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Aldose reductase inhibitor counteracts the enhanced expression of matrix metalloproteinase-10 and improves corneal wound healing in galactose-fed rats

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Purpose: We investigated the effect of an aldose reductase inhibitor (ARI) and the role of matrix metalloproteinase (MMP)-10 on recovery after corneal epithelium removal in a rat diabetic keratopathy model.
Methods: Three-week-old Sprague-Dawley rats were fed the following diets for 6 weeks: normal MF chow (MF), 50% galactose (Gal), and 50% Gal containing 0.01% ARI (Gal +ARI). The corneal epithelium was removed using n-heptanol, and the area of epithelial defects was photographed and measured every 24 h. Real-time reverse transcriptase PCR, western blotting, and immunohistochemistry were used to determine the expression profile of MMP-10 and integrin α3.
Results: Compared to the MF control group, the amount of galactitol in the Gal group increased approximately 200-fold, which was reduced to sevenfold by ARI treatment. The area of corneal erosion in the Gal group was significantly larger than in the MF group at 72 h and thereafter ($p<0.01$, unpaired *t* test). The expression level of MMP-10 was enhanced at both the protein and mRNA levels by exposure to a high concentration of Gal, while integrin α3 expression decreased at the protein level but remained unchanged at the mRNA level. Delayed epithelial wound healing and alterations in the expression levels of MMP-10 and integrin α3 were normalized by ARI. The corneal erosion closure rate was significantly decreased with topical recombinant MMP-10.
Conclusions: These studies confirm that the increased expression of MMP-10 induced by Gal feeding is counteracted by ARI treatment and suggest a role of MMP-10 in modulating corneal epithelial wound healing.

Although diabetic retinopathy (DR) leads to severe vision loss and remains the major cause of blindness in the world, keratopathy is also recognized as a major complication in patients with diabetes [1]. Diabetic keratopathy involves delayed epithelial wound healing, superficial punctate keratitis, recurrent erosions, and ulcers with epithelial detachment [2]. These corneal problems frequently develop following intraocular surgical procedures, such as cataract extraction or vitrectomy [3-5]. Several mechanisms for diabetic keratopathy have been proposed, including activation of the polyol pathway [6-8], accumulation of advanced glycation end products (AGEs) [9-11], and increased osmotic stress [12,13].

To elucidate the mechanisms underlying disorders of corneal epithelial cells (CECs), galactose-fed animals have been used as a practical model of diabetic keratopathy [14,15]. These laboratory animals exhibit delays in corneal wound healing and development of corneal opacities [16-18]. Histological studies have shown abnormalities in the corneal basement membrane of galactosemic rats. In addition, corneal epithelial barrier function is decreased in these animal models [19]. Several studies have demonstrated diabetic abnormalities, such as delayed wound healing and decreased sensitivity, could be counteracted by inhibitors of aldose reductase (AR), the first enzyme in the polyol pathway [20,21]. Moreover, clinical data also indicate that topical or oral administration of AR inhibitor (ARI) improves corneal sensation [22] and epithelial barrier function [21,23]. These reports suggest that AR plays an important role in the pathogenesis of diabetic keratopathy in humans. Although polyol accumulation induced by galactose exposure has been shown to be inhibited by oral ARI, the mechanisms by which the inhibition of AR achieves a therapeutic effect on corneal epithelial abnormalities remains unclear.

Since many diabetic corneal abnormalities are apparently related to changes in cell adhesion and tissue repair, they are likely to be associated with alterations in the adhesive molecules of the extracellular matrix (ECM) and basement membrane (BM) [24]. Matrix metalloproteinases (MMPs) are collectively capable of hydrolyzing essentially all the components of the ECM and BM. MMP-10/stromelysin-2 is specifically upregulated in the corneal epithelial layer and stroma of patients with DR [25]. It has also been shown that

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corneas of patients with DR show significantly decreased immunostaining for major epithelial BM components, such as nidogen-1/entactin, laminins, and binding partner integrin α3β1 [25,26]. Alteration of the BM and epithelial integrins in diabetic human corneas could occur through proteolytic degradation by MMPs [25]. Recently we found that cultured human CECs (hCECs) in the presence of a high glucose concentration enhanced the expression of MMP-10, decreased the expression of integrin α3β1, and attenuated cell adhesion [27]. The addition of ARI into the culture medium counteracted these alterations in expression levels and decreased cell adhesion.

Based on these findings, it is possible that polyol accumulation via AR leads to the upregulation of MMP-10, resulting in the degradation of integrin subunits, thus attenuating cell adhesion. In this study, we investigated the expression levels of MMP-10 and integrin α3 in the corneas of rats fed a 50% galactose diet in the presence or absence of ARI to investigate the role of AR.

