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Effect of IOP Elevation on Matrix Metalloproteinase-2 in Rabbit Anterior Chamber

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To investigate changes in the level of matrix metalloproteinase-2 (MMP-2) in the anterior chamber of rabbit with intraocular pressure (IOP) elevation. The IOP was elevated with scleral encircling in 12 rabbits. In the control group (4 rabbits), IOP was not changed after scleral encircling, and in group1 (4 rabbits) and 2 (4 rabbits), IOP was elevated about 10 and 20mmHg respectively after scleral encircling. At 2 days after scleral encircling, aqueous sampling was performed and levels of MMP-2 were checked by Western blots and gelatin zymograms. The greater the IOP elevation, the more MMP-2 expression in the anterior chmaber by Western blots and gelatin zymograms. The increase in MMP-2 expression in response to IOP elevation may have important implications for the IOP feedback control mechanism.

Key words: anterior chamber, IOP, MMP-2, rabbit

INTRODUCTION

Extracellular matrix (ECM) components in the trabecular meshwork are believed to play a major role in the regulation of normal outflow resistance through the trabecular meshwork.[1-4] The regulation of the trabecular meshwork ECM is likely to involve a family of proteinases, called the matrix metalloproteinases (MMPs).[5-6]

The MMPs found in the anterior chamber were interstitial collagenase (MMP-1), gelatinase A (MMP-2), gelatinase B (MMP-9), and stromelysin (MMP-3).[7] MMP-1 is active against native types I, II, and III collagens; MMP-2 and MMP-9 are active against denatured interstitial collagens (gelatins) and native type IV collagen, laminin, and fibronectin; and MMP-3 is active against proteoglycan core proteins, laminin, fibronectin, type IV collagen, and a number of globular proteins.[2,5,6] The expression of these MMPs in other tissue is regulated by a variety of cellular modulators including growth factors, oncogenic activation and cellular transformation, retinoids, glucocorticoids, cytokines, and transcriptional activators.[2,5,6] The modulation of ECM in the trabecular meshwork by some substances such as glucocorticoids, interlekin-1 and laser trabeculoplasty has been demonstrated.[3,4,8] ECM modulation by growth factors and mechanical forces, including pressure and flows, still needs to be addressed.[9] ECM and MMPs in the trabecular meshwork may possibly be changed by intraocular pressure (IOP) changes as a feed back mechanism. In this study we investigated the effect of IOP elevation on MMP-2

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known as a major MMP in rabbit anterior chamber.

MATERIALS AND METHODS

IOP elevation and aqueous humor preparation

Twelve New Zealand White rabbits, each weighing 2 to 2.5 kg, were used in this study. The rabbit eyes were healthy with no previous history of surgical, laser, or experimental drug treatment. The animals were anesthetized with 50 mg/kg ketamine hydrochloride intramuscularly and 30 mg/kg pentobarbital sodium intravenously. IOP was measured with a Tonopen (Mentor, Norwell, MA, USA). For IOP elevation, scleral encircling with 3 mm sponge was performed in the one eye of each of the rabbits. Under intravenous anesthesia, 360 degree periotomy and sponge encircling was performed under the four rectus muscles. All sclera encircling in this study were performed on one eye of animals by one of the authors (CY Kim) using the same methods. In the control group (4 eyes of 4 rabbits), the sclera sponge was loosely sutured for IOP maintainance. In group 1 (4 eyes of 4 rabbits), the sclera sponge was tightened for about a 10mmHg elevation of IOP. In group 2 (4 eyes of 4 rabbits), the sclera sponge was tightened for an IOP elevation about 20mmHg. Postoperative IOP was checked immediately postoperative, 1day, and 2days after scleral encircling. At 2 days after sclera encircling, aqueous humors were obtained with a 26-gauge and a 1ml tuberculin syringe from rabbits killed by the intravenous injection of sodium pentobarbital just prior to harvesting the materials.

Western blots

Aqueous samples were subjected to 15% SDS-polyacrylamide gel electrophoresis by the usual methods. Proteins were then electrophoretically transferred to nitrocellulose in transfer buffer composed of 25 mM Tris, 192 mM glycine, and 20% methanol (pH 8.8) for 30minutes at 50 Volts. The nitrocellulose membrane was then incubated with a poly clonal rabbit IgG against MMP-2 (Triple Point Biologics, U.S.A). Immunoreactive bands were visualized by enhanced chemo luminance system (ECL system, Amersham, Arlington Heights, IL, U.S.A.).

