

Chapter 19

Role of Metalloproteases in Retinal Degeneration Induced by Violet and Blue Light

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Abstract

Introduction: An essential role for metalloproteases (MMPs) has been described in blood vessel neof ormation and the removal of cell debris. MMPs also play a key role in degenerative processes and in tumors. The participation of these enzymes in light-induced phototoxic processes is supported by both experimental and clinical data. Given that patients with age-related macular degeneration often show deposits, or drusen, these deposits could be the consequence of deficient MMP production by the pigment epithelium.

Objective: To gain insight into the regulation of metalloproteases in the pathogenesis of retinal degeneration induced by light.

Materials and Methods: We examined the eyes of experimental rabbits exposed for 2 years to circadian cycles of white light, blue light and white light lacking short wavelengths. For the trial the animals had been implanted with a transparent intraocular lens (IOL) and a yellow AcrySof® IOL, one in each eye. After sacrificing the animals, the retinal layer was dissected from the eye and processed for gene expression analyses in which we examined the behavior of MMP-2, MMP-3 and MMP-9.

Results: MMP-2 expression was unaffected by the light received and type of IOL. However, animals exposed to white light devoid of short wavelengths or those fitted with a yellow IOL showed 2.9- and 3.6-fold increases in MMP-3 expression, respectively compared to controls. MMP-9 expression levels were also 3.1 times higher following exposure to blue light and 4.6 times higher following exposure to white light lacking short wavelengths or 4.2 times higher in eyes implanted with a yellow IOL.

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Conclusion: Exposure to long periods of light irrespective of its characteristics leads to the increased expression of some MMPs. This alteration could indicate damage to the extracellular matrix and have detrimental effects on the retina.

19.1 Introduction

The exposition to light radiation causes 3 types of damages on the retina: photomechanical, photothermal and photochemical damages. Light-induced damages mechanism is not exactly known, although there is a lot of references about the effects of light on the retina (Wenzel et al. 2001, Wu et al. 2006), also the mechanism to avoid these damages. In order to gain insight into the knowledge of the phototoxic damages causes by light radiation, it is necessary to focus the analysis in the level of the genetic expression of some possible mechanisms that are involved into the light-induced phototoxic processes. One of the possible mechanisms, which affects the degenerative processes, is the one in which are involved, a serial of proteins called metalloproteases (MMPs).

MMPs are a family of proteins that degraded, in a very selective way, the components of the basal layer and the extracellular matrix. An essential role for metalloproteases (MMPs) has been described in blood vessel neof ormation and the removal of cell debris. MMPs also play a key role in degenerative processes and in tumors. MMPs are involved in every process that concerns the extracellular matrix restructuring and they act in a balanced way with their *endogenous inhibitors*, the TIMs (tissue inhibitor of MMPs).

Actually, 20 MMPs, grouped in 4 families: (1) collagenases (MMP-1, 8 and 13), which hydrolyze the interstitial collagen; (2) gelatinases (MMP-2 and 9), which hydrolyze the denatured collagen and some non-fibrillar proteins; (3) the family of stromelysin (MMP-3, 7, 10, 11 and 12) and 4) MMPs jointed to membranes (MMP-14, 15, 16 and 17). In this study 3 types of MMPs have been analyzed: MMP-2, MMP-3 y MMP-9.

These enzymes role in light-induced phototoxic processes is supported by both experimental and clinical data. Plantner (Plantner et al. 1991; Plantner 1992; Plantner and Drew, 1994) was the first on describing the presence of MMPs, specifically MMP-1, MMP-3 and MMP-9, on the matrix situated between the photoreceptors; later, the presence of MMP-2 was confirmed to increase due to light exposure (Plantner et al. 1998a). Light-induced retina's overstimulation causes an increase in the expression of the MMP-9 or gelatinase B, regardless of whether there's a lost of photoreceptors or not (Papp et al. 2007). In the other hand, laser's photocoagulation makes the pigment epithelium to produce MMP-2, MMP-3 and MMP-9 (Flaxel et al. 2007).

Given that patients with age-related macular degeneration (AMD) often show deposits, or drusen, these deposits could be the consequence of deficient MMP production by the pigment epithelium (Elliot et al. 2006). Also, there are many cases of patients with AMD and blood vessel neof ormation, what suggests also the MMPs influence in the process.

In a general way, proteases from MMPs family are involved in every biological process that entails the extracellular matrix restructuring of basal layers and blood vessel neoformation. One of the characteristics of light-induced pathologies in humans, also in experimental models, is the appearance of not-well-characterized deposits, drusen. Because of this, these deposits could be a consequence of a reduction on the expression of MMPs, also this could shown the inability of the MMPS to degrade these deposits, that shape the drusen.

Because of and in order to contribute to a better knowledge of permanent and long-term (2 years) lighting effect on the retina, rabbit eyes (clear and yellow intraocular lenses, IOLs have been implanted) under different permanent lighting conditions have been studied: white light, blue light and white light without the blue-light part (called here yellow light).

19.2 Objective

To analyze the phototoxic effect of light on the retina and its prevention by using blue-light filtering IOLs. More specifically, to gain insight into the regulation of metalloproteases in the pathogenia of retinal degeneration induced by light.

19.3 Materials and Methods

Experimental rabbits eyes exposed for 2 years to circadian cycles of white light, blue light and white light lacking short wavelengths were examined. Also, animals had been implanted with a clear IOL (left eye) and a yellow AcrySof[®] IOL (right eye), one in each eye (Table 19.1). After sacrificing the animals, the retinal layer was dissected from the eye and processed for gene expression analyses in which we examined the behavior of MMP-2, MMP-3 and MMP-9 (Fig. 19.1 and Table 19.2).