METHODS

Animal and corneal wounds: All experimental procedures conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Three-week-old male Sprague-Dawley rats were fed a 50% galactose-supplemented diet in the absence (Gal group) or presence (Gal + ARI group) of a newly developed ARI, 0.025% ranirestat (Dainippon Sumitomo Pharma Company Ltd, Osaka, Japan) for 4 weeks. Control rats were fed a normal cereal diet (MF; Oriental Yeast Company Ltd, Tokyo, Japan). CECs from six rats in each group were removed by n-heptanol treatment as follows [28]. Prior to corneal abrasion, the rat was anesthetized with an injection of sodium pentobarbital into the abdominal cavity (50 mg/kg body weight). A small cotton swab dipped into n-heptanol was placed on the corneas of both eyes under a dissection microscope and rubbed gently from limbus to limbus for 60 s to remove CECs. Other ocular tissues did not come into contact with n-heptanol. The cornea was then washed with 10 ml of saline for 2 min. Removal of CECs was confirmed with fluorescent staining. Corneal photographs were taken every 12 h using a GENESIS hand-held fundus camera (Kowa Company Ltd, Tokyo, Japan) with a cobalt blue filter for better demonstration of re-epithelialization. Re-epithelialization rates were calculated with Adobe Photoshop CS6 Extended (Adobe Systems Inc., San Jose, CA) by measuring the ratio of abrasion area to entire CEC area over time. This study was designed as a double-masked study. The removal of corneal epithelium, corneal photography, and the computerized calculations of abraded areas were performed by research assistants (T. T.).

Recombinant MMP-10, MMP-2, or MMP-9 protein (R&D Systems, Minneapolis, MN) was dissolved 0.1 M "phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4; pH 7.4), and the concentration was adjusted to 50 µg/ml. These recombinant proteins were activated by heating at 37 °C for 2 h. Seven-week-old rats fed with MF received eye drops of PBS with MMP-10, five times per day until wound closure was achieved. The same solution was applied to rats fed with MF in the presence of 0.025% ARI for 4 weeks before epithelial debridement. As a control, the same volume of PBS was topically applied to rats fed with MF. Six rats were used in each group

Measurement of galactitol in corneal tissue: Galactitol levels in the entire sample of corneal tissue were determined by the method of Liang et al. [29] described as follows. After 4 weeks of feeding, 12 corneas of six rats in each diet group were dissected. Galactitol was detected by the liquid chromatography with tandem mass spectrometry detection (LC/MS/MS) system that consisted of a 1200 Series (Agilent Technologies Inc., Santa Clara, CA) and an API4000 tandem mass spectrometer (Applied Biosystems/MDX SCIEX, Framingham, MA) with atmospheric pressure chemical ionization. The column and autosampler temperatures were 40 °C and 10 °C, respectively. The analytical run time was 5 min. The monitored ion was used for 181.1 m/z → 101.1 m/z. Protein concentrations were determined using the method described by Bradford [30].

Immunohistochemical staining: Twelve rats (four rats from each diet group) were sacrificed with an overdose of intravenous sodium pentobarbital (100 mg/kg body weight). The corneas were dissected and immersed in 4% paraformaldehyde in 0.1 M PBS for 24 h and then embedded in paraffin and sectioned at 5 µm. The sections were deparaffinized in graded ethanol and xylene. After washing in PBS, histochemical staining for MMP-10 was performed using ImmunoCruz Staining Systems (Santa Cruz Biotechnology, Inc., Dallas, TX). The sections were incubated with serum block solution for 20 min and then with primary antibody for 2 h. Primary antibodies used to detect MMP-10 and integrin α3 included rabbit polyclonal antibodies NB100–92182 (diluted 1:100; Novus Biologicals, Littleton, CO) and ab131055 (diluted 1:500; Abcam, Cambridge, UK), respectively. The specimens were washed and incubated in biotinylated secondary antibody solution for 30 min, then washed and treated with horseradish peroxidase (HRP)–streptavidin complex for 30 min. All incubations were performed at room temperature.

Immunoreaction of MMP-10 and integrin α3 was visualized using a 0.02% 3, 3′-diaminobenzidine tetrahydrochloride solution (Nakarai, Company, Kyoto, Japan) containing 0.05% hydrogen peroxide.

Western blot analysis: For the preparation of each lane, protein extracts from six samples of corneal tissue obtained from six rats were used. Protein extracts were homogenized with radioimmunoprecipitation assay (RIPA) buffer (1% Nonidet P40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate-polyacrylamide [10] in PBS). Equal amounts (20 µg/lane) of protein were subjected to a 10%–20% sodium dodecyl sulfate-polyaclylamidegel electrophoresis (SDS-PAGE) gradient and blotted onto polyvinylidene difluoride membranes (Immobilon-P; Millipore Corporation, Billerica, MA). Blocking was done with 5% nonfat dry milk in 0.1% Tween-20 in PBS and then incubated overnight at 4 °C with anti-MMP-10 antibody (NeoMarkers, Fremont, CA) at 1:200 dilution. After four washes with PBS with Tween 20 (PBS-T), the membranes were incubated with HRP-labeled anti-mouse immunoglobulin G. The specific band was visualized with Luminol reagent (Santa Cruz Biotechnology). The membranes were then stripped and reprobed with anti-integrin α3, anti-integrin α6, anti-integrin β4 or anti-β-actin antibodies (Santa Cruz Biotechnology). The density of each band was analyzed using a LAS-1000 UVmini imager (FujiFilm, Tokyo, Japan).

