic light conditions (12 hours on/12 hours off) were used. Topical administration of antiglaucoma drugs (drop volume, 20 μL) β-blockers (0.5% timolol, 0.5% betaxolol, 2% carteolol), α/β-
blockers (0.25% npipradilol, 0.5% levobunolol), α1-blocker (1% bunazo-
cin HCl), α2-agonist (1% brimonidine), or prostaglandin derivative (0.005% latanoprost) were applied to SD rat eyes daily for 2 weeks using a micropippette. Drug concentrations corresponded with those used in clinical practice. As a control, phosphate-buffered saline (PBS) containing 0.02% benzalkonium hydrochloride, the highest concentration of preservative contained in the antiglaucoma drugs used in these studies, was administrated as described. Eyes were enucleated, and conjunctival and subconjunctival tissues were subjected to biochemical and morphologic examination, as described. Timolol, bunazocin HCl, and brimonidine were purchased from Sigma (St. Louis, MO). Betaxolol was obtained from Alcon Japan Ltd. (Tokyo, Japan). Npipradilol and levobunolol were obtained from Kowa Co. Ltd. (Tokyo, Japan), and Kaken Co. Ltd. (Tokyo, Japan), respectively, and latanoprost was from obtained from Pfizer Japan Inc. (Tokyo, Japan).

Collagenolytic, β-Caseinolytic and Gelatinolytic Activity Assays

Freshly prepared cell lysate or isolated culture supernatants were assayed for collagenolytic, β-caseinolytic, and gelatinolytic activity on the basis of the cleavage of fluorescently labeled substrates (Type I Collagenase Assay Kit, Stromelysin Activity Assay Kit and Type IV Collagenase Assay Kit; Yagi Corp., Yamagata, Japan), respectively, according to the manufacturer’s instructions.

Taqman Quantitative RT-PCR Analysis

Total RNA from conjunctival and subconjunctival tissues was isolated using reagent according to the procedure recommended by the manu-
facturer (Isogen; Nippon Gene, Tokyo, Japan). The cDNAs were isolated from 2 μg RNA in a 12-μL reaction using 1 μL oligo(dt) primer (0.5 mg/mL; Gibco-BRL, Life Technologies, Inc., Rockville, MD). The reaction mix was denatured at 70°C for 10 minutes. Four microliters of first-strand buffer (250 mM Tris HCl, 375 mM KCI, 15 mM MgCl₂; Superscript; Gibco-BRL), 2 μL dithiothreitol (0.1 M; DTT; Gibco), 1 μL deoxyribonucleoside triphosphate (10 mM; dNTP; Gibco-BRL), 1 μL RNase inhibitor (40 U/μL; RNase inhibitor; Gibco-BRL), and 1 μL reverse transcriptase (200 U/μL; Superscript II; Gibco-BRL) were added to the mix. The incubation was carried out at 42°C for 50 minutes and at 70°C for 15 minutes. PCR amplifications were performed using 4 μL from the RT reaction, 5 μL 10 × PCR buffer (200 mM Tris HCl, 500 mM KCl), 2 μL MgCl₂ (50 mM), 1 μL dNTP, 5 μL sense and antisense primers (10 pM/μL) and, 0.5 μL Taq polymerase (5 U/μL; Gibco). The PCR mix was denatured at 94°C for 4 minutes and then run for 30 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes.