Table 19.1 Lighting conditions diagram (yellow, white and blue) in combination with IOL's (clear/left eye, yellow/right eye) implanted in rabbits of the sample. IOL's and surgery used characteristics follows the protocol established for the Escuela de Óptica (UCM)

ID	Surgery (eye)		Light	Time (years)
	Left	Right		
13	Clear	Yellow	Yellow	2
G1	Clear	Clear	Non exposure	2
G3	Clear	Yellow	White	2
G5	Clear	Yellow	Yellow	2
G6	Clear	Yellow	Blue	2
E2	Clear	Yellow	Blue	2,5

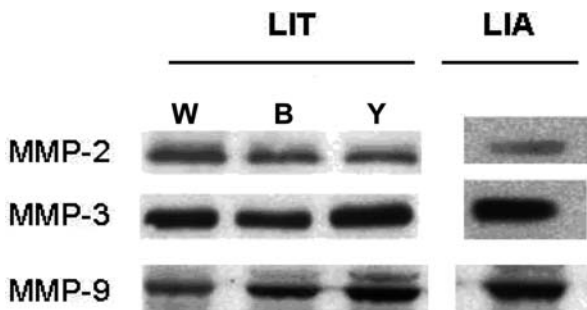


Fig. 19.1 Both eyes hemi-retinas of animals exposed to the same lighting levels were processed together. Total mRNA was extracted from the different experimental group's retinas by using the TRI reagent (Sigma; St. Louis, MO), also RNA integrity was quantified with agaroses gel electrophoresis. RNA extracted was processed for inverse transcription with the tampon solution provided by the laboratory Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, UK). Primers sequences used are base on the sequences published for mouse (GenBank accession). Reaction was made on a cyclator (Hyband Th. Cyclor). PCR final product was visualized by using a tinction of bromure ethidium under UV light follows by electrophoresis in agaroses gel (2%). Products were quantified in a PhosphorImager (Fuji)

Table 19.2 Primers used in the study, which have been manufactured following previous bibliography, concerning genetic sondas were made similar as mouse's genome. Every hybridation's results were satisfactory

Metalloproteases	
•	MMP-2: up: 5'-CCA CTG CCT TCG ATA CAC-3', down: 5'-GAG CCA CTC TCT GGA ATC TTC AAA-3'
•	MMP-3: up:5'-GCT TTG AAG GTC TGG GAG GAG GTG-3',down: 5-CAG CTA TCT TCC TGG GAA ATC CTG-3'
•	MMP-9: up: 5'-GTT CCC GGA GTG AGT TGA-3', down: 5'-TTT ACA TGG CAC TGC AAA GC-3'

19.4 Results

These study findings indicate that exposure to long periods of light increases the expression of some MMPs and this could have harmful effects on the retina since it indicates damage to the extracellular matrix. Increased MMP expression could determine the faster turnover of the extracellular matrix to avoid the formation of matrix deposits.

Light exposure or the intraocular implant of a yellow lens does not modify MMP-2 expression. In animals exposed to light, lacking the blue portion of the spectrum, and in animals implanted with a yellow IOL, MMP-3 expression was 2.9 and 3.6 times higher than in controls, respectively. Similar behaviour was observed for MMP-9 expression which was upregulated in: animals exposed to blue light (3.1 times), animals exposed to white light lacking the blue portion of the spectrum (4.6 times) and animals fitted with a yellow intraocular lens (4.2 times). Light

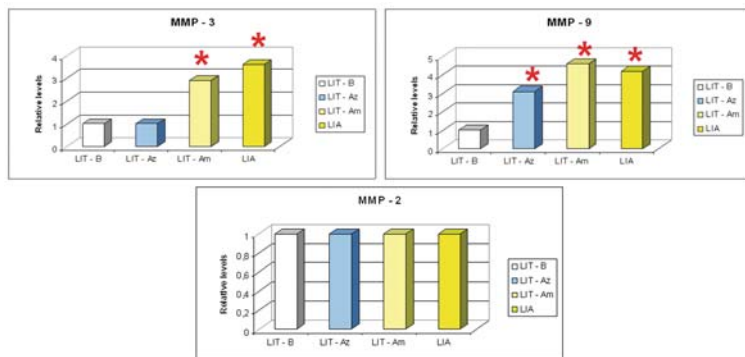


Fig. 19.2 Results obtained for MMP-2, MMP-3 and MMP-9. LIT-B: white IOL; LIT-Az: blue-light IOL; LIT-Am: yellow (white light without the blue-light part); LIO: yellow IOL

exposure results in no changes in the expression for MMP-2, whereas MMP-3 and MMP-9 were up regulated, especially in the animals exposed to white-filtered light and carrying a yellow intraocular lens (Fig. 19.2).

These results agree partially with other animal model trials published before. There no modifications in MMP-2 expression, but Plantner (Plantner et al. 1998) found it increased in animals exposed to light. In the other hand, data concerning MMP-9 expression are coincident with the obtained for Papp (Papp et al. 2007). In general, these result can't support the hypothesis that drusen are a consequence of MMPs production drop in pigment epithelium (Elliot et al. 2006).

These results analysis can be made in 2 ways. First, long-term lighting exposure, irrespective of its characteristics, increases some MMPs expression and that could damage the retina, because this would indicate extracellular matrix injuries. In the other hand, the increase in the expression of the MMPs would be related with an accelerated turnover of the matrix to avoid the appearance of deposits that give rise to drusen.

19.5 Conclusion

Exposure to long periods of light irrespective of its characteristics leads to the increased expression of some MMPs. This alteration could indicate damage to the extracellular matrix and have detrimental effects on the retina.

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