Real-time reverse transcriptase–PCR: Total cellular RNA was isolated from rat corneal tissue using an RNeasy Mini Kit (Qiagen Inc., Hilden, Germany). Rat cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems) in accordance with the manufacturer's protocol. Six rats for each diet group were used. To assess the level of MMP-10 and integrin α3 transcripts, we synthesized a pair of sense 5′-ACC TGG CCC TGG ATT TTA TGG A-3′ and antisense 5′-CTT CAG GCT TAG ATG CTG CCT ATG A-3′ primers for MMP-10 and a pair of sense 5′-TAC TAC TTC GAA CGG AAA GAG GAG G-3′ and antisense 5′-CAG GTC TGG GTA TAA GTT TTC ATC-3′ primers for integrin α3. Quantitative real-time PCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems) using a 7300 Real-Time PCR system (Applied Biosystems). The following PCR cycling conditions were used: 95 °C for 10 min, with 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The relative quantities of MMP-10 and integrin α3 mRNA were automatically assessed by the comparative cycle threshold method and normalized to the β-actin/ribosomal mRNA level as an endogenous control.

Statistical analyses: Statistical analyses were performed using JMP 10 (SAS institute Inc., Tokyo, Japan). The data were shown as mean±standard error of the mean. Bartlett's test was used to examine equal variances across samples. After inspecting the normal distribution of the data, we assessed the statistical significances between the groups by unpaired Student *t* test. For all statistical tests, $p<0.05$ was considered the significance level.

RESULTS

Aldose reductase inhibitor treatment for attenuated corneal wound healing induced by galactose exposure: To test whether ARI counteracts abnormal wound healing in the corneas of galactose-fed rats, the healing process after epithelial debridement by n-heptanol was initially monitored every 24 h (Figure 1). Normal corneas completely closed epithelial defects in approximately 116±14 h on average, while the wounded corneas of rats fed 50% galactose healed significantly slower on average, with a mean healing time of 152±18 h ($p<0.05$ Figure 2). The wounded corneas of rats fed 50% galactose with ARI healed in approximately 120±12 h on average, with no significant difference from controls. The area of corneal erosion in galactose-fed rats was significantly larger than in controls at 72 h and thereafter. In ARI-treated rats, the corneal erosion area was smaller than in galactose-fed rats at 96 h and thereafter ($p<0.01$).

To test whether AR affected the activity of the polyol pathway, we measured the sorbitol concentration in corneal tissue (Figure 3). The intracellular galactitol concentration of galactose-fed rats was 4953.8±598.6 nmol/mg protein, approximately 200-fold higher than in controls (25.3±3.8 nmol/mg protein; $p<0.05$). Treatment with ARI strongly prevented the accumulation of sorbitol (172.7±30.9 nmol/mg protein; $p<0.05$).

Increased expression of matrix metalloproteinase-10 in galactose-fed rats was normalized by aldose reductase inhibitor treatment: To determine whether galactose feeding alters the expression levels of MMP-10, we performed western blot analyses after 50% galactose-feeding with or without ARI (Figure 4). As a proportion of total corneal protein, the expression level of MMP-10 protein in rats fed 50% galactose was approximately threefold higher than that of controls. This increase was significantly inhibited by ARI treatment, and there was no significant difference between ARI-treated rats and controls. On the other hand, the expression levels of integrin α3 subunits were significantly decreased by galactose exposure, and this effect was counteracted by the administration of ARI.

To confirm the results of western blot analysis and to localize MMP-10 expression, immunohistochemical staining was performed (Figure 5). MMP-10 was weakly expressed in normal corneal epithelium. Galactose feeding enhanced