For Taqman PCR analysis, primers and probes were designed (Primer Express software; Applied Biosystems, Foster City, CA) as follows: Rat MMP-3—forward, 5'-AGTCCTTTCGTGAGAA-CATG-3'; reverse, 5'-GAATCATGTTGTTTATGCTC-3'; detection probe, 5'-AGGAGTTTGCTGCTGAGGAA-3'. Rat TIMP-1—forward, 5'-ATGATCTCGAGGTGCTGA-3'; reverse, 5'-GGCTGTCGTTTCCAAGAGA-3'; detection probe, 5'-CTCGAGACTGATTCCTGCACTGGA-3'. Rat TIMP-2—forward, 5'-TCTAGATCTCATGAGGCTC-3'; reverse, 5'-CTGTCTGACCTTCTTCTGAGGGA-3'; detection probe, 5'-CTCGAGCTGATCCTCCAAGAGA-3'. Rat TIMP-3—forward, 5'-CTGGAATGCCTGACAAGGG-3'; reverse, 5'-CTGGAATGCCTGACAAGGG-3'; detection probe, 5'-CTGGAATGCCTGACAAGGG-3'. Rat TIMP-4—forward, 5'-GAAGCAGATCTGAGAGAGG-3'; reverse, 5'-GAAGCAGATCTGAGAGAGG-3'; detection probe, 5'-GAAGCAGATCTGAGAGAGG-3'.

GAPDH as an internal control was amplification was done using a commer-
cially available kit (Applied Biosystems) at the same time. The PCR mix (total, 50 μL) contained 1 μL cDNA template, 1X Taqman buffer A, 8% glycerol, 5 mM MgCl₂, 200 μM each dATP, dCTP, and dGTP, 400 μM dUTP, 1.25 U AmpliTag Gold DNA polymerase, 0.25 U AmpliEraseUNG, and 300 nM each primer. Standard reactions were performed (PRISM Sequence Detection System; Applied Biosystems). All experiments were performed in triplicate.

Detection of MMP-3 by Zymography and Western Blot Analysis

Conjunctival tissue (approximately 10 mg from two eyes) obtained from rats and treated with several antiglaucoma drops, as described, were homogenized in 500 μL of 50 mM Tris HCl buffer, pH 7.5, containing 150 mM NaCl, 10 mM CaCl₂, and 0.5% Triton X-100 and were subjected to zymography, also as described. Protein sample (50 μg) was subjected to 12% to 15% SDS-PAGE gel containing 0.1% casein. The gel was stained with Coomassie blue R250 and destained with 40% methanol and 10% acetic acid. MMP-2 and MMP-3 bands were confirmed by comparison with commercially available purified MMP-2 and MMP-3 (Yagai Science Co., Yamagata, Japan).
Several changes in conjunctival tissues, including a decrease in the number of epithelial goblet cells,\textsuperscript{30} an increase in subepithelial collagen deposition,\textsuperscript{31} and a high number of macrophages, fibrocytes, lymphocytes, and mast cells in the substantia propria,\textsuperscript{52–54} have been reported with regard to the effects of long-term topical antiglaucoma therapy toward ocular surface tissue. In addition, antiglaucoma drugs delay corneal epithelial regeneration\textsuperscript{55} and decrease the mucous layer of the tear film.\textsuperscript{36} These effects on ocular surface tissue may be caused in part by preservatives usually applied with the therapeutic agent. In an in vitro study, Tenon’s fibroblast proliferation was not altered by exposure to β-blocker without preservatives but was by exposure to β-blockers with preservatives.\textsuperscript{57} Alternatively, in an experiment using rabbits, mild increases of subepithelial fibrosis were detected in eyes treated by 0.5% levobunolol, 0.25% nipradilol, 1% bunazocin HCl, 1% brimonidine, and 0.005% latanoprost, whereas no changes were observed in other specimens. Antiglaucoma drugs were also administrated to cultured human fibroblasts and human keratocytes, and changes in their MMP activities were analyzed by zymography. As shown in Figure 3, the administration of timolol caused significant and relative decreases in the expression of MMP-3 and MMP-2 in a dose-dependent manner. In contrast, nipradilol, brimonidine, and bunazocin caused relative enhancement of MMP-3 expression. In enzymatic assays, stromelysin activities were significantly enhanced by the presence of nipradilol, brimonidine, and bunazocin but were suppressed by timolol, whereas types 1 and 4 collagenase activities were not affected by any of these drugs (Fig. 4). Therefore, ECM metabolism of ocular surface tissues can be modified by the topical administration of antiglaucoma drugs that alter their MMP and TIMP balance.

