Chapter 2

Trabecular Meshwork and Intraocular Pressure Dynamics: Oxidative Stress-Induced Changes

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66138

Abstract

Glaucoma is known as progressive neurodegenerative disease with irreversible loss of vision. Next to perimetric visual field defects, morphological alterations of the optic nerve and an increased intraocular pressure (IOP) occur. IOP is a fine regulated, complex homeostasis of production of aqueous humor (AH) in the non-pigmented ciliary body and its outflow. About 80% of AH is drained throughout the trabecular meshwork (TM). Any outflow resistance, with consecutive increase in IOP, can be generated by a decreased pore size or any other alterations of TM, making it more rigid. Oxidative stress, a disbalance of oxidants and antioxidants, is one mechanism, causing an altered extracellular matrix (ECM), and seems to play a key role in the pathogenesis of glaucomatous nerve atrophy. Damage of DNA, caused by oxidative stress, was shown in TM cells of glaucoma patients. This chapter gives a review about oxidative stress and its pathological alterations in the main outflow pathway—the trabecular meshwork—in glaucoma patients.

Keywords: glaucoma, trabecular meshwork, intraocular pressure, oxidative stress

1. Introduction

Glaucoma is known as a progressive neurodegenerative disease with irreversible loss of vision. Next to perimetric visual field defects, morphological alterations of the optic nerve and an increased intraocular pressure (IOP) occur. Additionally, special examinations were investigated in the last years for glaucoma diagnosis and follow-up [1–4].

IOP is a fine regulated, complex homeostasis of production of aqueous humor (AH) in the non-pigmented ciliary body and its outflow. About 80% of AH is drained throughout the trabecular meshwork (TM) [5], a sponge-like network of TM cells with glycoproteins, hyaluronic acid, elastic fibers, collagen and extracellular matrix (ECM). Any outflow resistance can be generated by a decreased pore size or any other alterations of TM, making it more rigid, resulting in an increased IOP. TM cells have the ability of different functions. Up to date it is not known, if cellular or intracellular disturbances are the origin of the altered TM in glaucoma. It is also conceivable that both mechanisms are impaired.

Oxidative stress is one mechanism, causing an altered extracellular matrix (ECM) [6] due to a disbalance of oxidants (free radicals, ROS) and antioxidants. If there is any imbalance between both with preponderance of oxidants, an irreversible cell loss is induced. Oxidative stress seems to have a key role in the pathogenesis of glaucomatous nerve atrophy [7, 8]. Damage of DNA, caused by oxidative stress, correlates significantly with IOP as well as with visual field defects [9, 10], and could be shown in TM cells in glaucoma patients [11]. Additionally, an increased lipid peroxidation, being the result of a ROS-induced cell damage, was noticed [12] as well as an impairment of metalloproteinases-2 (MMP-2) expression, resulting in an accumulation of ECM [13]. This accumulation (i.e., 'plaques') in TM could be seen several years before by electron microscopy studies [14, 15]. Additionally, actin expression can be induced by oxidative stress [16, 17], which is responsible for the rigidity of TM—the more amount of actin, the more rigid the TM [18].

In which way is oxidative stress induced, especially in glaucoma patients? Certainly, endogenous and exogenous factors contribute together. For example, daylight (especially ultraviolet light) can induce ROS in AH [19, 20]. Further on a vascular dysregulation [21] is discussed in glaucoma pathogenesis. An impaired blood flow with decreased nutritive supply can result in hypoxia, the main factor of inducing oxidative stress [22]. This hypothesis is supported by an increased concentration of vascular endothelial growth factor (VEGF) and erythropoietin in samples of AH in glaucoma patients [23, 24].

