Advances in Medical Sciences 61 (2016) 6–10

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Metalloproteinase 9 and TIMP-1 expression in retina and optic nerve in absolute angle closure glaucoma

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ARTICLE INFO

Article history:
Received 10 November 2014
Accepted 24 July 2015
Available online 9 August 2015

Keywords:
Glaucoma
MMP-9
TIMP-1
Retina
Optic nerve

ABSTRACT

Purpose: Glaucoma is one of the most important reason causes of the blindness, associated with retinal ganglion cells (RGC) death. This process is not fully understood, however apoptosis due to hypoxia is one of the most important processes leading to RGC death. Glaucomatous optic neuropathy is characterized by remodeling of the extracellular matrix due to metalloproteinase activation, which leads to loss of RGC and axons at the optic nerve head.

The aim of the study was to evaluate metalloproteinase 9 (MMP-9) and tissue metalloproteinase inhibitor-1 (TIMP-1) expression in the retinal ganglion cells and optic nerve axons in 33 eyes with absolute primary glaucoma.

Material/methods: To evaluate MMP-9 and TIMP-1 expression primary polyclonal goat antibodies against MMP-9 and TIMP-1 were used. The control group was composed of 8 cases of eyes enucleated and fixed in the first day after trauma.

Results: MMP-9 expression was observed in retinal ganglion cells and in the inner nuclear layer of the retina in all the examined cases. In 28 out of 33 glaucomatous eyes, MMP-9 expression was observed in the proliferating glial cells surrounding the optic nerve axons. TIMP-1 expression was observed in 10 out of 33 glaucomatous eyes, only in retinal ganglion cells. None of the examined injured eyes showed MMP-9 and TIMP-1 expression.

Conclusions: MMP-9 activation rather than TIMP-1 may by associated with the pathomechanism of retinal ganglion cell and optic nerve damage in absolute glaucoma.

1. Introduction

Some eye diseases are associated with damage of the optic nerve axons as well as retinal ganglion cells. Glaucoma is one of the leading causes of blindness. Irreversible vision loss in glaucoma is attributed to retinal ganglion cell (RGC) death connected with apoptosis. RGC death is not fully understood, because the processes of RGC (retinal ganglion cells) apoptosis is heavily complicated [1]. Glaucomatous optic neuropathy is multifactorial in etiology [2]. The mechanism which is responsible for RGC apoptosis is associated with hypoxia, which is most frequently a consequence of increased intraocular pressure [3]. Glaucomatous optic neuropathy is characterized by remodeling of the extracellular matrix and loss of retinal ganglion cell axons at the optic nerve head level. The integrity and turnover of the extracellular matrix are influenced by many factors, including matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [4].

Matrix metalloproteinase 9 (MMP-9), also known as 92 kDa type IV collagenase, 92 kD A gelatinase or gelatinase B (GELB), is an enzyme encoded by the MMP9 gene. The enzyme encoded by this gene degrades type IV and V collagens and other extracellular matrix proteins like fibronectin, laminin, and other glycoproteins [5,6]. Among the many subtypes of metalloproteinases, some are secreted constantly, some only in certain circumstances. This is due to the regulation of the expression of tissue inhibitors of metalloproteinases (TIMPs) [6,7]. Recent studies show abnormal activation of matrix metalloproteinases, in particular MMP-9, which triggers an extracellular signaling cascade leading to apoptosis [6,8–11].

Tissue inhibitors of metalloproteinases (TIMP) are the inhibitors of matrix metalloproteinases that participate in controlling the local activities of MMPs in tissues. So far little is known about

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http://dx.doi.org/10.1016/j.advms.2015.07.007
1896-1126/

MMP-9 inhibition by TIMP-1. Data show that TIMP activity is responsible for tumor growth reduction, and inhibition of endothelial cell growth induced by basic fibroblast growth factor. TIMP-1 and TIMP-2 have antiapoptotic activity [12].

Remodeling of the optic nerve head in glaucoma involves astrocyte response and changes in the extracellular matrix composition and distribution. The MMP family has been implicated in the cascade of events leading to neuronal apoptosis in the central nervous system. MMP substrates include essentially all extracellular matrix components as well as a wide array of molecules involved in intracellular adhesion, cell–matrix interaction, and cell signaling [13–16]. MMP1, MMP2 and MMP-9 have previously been implicated in the pathogenesis of primary open angle glaucoma and open angle glaucoma secondary to exfoliation syndrome, respectively; matrix metalloproteinase-9 (MMP9) gene was investigated for association with primary angle closure glaucoma [5,17,20,22]. Classic glaucoma treatment focuses on intraocular pressure reduction. However, recent knowledge about the pathogenesis of glaucoma has opened up new therapeutic approaches. Some of the investigators suggest a pivotal role of MMP inhibition in glaucoma treatment. Inhibition of MMP-9 could inhibit the apoptosis of retinal ganglion cells and tissue remodeling [15].

The aim of the study was to evaluate metalloproteinase 9 (MMP-9) and TIMP-1 expression in retina and optic nerve in eyeballs with the primary glaucoma.