**DISCUSSION**

Several changes in conjunctival tissues, including a decrease in the number of epithelial goblet cells,\textsuperscript{30} an increase in subepithelial collagen deposition,\textsuperscript{31} and a high number of macrophages, fibrocytes, lymphocytes, and mast cells in the substantia propria,\textsuperscript{52–54} have been reported with regard to the effects of long-term topical antiglaucoma therapy toward ocular surface tissue. In addition, antiglaucoma drugs delay corneal epithelial regeneration\textsuperscript{55} and decrease the mucous layer of the tear film.\textsuperscript{36} These effects on ocular surface tissue may be caused in part by preservatives usually applied with the therapeutic agent. In an in vitro study, Tenon’s fibroblast proliferation was not altered by exposure to β-blocker without preservatives but was by exposure to β-blockers with preservatives.\textsuperscript{57} Alternatively, in an experiment using rabbits, mild increases of subepithelial fibrosis were detected in eyes treated by 0.5% levobunolol, 0.25% nipradilol, 1% bunazocin HCl, 1% brimonidine, and 0.005% latanoprost, whereas no changes were observed in other specimens. Antiglaucoma drugs were also administrated to cultured human fibroblasts and human keratocytes, and changes in their MMP activities were analyzed by zymography. As shown in Figure 3, the administration of timolol caused significant and relative decreases in the expression of MMP-3 and MMP-2 in a dose-dependent manner. In contrast, nipradilol, brimonidine, and bunazocin caused relative enhancement of MMP-3 expression. In enzymatic assays, stromelysin activities were significantly enhanced by the presence of nipradilol, brimonidine, and bunazocin but were suppressed by timolol, whereas types 1 and 4 collagenase activities were not affected by any of these drugs (Fig. 4). Therefore, ECM metabolism of ocular surface tissues can be modified by the topical administration of antiglaucoma drugs that alter their MMP and TIMP balance.
treated with antiglaucoma drugs and preservatives, and collagen type 4 staining and staining for α-smooth muscle actin increased in that area. This evidence has suggested that long-term topical administration may affect ECM metabolism within ocular surface tissue. In fact, in monkey eyes, MMP-2 and MMP-3 expression increased in the anterior part of the ciliary muscle after the application of latanoprost, possibly correlating with its powerful effect on lowering IOP through the increased uveoscleral outflow pathway of aqueous humor. Mietz et al. recently reported that a significant increase of subepithelial collagen density was present in timolol-treated eyes, but this finding was not apparent in latanoprost-treated eyes. These observations indicated that the effects of ECM metabolism within ocular surface tissue depend not only on the types of antiglaucoma drugs but potentially also on the preservatives. Thus, systematic study of the effects of antiglaucoma drugs toward ECM metabolism within ocular surface tissue should be accomplished as a basis for their selection. Nevertheless, almost no study has been available. In the present study, we investigated the effects of several antiglaucoma drugs toward ECM metabolism of ocular surface tissue and made several observations. First, significantly upregulated and downregulated expression of MMP and TIMP, respectively, were found in rat conjunctival tissue on the administration of α/β-blockers, α1-blocker, α2-agonist, and prostaglandin derivative compared with PBS with preservative. Second, in contrast, β-blocker administration caused the upregulation and downregulation, respectively, of TIMP and MMP expression.

Third, enzymatic activities of MMP in rat conjunctival tissues analyzed by biochemical assay and zymography showed corresponding results. Fourth, effects similar to those produced by these antiglaucoma drugs were also observed in cultured human corneal cells and human fibroblasts. These observations suggest that the effects of MMP and TIMP on ECM metabolism within ocular surface tissue were significantly modulated by topical antiglaucoma medications and that they depended exclusively on the type of drug used.