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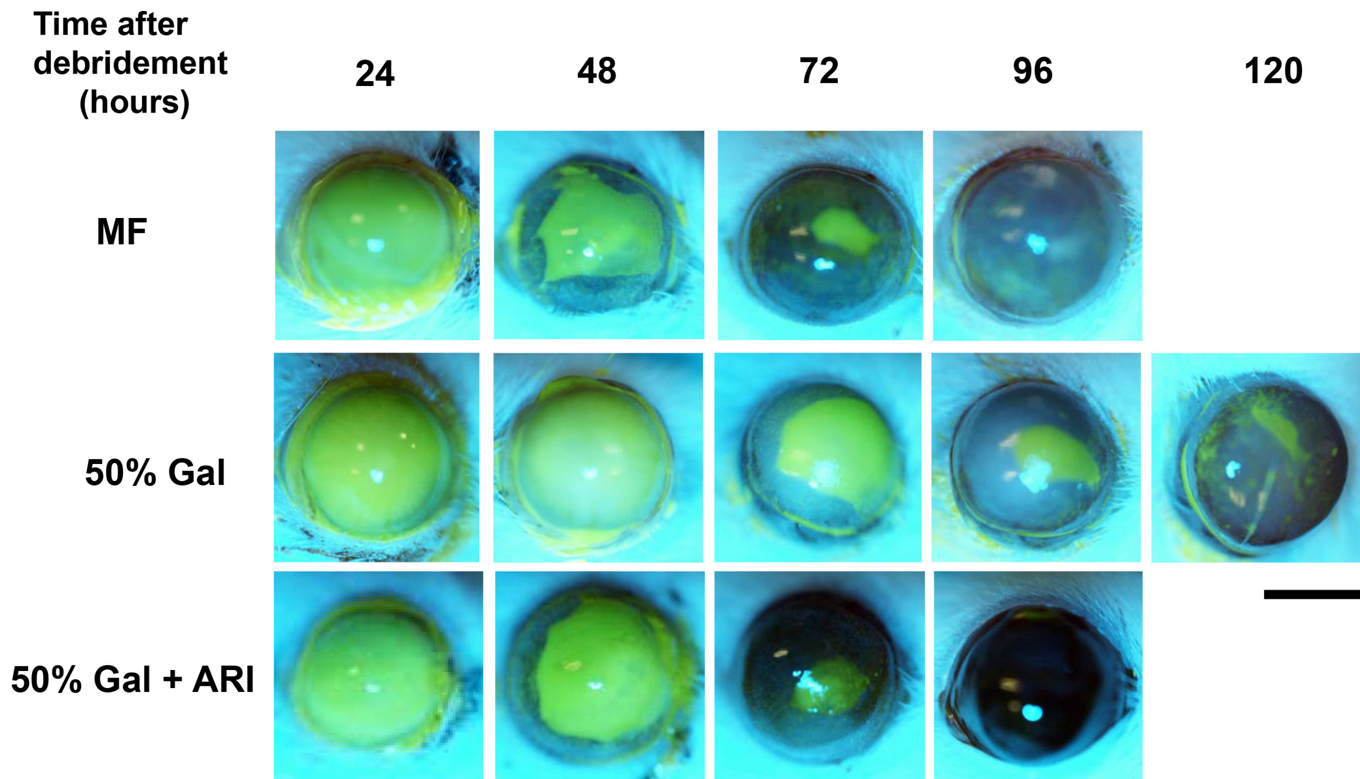

Figure 1. Representative photographs of corneas stained with fluorescein show wound healing dynamics of rats fed normal chow or 50% galactose in the presence or absence of aldose reductase inhibitor. The scale bar indicates 3.0 mm.

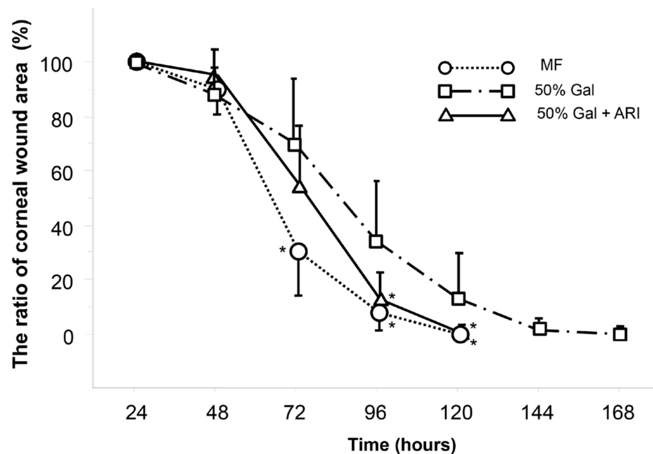

Figure 2. The delayed corneal wound healing of rats fed 50% galactose was counteracted with the treatment of aldose reductase inhibitor. The values are mean±standard error of the mean; *p<0.01.

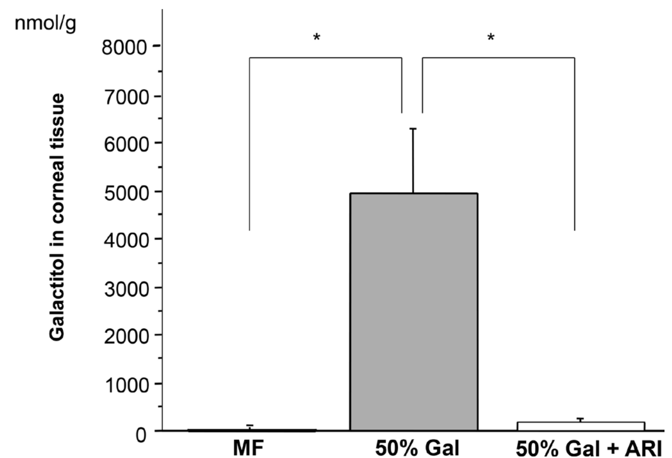

Figure 3. The amount of galactitol accumulating in the corneas of rats fed normal chow or 50% galactose in the presence or absence of aldose reductase inhibitor was measured. Compared to the control group, the amount of galactitol in the 50% Gal group increased approximately 200-fold, which was reduced to seven-fold by treatment with aldose reductase inhibitor. The values are mean±standard error of the mean; *p<0.05.