2. Oxidative stress and glaucoma

2.1. Oxidative stress: ROS and RNS

Oxidative stress was first described by Sies [25]. It is the result of an imbalance between oxidant (free radicals, reactive oxygen species and ROS) and antioxidant agents. Commonly, it is assumed that an excess of ROS, which follows from an increased production or decreased depletion, is the main reason for oxidative stress. Furthermore, a reduced antioxidant level can cause elevated ROS levels [26]. This imbalance with a preponderance of ROS results in a cytotoxicity with an irreversible cell loss.

The molecular basic for this damage can be found the reactivity of ROS. Free radicals are very aggressive molecules due to their unpaired electron [27]. They are generated under aerobic conditions. Excessive physical activities, infections or toxicity (e.g. cigarette smoke, UV

radiation), are only some of the ROS causing agents. Even microwave radiation was found to induce oxidative stress [28]. ROS production can be found mainly in mitochondria [29]. Electrons (e^-) and protons (H^+), which were released by oxidation of fats and carbohydrates, enter the 'electron transfer chain.' In this chain, several consecutive reactions transfer electrons from NAPH to O_2 (oxygen) via respiratory enzyme complexes [30]. 'NADH dehydrogenase complex' transfers the electrons from NADH via flavin and iron-sulfur centers to ubiquinone. At this point, the second complex 'cytochrome b-c1' passes the electrons to cytochrome c, containing the 'cytochrome oxidase complex'. This step forwards the electrons to react with O_2 [30]. Almost 98% of O_2 is reduced to H_2O. The remaining 2% were reduced only incompletely, resulting in the production of ROS [31]. Considering this fact, it is of interest that not only the biochemical alterations of free radicals, but also the cellular energy level (redox status) is important, when talking about oxidative stress [32].

Several molecules are subsumed in the ROS group, which are all intermediate products, originated from O_2: hydrogen peroxide (H_2O_2), singlet oxygen (½ O_2), hydroxyl radical (HO) and superoxide (O_2^-). Molecular O_2 reacts with a single electron, followed by the uptake of several protons. This reaction produces the reactive oxygen species [33]:

$$O_2 + e^- \rightarrow \mathbf{O_2}\cdot^- \tag{1}$$

$$O_2\cdot^- + H^+ \rightarrow HO_2 \tag{2}$$

$$HO_2\cdot + e^- + H^+ \rightarrow \mathbf{H_2O_2} \tag{3}$$

$$H_2O_2 + e^- + H^+ \rightarrow \mathbf{HO}\cdot + H_2O \tag{4}$$

$$HO\cdot + e^- + H^+ \rightarrow H_2O \tag{5}$$

If iron reacts with ROS, the production of further ROS is catalyzed (Fenton reaction, [33]).

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + FeO^{2+} + H^+ \rightarrow \mathbf{HO}\cdot + HO^- + Fe^{3+} \tag{6}$$

The hydroxyl radical is the most powerful radical, which is known up to now. It can cause membrane peroxidation and protein carbonylation [33], dectract or add electrons and hydrogen to the DNA, resulting in further radicals [34–36].

Organisms show diverse cellular escape mechanisms against ROS, which eliminates superoxide under normal conditions [29, 33, 37–39]:

$$2\mathbf{O_2}\cdot^- \rightarrow \text{½ } O_2 \tag{7}$$

$$2\mathbf{O_2}\cdot^- + \text{SOD} \rightarrow H_2O_2 + \ O_2 \tag{8}$$

$$\mathbf{H_2O_2} + \mathbf{O_2}^- \rightarrow O_2 + \text{HO}\cdot + \text{HO}^- \tag{9}$$

$$\mathbf{H_2O_2} + \text{Katalase} \rightarrow H_2O + \text{½ } O_2 \tag{10}$$

$$\mathbf{H_2O_2} + 2\ \text{GSH} + \text{GSH} - \text{Peroxidase} \rightarrow \text{GSSG} + 2\ H_2O \tag{11}$$

SOD superoxide dismutase

GSH glutathione

GSSG glutathione disulfide

At 'normal' ROS levels, these free radicals are interacting with other cellular molecules in cell regulation, transcription and cell proliferation. Additionally, they are very important as protective mechanism against distinct bacteria or fungi [26, 29, 33]. However with increasing amounts, ROS changes its function from defensive to aggressive.