Thus far, 24 MMPs have been identified in vertebrates and grouped into four main subfamilies based on their specificity for different extracellular matrix components, among them the collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10, -11), membrane-type MMPs, and others including matrilysin (MMP-7) and metalloesterase (MMP-12). The collagenases degrade fibrillar collagens types 1, 2, and 3; the gelatinases cleave denatured collagens (gelatins) and proteolytically collagen types 4, 5, and 7 and also elastin and vitronectin; stromelysins cleave type 4 collagen, proteoglycans, fibronectin, laminin, and elastin. MMPs are synthesized and secreted as inactive proenzymes and are activated by proteolysis. MMP activities are strictly regulated by unspecific plasma inhibitor (α-macroglobulins) and specific TIMPs. Four TIMPs have been identified that have the capacity to inhibit all active MMPs. TIMP-1 controls most MMPs, particularly MMP-1, whereas TIMP-2 is the major inhibitor of MMP-2. Functionally, MMPs have been implicated in the normal matrix remodeling process, such as embryonic development, morphogenesis, tissue homeostasis, and wound healing. In contrast, a lack of balance between the activities of MMPs and those of TIMPs has been associated with a number of pathologic conditions, including inflammatory diseases, cancer, cardiovascular disease, neurologic disease, fibrotic conditions, and several eye diseases, among them glaucoma. In terms of roles of ECM metabolism in the pathogenesis of glaucoma, Huang et al. examined normal aqueous humor by zymography and found several active forms of MMPs present in the aqueous and their endogenous inhibitors, TIMPs. In a different study, it was revealed that an increase of TIMPs was found only in the aqueous humor of POAG-affected eyes among aqueous humor specimens from eyes with POAG, eyes with secondary forms of glaucoma, and control eyes. Furthermore, adding aqueous samples to the tissue cultures significantly increased collagen synthesis. Based on these data, the author suggested that this effect might have contributed to an increased deposition of collagen in the trabecular meshwork in the pathogenesis of POAG. Alternatively, increased MMP-1 expression was found in retinal ganglion cells of glaucomatous eyes, and MMP-1, -2, and -3 were found all over the optic nerve heads and in the postlaminar region, especially in eyes with normal pressure glaucoma compared with control eyes. In terms of MMP ex-

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**FIGURE 2.** Detection of MMP-2 and MMP-3 by zymography. Enzyme activities of MMP-2 and MMP-3 within conjunctival tissues treated by several antiglaucoma drugs were analyzed by zymography (A). Their staining densities were evaluated by densitometric analysis and were plotted (B). Experiments were repeated three times using different preparations (n = 3 rats; 3 eyes in each condition) and identical results were obtained. Lane 1, PBS; lane 2, 0.5% timolol; lane 3, 0.5% betaxolol; lane 4, 2% carteolol; lane 5, 0.5% levobunolol; lane 6, 0.25% nipradilol; lane 7, 1% bunazocin HCl; lane 8, 1% brimonidine; lane 9, 0.005% latanoprost.
expression within ocular surface tissue, Li et al. reported the overexpression of MMP-1 and -3 in conjunctivochalasis fibroblast and suggested that this caused excessive degradation of conjunctival matrix and Tenon's capsule, resulting in the looseness of conjunctival tissue characteristic of this disease. In our recent study, the introduction of MMP-3 cDNA into conjunctiva caused significant effects on postoperative bleb survival and effectively maintained lower IOP levels. Furthermore, such MMP-3 transfection by EP caused effective expression within conjunctival epithelium until at least 30 days after transfection. These observations suggested that the reduction of subepithelial collagen density and the accumulation of ECM by the upregulation of MMP expression may have a beneficial effect on the survival of postoperative filtering blebs. Mietz et al. reported that compared with timolol administration, latanoprost administration caused the upregulation of MMP-3 and histopathologic changes showing decreased subepithelial density in rabbit conjunctiva. Our present study revealed that, in addition to latanoprost, carteolol, nipradilol, levobunolol, bunazocin HCl, and brimonidine demonstrated upregulation and downregulation of MMP and TIMP expression. Therefore, these antiglaucoma drugs may have beneficial effects on glaucoma-filtering surgery by modulating ECM metabolism.