the intensity of staining; strong expression was observed on the side of the basal cells. This increased expression was inhibited by ARI, resulting in MMP-10 staining intensity that was similar to background levels in normal corneas. Integrin

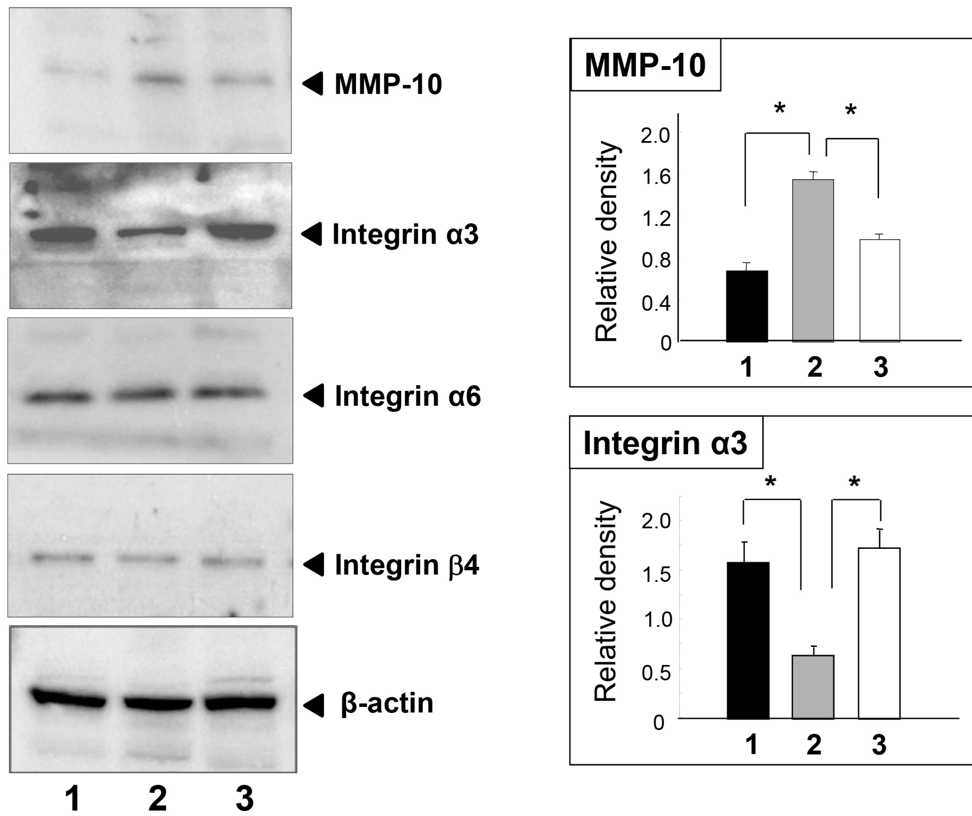

Figure 4. Matrix metalloproteinases (MMP)-10 and integrin α3 protein levels in the corneas of rats fed normal chow (lane 1) or 50% galactose in the absence (lane 2) or presence (lane 3) of aldose reductase inhibitor were analyzed by immunoblotting. The protein expression level of MMP-10 was enhanced by exposure to 50% galactose, while integrin α3 expression decreased. These changes were normalized with the treatment of aldose reductase inhibitor. No significant change was found in the expression levels of integrin α6 or β4. The bar graphs illustrate the intensities of the bands relative to β-actin. The values are mean±standard error of the mean; *$p<0.05$.

α3 was diffusely immunopositive in normal corneal epithelium, however, the intensity of staining in rats with galactose exposure was decreased, and this decrease in expression was prevented by the administration of ARI. On the other hand, the expression levels of integrin α6 or integrin β4 proteins did not change significantly in corneas of rats fed either galactose with or without ARI compared to normal corneas.