2. Materials and methods

Thirty-three eyeballs were examined and enucleated in the Department of Ophthalmology of the Medical University of Bialystok over the period 1991–2013. Patients were eligible for study participation if they had primary angle closure glaucoma (PACG) – 33 patients – and met the following criteria: blind eye, corneal edema, dilated unreactive pupil, and IOP (intraocular pressure) >50 mmHg using Goldmann aplanation tonometry. All eyeballs were removed from patients with absolute glaucoma, who suffered from severe ophthalmalgia. The study was approved by the Bioethical Committee in Medical University of Bialystok and was performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki. All patients signed an agreement form before their inclusion in the study. Thirty-three eyeballs were removed from patients with absolute glaucoma (blind eyes), who suffered from severe ophthalmalgia due to exceptionally high intraocular pressure. All of the cases were angle closure glaucoma. After enucleation, the eyeballs were fixed in a 10% buffered formalin solution on the same day of enucleation, embedded in paraffin at 56 °C, then cut into 5 μm slices and stained with hematoxilin and eosin (H+E). Following deparaffinization, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 30 min. The sections were then incubated with goat polyclonal antibody for MMP-9 (MMP-9 Antibody (C-20): sc-6840, Santa Cruz Biotechnology, Santa Cruz, CA) in 1:100 dilution, and with TIMP-1 antibody (TIMP-1 Antibody (C-20): sc-6832 Santa Cruz Biotechnology) in 1:50 dilution for a whole night at 4 °C, and labeled EnVision (DAKO) enzyme reagent and diaminobenzidine (DAB) chromogen for 5 min.

In each routine of staining, adjacent sections that were incubated without primary antibody were prepared as the negative control. Knowing MMP-9 strong expression in joint degradation in rheumatoid arthritis, synovial specimens were taken as positive controls. For TIMP-1 human prostate tissue showing TIMP-1 cytoplasmic and membrane staining of glandular cells was used as a positive control.

As a control group, we chose 8 eyeballs after severe trauma (all were closure injury). All were enucleated 1 day after the trauma, and then fixed in 10% buffered formalin. The sections were taken from the optic nerve head (1 section) and retina (3 cross-sections), and stained with H+E. Also, MMP-9 and TIMP-1 expression was evaluated using immunohistochemistry.

Two independent pathologists performed the immunohistochemical evaluations of MMP-9 and TIMP-1. The estimation of immunostaining was done under a light microscope in representative fields under magnification of 20×. The score for immunohistochemistry was as follows (as shown in Table 1,2): negative (−) if less than 10% of the examined cells were positive, (+) if 10–50% of cells were positive, and if more than 50% of the examined cells showed staining the evaluation was (++).

The obtained results were statistically analyzed using Spearman's and Pearson's tests.

3. Results

The mean age of the glaucoma group ranged from 54 to 88 years (mean age 69, SD ±12.4), with 13 male and 20 female patients. In the control injury group, age ranged from 21–77 (45, SD ±17.8) years, with 6 male and 2 female patients. Ten patients from the glaucoma group had hypertension, and six had coronary disease. Two patients from the trauma group had hypertension. There was no correlation between age and sex in both examined groups ($p = 0.983$).

3.1. MMP-9 expression in glaucomatous eyes

MMP-9 expression was observed in the perinuclear area of the examined cells.

28 out of 33 glaucomatous eyes presented MMP-9 expression in the optic nerve. It was mainly observed within the proliferating astrocytes surrounding the optic nerve axons; in 8 cases the expression was very strong and diffused (Fig. 1A and B). The results are summarized in Table 1.

All glaucomatous eyes presented MMP-9 expression in the retina. MMP-9 staining was observed both in the inner nuclear layer of the retina (Fig. 1C) and in the layer of the retinal ganglion cells (Fig. 1D).

All cases with MMP-9 expression localized in the optic nerve astrocytes also presented MMP-9 expression in retinal ganglion cells and the inner nuclear layer of the retina.

We did not observe MMP-9 expression in retinal ganglion cells, in the inner layer, and optic nerve in all eyes after trauma.

3.2. TIMP-1 expression

TIMP-1 expression was observed as cytoplasmic staining.

TIMP-1 expression was observed only in 10 out of 33 glaucomatous eyeballs with angle closure glaucoma. The expression was not observed in the inner nuclear layer of the retina; in all 10 cases the staining was observed in the retinal ganglion cell layer (Fig. 2A and B). None of the glaucomatous eyeballs showed TIMP-1 expression, neither in the optic nerve axons or proliferating astroglia (Fig. 2C and D). The results are shown in Table 2.

None of the injured eyeballs showed TIMP-1 expression, neither in the retina nor the optic nerve.

Table 1
MMP-9 expression in the glaucomatous eyes.

MMP-9 expression	Negative No %	(+) No %	(++) No %	p value
Optic nerve axons	5 15.1%	20 60.6%	8 24.3%	0.736
Retinal ganglion cells	0 0.0	28 84.8%	5 15.2%	
Inner nuclear layer of the retina	0 0.0%	28 84.8%	5 15.2%	