Next to ROS, N-containing radical molecules were known to have oxidative power [40, 41]. Reactive nitrogen species (RNS) are derived from the nitric oxide (NO). Free radicals react rapidly with NO, forming RNS [42, 43]:

$$O_2\cdot^- + \text{NO}\cdot \rightarrow \mathbf{ONOO}^- \tag{12}$$

$ONOO^-$ peroxynitrite

Superoxide can be built by diverse reactions (see above) under different conditions. High concentrations of lipoproteins disturb the NOS pathway, which cause an increased amount of superoxide [25]. Peroxynitrite is not a stable molecule. It reacts very fast with almost all classes of biomolecules, thus being a potential cytotoxic agent [42]. It works similar to hydroxyl radicals, thus inducing DNA damages with breaks, nitrations and oxidations [36]. Unlike H_2O_2 peroxynitrite can be degraded without metal ions by inducing radicals [44, 45]:

$$ONOO^- + H^+ \rightarrow \text{OH}\cdot + \cdot NO_2 \tag{13}$$

Peroxynitrite can react with several molecules. Next to molecules with aromatic structures (e.g. alpha-tocopherol [46]), thiols [47] can be modified. These reactions generate further radicals, which can induce themselves peroxidation in lipids [48].

In which way does ROS or RNS accumulate? Is there an alteration at the protein or even gene level, which results in this imbalance? Which external factors induce this 'program?' The final answer to all these questions is open up to now. There are evident hints that several transcription factors are involved in this complex network (e.g. p53, [49]).

2.2. Oxidative stress: molecular interactions

ROS and RNS can react with even each biochemical molecule (e.g. lipids, proteins). Cell membranes, containing a high amount of phospholipids and triglycerides, are one of the targets [50, 51]. After peroxidation of the lipids, bond arrangement and nonenzymatic Hock cleavage start. The produced α, β polyunsaturated lipid aldehydes act as radicals in turn [52]. Thus, a reaction chain is triggered. Additionally, the membrane structure and integrity are disturbed [53]. In particular, nerve structures are even more sensitive for oxidative stress as showing a large amount of lipids and high energy requirement [54].

Additionally, free radicals attack the ion channels within the cellular [55] as well as intracellular membranes [56]. The conductance is significantly altered, resulting in an altered excitability of the membranes. After free radical attacks proteins change their backbone and/or side chain structure in that way, that it reacts itself with other amino acid side chains to build carbonyl chains. Unfolding, misfolding and consecutive loss of activity remains [57].

As third target, ROS/RNS attack DNA by purine or pyridine bases structure changes. Yet, protein cross-linking and breaks in the DNA string were detected [39, 58]. These mutations can concern nuclear as well as mitochondrial DNA (mtDNA). However, in particular, mitochondrial DNA is affected, resulting in an impaired energy production [59, 60].

Why are mutations more dangerous, when affecting mitochondrial than nuclear DNA? Several hypotheses were discussed in this context. It is assumed that because of the local closeness of the enzyme complexes, which are involved in the electron transfer chain, next to the mitochondrial DNA, the produced ROS can attack the mtDNA more severe [61–63]. Additionally, mtDNA can replicate independently of the S-Phase, thus being more sensitive for oxidative agents, when the redox level is more oxidative [64], and free nucleotides are low [65]. Recent reports even suggest that hPrimpol1/CCDC111 (human primase-polymerase 1) is involved in DNA reparation due to its primase and DNA polymerase activity [66].

2.3. Oxidative stress: glaucoma

The influence of oxidative stress is discussed in the pathogenesis of several neurodegenerative diseases, like Morbus Parkinson or Morbus Alzheimer, as well as ophthalmic disorders, for example, glaucoma. The pathogenesis of glaucoma, being the second cause for blindness [67], is unknown up to now.