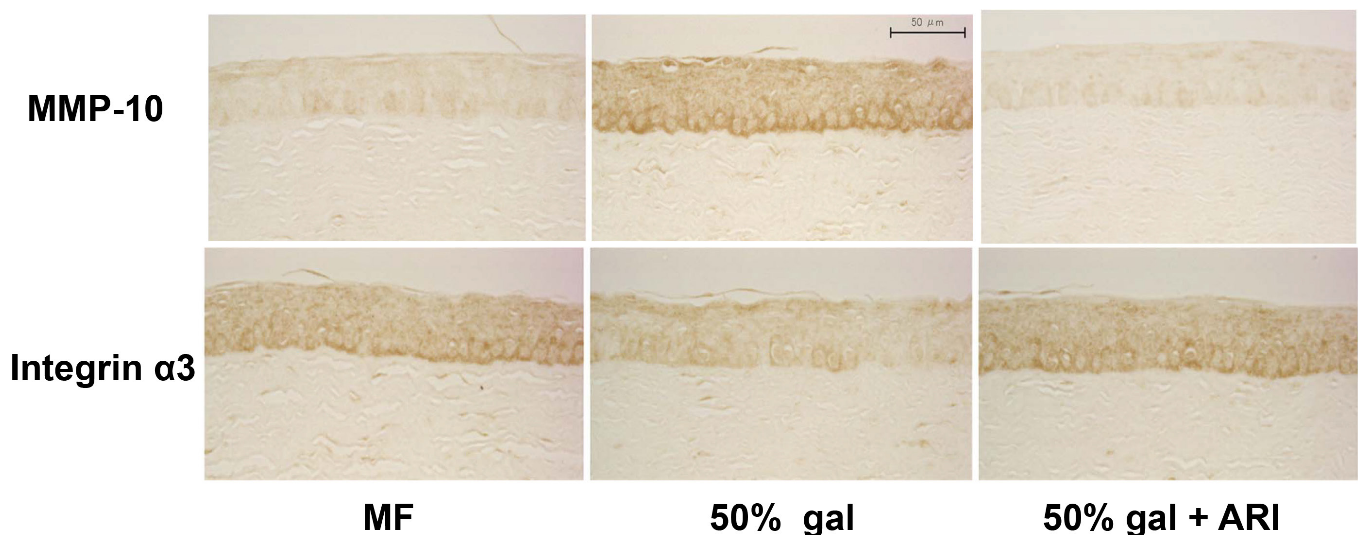

Figure 5. Immunohistochemical staining showed that the intensity of MMP-10 staining (upper panel) was increased by exposure to 50% galactose, while integrin α3 expression (lower panel) decreased. These changes were normalized with treatment of aldose reductase inhibitor. The scale bar indicates 50 µm.

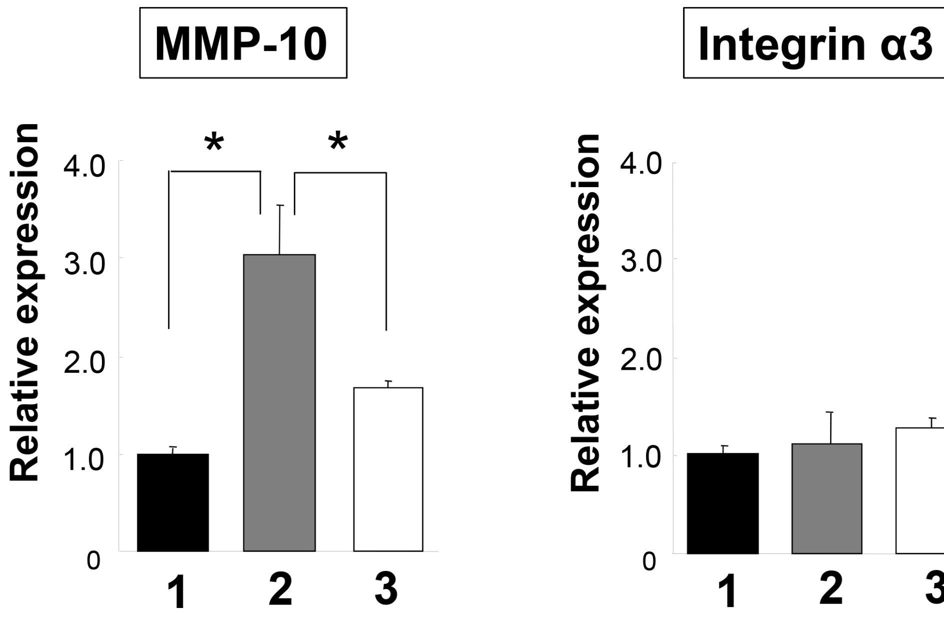

Figure 6. Histograms show the levels of matrix metalloproteinases (MMP)-10 and integrin α3 subunit mRNA isolated from the corneas of rats fed normal chow (lane 1) or 50% galactose in the absence (lane 2) or presence (lane 3) of aldose reductase inhibitor analyzed by real-time reverse transcriptase-PCR. MMP-10 mRNA levels were elevated by exposure to high levels of galactose, while mRNA expression levels of integrin α3 subunits were unaffected. The values are mean±standard error of the mean; *p<0.05.