Prevention of wound healing in glaucoma-filtering surgery is an important factor for the long-term survival of filtering blebs. To prevent scar formation, antiproliferative agents such as mitomycin C (MMC) are often used for trabeculectomy, allowing longer survival of the filtering bleb and maintaining lower IOP levels. Nevertheless, serious complications of MMC treatment, such as postoperative infection caused by the leaking filtering bleb, often occur in combination with trabeculectomy. Therefore, alternative techniques have been needed for the long-term maintenance of the filtering bleb after glaucoma surgery. For this purpose, several new methods have been considered. It was reported that adenovirus-mediated gene therapy using the human p21 gene, which is identified as an inhibitor of cell proliferation by the suppression of cyclin-dependent kinase, significantly reduced scarring in a rabbit model of glaucoma surgery. However, although these effects contributing to survival of the filter bleb and low IOP levels were significantly longer than those in control, they were shorter than those in MMP combined with surgery. Sakamoto et al. reported that delivery of an anti-proliferative drug, such as bleomycin, by EP to the glaucoma-filtering bleb decreased IOP more significantly than EP or antiproliferative drug treatment alone. Therefore, they emphasized that this combination, including a minimum dose of antiproliferative drug, may be an effective means to maintain the glaucoma-filtering bleb. EP is already used in several clinical fields, such as transdermal drug delivery to cutaneous melanoma, and its clinical application has been considered useful. In fact, our recent preliminary trial of MMP-3 gene transfection to glaucoma-filtering blebs caused significant long-term survival of postoperative filtering blebs, suggesting that MMP-3 upregulation is beneficial for maintaining the glaucoma-filtering bleb, as evidenced by its proteolytic activities on the ECM.

In the present study of in vivo experimental methods, we studied major antiglaucoma drops used as the first or second choice for patients with OAG and found that the balance of effects on ECM metabolism by MMP and TIMP could be modulated by topical administration of some antiglaucoma drugs and that changes in MMP and TIMP expression in subconjunctival tissues were influenced by the drugs used. The results of zymography (Figure 3) showed that the enzyme activities of MMP-2 and MMP-3 in human fibroblast and keratocyte were regulated by different antiglaucoma drugs. The densitometric analysis of zymography revealed that the staining densities of MMP-2 and MMP-3 were significantly increased by latanoprost, carteolol, nipradilol, levobunolol, bunazocin HCl, and brimonidine, indicating their upregulation in the subconjunctival tissues. These findings suggest that the balance of MMP and TIMP expression in subconjunctival tissues can be modulated by different antiglaucoma drugs, and further studies are needed to determine the clinical relevance of these findings.

2, 10^{-6} M timolol; lane 3, 10^{-7} M timolol; lane 4, 10^{-6} M nipradilol; lane 5, 10^{-7} M nipradilol; lane 6, 10^{-6} M bunazocin HCl; lane 7, 10^{-7} M bunazocin HCl; lane 8, 10^{-6} M brimonidine; lane 9, 10^{-7} M brimonidine.

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tival tissues were altered on topical administration of antiglaucoma drugs. If changes in ECM metabolism can be controlled by some combinations of these antiglaucoma drugs, the effects will be longer survival of the postoperative filtering blebs after surgical intervention and new insight into antiglaucoma drug selection. However, before this evidence can be used to select antiglaucoma drugs for the clinical treatment of patients with glaucoma, we think the following additional effects must be examined and defined: effects on ECM metabolism of less frequently used antiglaucoma drops, such as carbonic anhydrase inhibitors and cholinergic agents, that were not included in this study; effects on ECM metabolism of several combinations of antiglaucoma drugs used in the ophthalmology clinic; and changes in MMP and TIMP expression and their enzymatic activities in human specimens obtained surgically from glaucoma patients using several antiglaucoma drops. These will form the basis of our next study.

References

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