Gelatin zymography

Gelatin was added to a standard Laemmli acrlamide polymeraization mixture to a final concentration of 1mg/ml. Samples of aqueous humor were electrophoresed on a SDS-polyacrylamide gel copolymerized with gelatin. Following electrophoresis under non-reducing conditions, the gel was soaked in 2.5% Triton X-100 for 30minutes at room temperature and incubated overnight at 37°C in substrate buffer (50 mM Tris-HCl buffer, pH8.0, 5 mM CaCl2, 0.02% NaN3). After incubation, the gel was stained for 15-30 minutes with 0.5% Coomassie blue R-250 in acetic acid-isopropyl alcohol, and destained in water.

Analysis

Western blots and zymogram activity was obtained by densitometer. Sample activities of samples were analyzed using the Wilcoxon-rank Sum test.

RESULTS

Preoperative mean IOP was 10.9 ± 1.7 mmHg in the control group, 10.6 ± 1.4 mmHg in group 1, and 10.9 ± 1.7 mmHg in group 2. After scleral encircling, the immediate postoperative mean IOP was 11.4 ± 1.2 mmHg, 19.5 ± 3.3 mmHg, and 29.6 ± 3.4 mmHg in the control, group 1, and group 2 respectively. IOP in group 1 and 2 increased significantly compared with the control group ($p<0.01$). At 2 days after scleral encircling, mean IOP was reduced to almost the level of preoperative IOP; 10.8 ±(1.4 mmHg in control, 11.7 ± 1.5 mmHg in group 1, and 11.9 ± 1.8 mmHg in group 2 ($p>0.01$) (Table 1).

MMP-2 levels in group 2 showed a statistically significant ($p<0.01$) increase compared with the control group and group 1. MMP-2 levels in group 1 were also significant higher than in the control group (Fig. 1).

On gelatin zymography, the activity of the 66kDa MMP-2 band in group 1 increased compared with that of the control group and group1 ($p<0.01$). In group 1 increased MMP-2 activity with zymogram was also observed compared to the control group (Fig. 2).

Table 1. IOP changes after scleral encircling in rabbit

Group \ Time	Preoperative (mmHg)	Immediate postoperative (mmHg)	2 days after scleral encircling (mmHg)
Control	10.9 ± 1.7	11.4 ± 1.2	10.8 ± 1.4
Group 1	10.6 ± 1.4	19.5 ± 3.3*	11.7 ± 1.5
Group 2	10.9 ± 1.7	29.6 ± 3.4*	11.9 ± 1.8

**p < 0.01 by Wilcoxon-rank sum test*

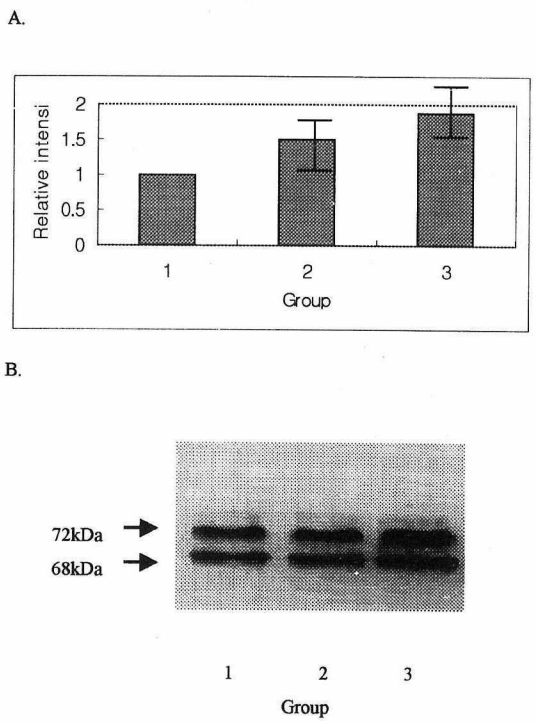

Fig. 1. MMP-2 levels in the rabbit anterior chamber by Western blots after IOP elevation by scleral encircling. (A) Relative intensity is defined as the ratio of the densitometer values of the appropriate group to the control group. The numbers 1, 2, and 3 in the X-axis refer to the control group, group 1, and 2 respectively. Bars represents the mean ± standard deviation of four experiments. (B) Western blots of MMP-2; MMP-2 level was increased upon IOP elevation.