Gene expression of matrix metalloproteinase-10 and integrin **α**3 subunits: Our finding of decreased expression of integrin α3 could be due to either decreased protein synthesis or increased degradation. To examine changes in protein synthesis, real-time RT–PCR was performed (Figure 6). Gene expression of MMP-10 was approximately threefold higher in the corneas of rats fed 50% galactose compared to controls (p=0.019). The increase in MMP-10 mRNA expression was significantly inhibited by ARI (p=0.021). In contrast, there were no significant differences among the 3 groups in integrin α3 gene expression.

Effect of topical matrix metalloproteinase-10 on corneal epithelial wound healing: To explore the role of MMP-10 in corneal wound healing, we applied recombinant MMP-10 topically after debridement of CECs (Figure 7). The wound area of eyes treated with topical MMP-10 was significantly larger than that of controls at 96 h. Oral administration of ARI for 4 weeks before the removal of the corneal epithelium had no effect on wound healing in rats treated with recombinant MMP-10. In contrast to MMP-10, the topical application of MMP-2 or MMP-9 recombinant protein did not influence on the wound area significantly at any time points.

DISCUSSION

In this study we focused on the expression of MMP-10, one of the main forms of MMPs, since our previous findings suggested that MMP-10 may play an important role in the pathogenesis of diabetic keratopathy. The addition of recombinant MMP-10 protein attenuated the adhesion of cultured hCECs, and increased expression of MMP-10 was induced in a medium with a high concentration of glucose [27]. We demonstrated that recombinant MMP-10 delayed wound closure in CECs, indicating that aberrant levels of MMP-10 have a deleterious effect on the healing process of the corneal epithelium. Saghizadeh et al. demonstrated that upon recombinant adenovirus-driven transduction of MMP-10 and cathepsin F, organ-cultured human corneas displayed slower corneal wound healing, similar to that observed in diabetic corneas [31]. They also reported that MMP-10 mRNA levels were increased in the corneal epithelium and stroma of

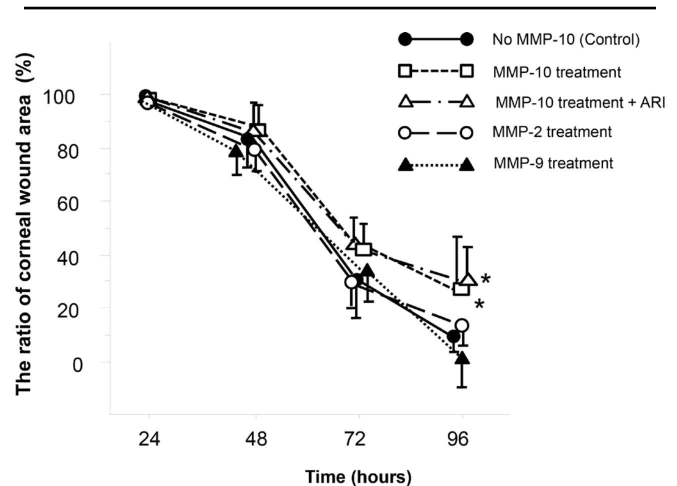

Figure 7. The effect of topical application of recombinant matrix metalloproteinases (MMP)-10 on corneal wound closure was examined. The corneal erosion closure rate was significantly decreased with topical recombinant MMP-10 but not with MMP-2 or MMP-9. The values are mean±standard error of the mean; *p<0.05.

patients with DR, whereas such elevation was not seen in corneas with keratoconus [25]. Expression of MMP-3 was also enhanced in patients with diabetes, but the distribution of this molecule is restricted to the corneal stroma and was not found in the epithelium [25]. MMP-2 and MMP-9 are expressed in tears and in corneal tissue during wound healing, so we also examined the effect of the topical application of these MMPs [32,33]. In contrast to MMP-10, no significant effects were found in the rate of corneal wound healing. These results suggest that especially MMP-10 plays an important role in delayed wound closure of corneal epithelium.

We found that the expression of MMP-10 was elevated and that of integrin α3 was decreased in the corneas of rats fed a diet containing galactose. This expression pattern of MMP-10 and integrin was confirmed by immunohistochemical staining and western blot analysis. Increased expression of MMP-10 was observed at both the mRNA and protein levels, indicating that MMP-10 protein synthesis was enhanced. The decrease in integrin expression could be due to either increased degradation or decreased synthesis. Since our data showed that mRNA levels of integrin α3 remained unchanged by galactose feeding, the decrease in integrin seems to be due to increased degradation of the affected components. This finding is consistent with the results of in vitro experiments using hCECs exposed to high concentrations of glucose [27]. Ljubimov et al. reported that corneas taken from patients with diabetes showed decreased immunostaining for laminins and integrin α3β1 [24]. However, gene expression of these molecules in the corneal epithelium of patients with diabetes was not significantly different from that in individuals without diabetes, whereas expression of MMP-10 mRNA was elevated [25]. Taken together, we consider that the alterations of epithelial integrin expression upon exposure to a high glucose concentration could occur because of proteolytic degradation by specific proteinases, especially MMP-10.

In contrast to integrin α3, the expression levels of integrin α6 or integrin β4 proteins were not affected by galactose feeding. Our results are similar to those of another study where the expression levels of integrin α3β1and their binding partner entactin and laminins, were decreased in the epithelium of corneas with DR but not in the bullous keratopathy; however, those of integrin α6 or integrin β4 was generally not altered [24]. Therefore, the decreased expression of integrin α3 may be a specific change in the pathogenesis of diabetic keratopathy.

AR is expressed in the epithelium and endothelium of the cornea. Polyols quickly accumulate in the corneal epithelium of rats fed a 50% galactose diet during the first week and reach a maximum concentration during the third week of feeding [15]. In agreement with that report, we showed that polyols accumulate after galactose exposure in rat corneas, but this is dramatically inhibited by treatment with an ARI. In addition, the oral administration of ARI counteracted the delay in epithelial wound healing induced by a high glucose diet. Previously we showed that the decreased adhesion of hCECs induced by a culture medium with a high concentration of glucose was recovered by treatment with an ARI [27]. These data indicate that polyol accumulation is involved in the abnormal behavior of the corneal epithelium and can form a part of the mechanism underlying diabetic keratopathy.

The delay of corneal wound healing by the topical application of recombinant MMP-10 was not altered by treatment with an ARI. These data indicate that an ARI has no pharmacological effect to inhibit the function of MMP-10 directly in the process of wound closure without galactose exposure. A high glucose or galactose condition is necessary to activate the polyol pathway via AR [6]. It is probable that the expressions of MMP-10 and integrin α3 were normalized in association with the inhibition of polyol accumulation by ARI, and thus the delay in wound healing was prevented. However, it remains unclear how activation of the polyol pathway results in the upregulation of MMP-10. Inflammatory cytokines, such as transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, and interleukin-1, have the potential to increase the expression of MMPs in hCECs [34,35]. The corneal epithelium is exposed to cytokines that are present in the tear film produced by stromal fibroblasts. These factors may be associated with the regulation of MMP expression.

In diabetic keratopathy, weak adhesion of CECs as well as delayed wound healing can frequently be problematic. Several morphological changes that may cause loose adhesions in diabetic corneas include thickening of the basement membrane and decreased penetration of anchoring fibrils into the basement membrane [36]. In our study, increased expression of MMP-10 and weak staining of integrin α3 were observed in CECs, especially in the basal cells of galactose-fed rats. We suggest that enhanced expression of MMP-10 leads to degradation of the epithelial anchoring system. Actually, we previously showed that MMP-10 is capable of attenuating the adhesion of hCECs in vitro [27].

Based on our data, it is likely that the osmotic stress caused by increased AR activity was responsible for delayed wound healing of the corneal epithelium that is similar to diabetic keratopathy. However, it should be noted that a high glucose concentration also triggers other processes, including oxidative stress [10,11] and the production of AGEs [12-14].

It is probable that these mechanisms are also involved in the pathogenesis of abnormal corneal epithelium behavior. Interdisciplinary studies will be necessary to clarify the mechanisms underlying diabetic keratoepitheliopathy.

Our in vivo analyses had limitations. We could not confirm the effect of MMP-10 specific inhibitor during corneal wound healing. Further in vitro studies using specific inhibitor or small interfering RNA (siRNA) of MMP-10 may strengthen our findings. Alternatively, MMP-10 knockout mice can be used. Indeed, the analysis using MMP-10 knockout mice with arterial thrombosis showed limited fibrinolysis, shifting the hemostatic equilibrium toward hypofibrinolysis [37]. However, it is known that mice show little progression of diabetic or galactose-induced complications and thus the effect of ARI is not apparent, while rats and dogs are prone to strongly develop these complications [38]. The reason for this is that rats and dogs have high levels of AR expression in ocular tissue, whereas mice have extremely low AR activity [39]. Therefore, it would be difficult to estimate the levels of diabetic keratoepitheliopathy using MMP-10 knockout mice.

In summary, topical application of MMP-10 delayed the wound healing of CECs, synthesis of MMP-10 was stimulated by galactose exposure, and the expression level of integrin α3 was downregulated, suggesting MMP-10 may be overproduced in diabetic keratopathy. Oral administration of ARI counteracts the expression levels of MMP-10, resulting in more normal behavior of CECs. At present, the recurrent and persistent epithelial defects that are frequently seen in the cornea of patients with diabetes are a major clinical problem. A delay in epithelial healing may increase the risk of developing corneal haze, infectious keratitis, and ulceration. ARI treatments may be effective to promote the re-epithelialization of wounds and prevent these corneal disorders. Alternatively, it may be valuable to investigate the effect of MMP-10-specific inhibitor in normalizing the aberrant expression of MMP-10; however, further study is needed.

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