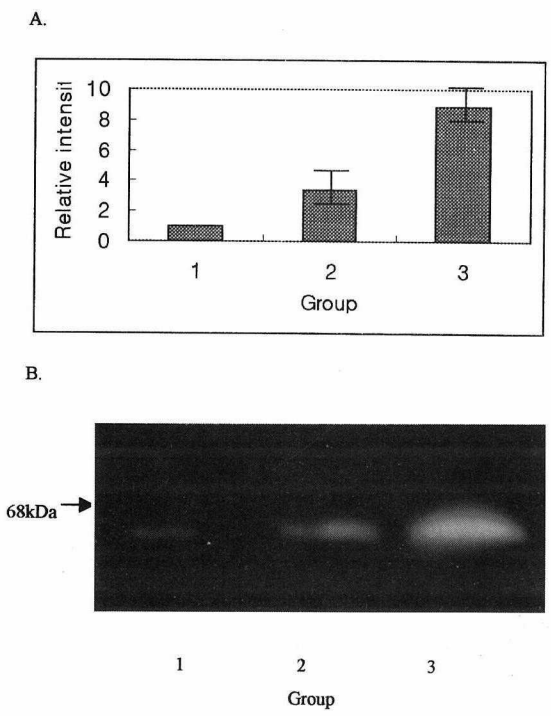

Fig. 2. MMP-2 activity in rabbit anterior chmaber with gelatin zymography after IOP elevation by scleral encircling. (A) Relative intensity is defined as the ratio of the densitometer values of the appropriate group to the control group. The numbers 1, 2, and 3 in the X-axis refer to the control group, group 1, and 2 respectively. Bars represents the mean ± standard deviation of four experiments. (B) Gelatin zymograms of MMP-2; MMP-2 activity increased upon IOP elevation.

DISCUSSION

Increased IOP in primary open angle glaucoma (POAG) is known to be caused by decreasing outflow facility in the trabecular meshwork.[10] Resistence in the trabecular meshwork is due to the extracellular matrix of Schlemm's canal and the trabecular meshwork cells.[1-4] MMP is known to degradate the ECM in trabecular meshwork,[5-6] but MMPs

and ECM modulation in trabecular meshwork still needs to be elucidated. Some type of regulation by aqueous out-flow rate or IOP can also be assumed.[1-2] Without some mechanism to couple intraocular pressure to ECM turnover, glaucoma would be a very common condition.[11] In this study we investigate the effect of IOP elevation on the levels and activity of MMP-2 in the anterior chamber by Western blots and zymograms. We elevated IOP in rabbit by scleral encircling, because the other methods of IOP elevation such as herpes virus injection,[12] protein injection[13] or prostaglandin[14] itself may alter the levels of MMPs in the anterior chamber. The scleral encircling method has a number of shortcomings in terms of IOP elevation. The amount of IOP elevation is difficult to achieve equal. Duration of the IOP elevation was not long enough, and it decreased to a preoperative level 2 days after scleral encircling. The possible mechanism of IOP elevation by scleral encircling are believed to be threefold; pupillary block,[15] impedance of venous drainage,[16] and simple mechanical indentation.[17] IOP return to preoperative levels, may occurr by increased outflow facility with increasing MMP-2 level. The possible roue of increased outflow is via the conventional pathway through the trabecular meshwork and the uveoscleral pathway. Which route is more important in terms of decreasing IOP after scleral encircling is unclear, and unfortunately the outflow facility was not checked in this study. Okada Y et al.[18] reported that MMP-2 was increased by mechanical stretching in trabecular meshwork cells, indicates that IOP elevation might increase the MMP level as part of the feedback mechanism. However other possibilities exist. Mechanical rearrangement without change of the out flow facility might influence the level of MMP-2, or inflammation after scleral encicling might change the level of MMP-2. But scleral fibroblasts seems to be too far to elevate MMP-2 in the anterior chamber, and inflammation after scleral encircling seems to be difficult to correlate well with IOP elevation as the results of this study.

Kee C et al[18] reported that abnormal expression of gelatinase A (MMP-2) in aqueous humor may be associated with the developement of POAG . If increased level of gelatinase A is a biologic response to combat elevated pressure, increased MMP-2 level in CACG would also be seen, but this wasn't found. Various MMPs in the anterior chmaber may relate in outflow facility in the trabeculat meshwork. Parshley et al[8] insisted that the argon laser trabeculoplasty effect is achieved by increasing MMP-3 in the trabecular meshwork. Which ECM in the trabecular meshwork mainly related to increased outflow facility[20] and which MMPs are related mainly to ECM degradation in trabecular meshwork are not unclear.

This study reveal the short-term effect of IOP elevation on MMP-2 level in rabbit anterior chmaber. This result implies that the increase in MMP-2 expression in response to IOP elevation may have important implications for an IOP feedback control mechanism, but more study concerning changes in MMPs and the long term effects of IOP elevation on MMP levels are required.

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