Glaucoma seems to be a multifactorial disorder. The most important risk factor for converting to glaucoma or glaucoma progression can be seen in the IOP [68, 69]. However, not all patients show a stable glaucoma disease after IOP regulation, and thus, several other factors seem to be involved. 'Vascular dysregulation' [21], glutamate exotoxicity [70], an interrupted retrograde transport of neutrophins [71] as well as ocular ischemia [72–74] and oxidative stress [7, 8, 75–77] are established in this discussion.

Glaucoma is characterized by an altered optic disc, visual field defects and an elevated intraocular pressure (IOP). As consequence of the increased IOP, retinal ganglion cells (RGC) and axons will die and cause the typical optic nerve appearance with glaucomatous excavation. Free radicals are known to induce oxidative stress in retinal ganglion cells, resulting in apoptosis of these cells [78]. Trapping ROS/RNS with NF-E2 related factor 2 (Nrf2), the death of RGC, seems to be prevented [79]. Several studies have been performed to investigate the potential influence of oxidative stress in glaucoma patients. It was shown that the L-arginine/nitric oxide system is altered in patients with glaucoma [80]. Increased concentrations of superoxide dismutase, malondialdehyde and glutathione peroxidase could be detected in aqueous humor of patients with primary open-angle glaucoma (POAG) [81, 82]. Additionally, antioxidant activity was shown to be decreased in POAG [82]. If systemic antioxidant reserve is low, more severe perimetric defects occurred [83, 84]. Further on the DNA damage, caused by oxidative stress, correlates significantly with glaucomatous visual field defects and IOP [9, 10].

Up to now, there are evident hints for a critical role of oxidative stress in the pathogenesis of glaucoma, yet there are several questions unanswered. However, oxidative stress induces change within different ocular structures, whereas alterations in tissues, influencing IOP, are of special interest.

3. Intraocular pressure

Intraocular pressure is the result of aqueous humor (AH) production and outflow through trabecular meshwork and uveoscleral. This circular flow is regulated until a steady state arises. The production of AH starts at the non-pigmented ciliary body. Plasma of the vascular plexus is filtrated by diffusion, ultrafiltration and active secretion to build the AH. First of all, lipid-soluble molecules diffuse according to a gradient. Second, water and water-soluble molecules are ultrafiltrated because of an osmotic gradient and hydrostatic pressure [85]. Active secretion completes the process of AH production [86]. Electrolytes flow due to an electrochemical gradient [87] into the interstitial space, whereas protein transporters, ion channels, aquaporins and enzymes work together to pass molecules, ions or ascorbic acid through the blood-aqueous barrier [88–91]. Liquid diffusion is a consecutive result of the movement of electric charge [92], which is completed by the work of aquaporins.

The produced AH is similar to plasma, yet proteins are 200× less and ascorbic acid 20× higher than in plasma [93]. Additionally, endothelin-1 [94], several growth factors [95], enzymes

(collagenase) [96] or Immunoglobulin G [97], intrinsic glycoproteins [98] and transferrin [99] can be detected.

After production in the posterior part of the eye, AH flows through the pupil. Convection effectuates the movement of AH in the anterior chamber. AH circulates with an upward trend at the cornea and downstream flow at the iris [100]. The outflow of AH is provided by two pathways: The trabecular meshwork in the anterior chamber angle drains off the AH into the Schlemm's canal. The fluid passes multiple intrascleral collector canals until drainage into episcleral veins, ciliary veins and veins of the extraocular muscles [101, 102]. The second draining pathway is the uveoscleral outflow. Through the uveal meshwork and the anterior parts of the ciliary muscle, AH flows toward suprachoroidal space and sclera, where it is resorbed by several veins [103–105].

To generate the IOP distinct, resistance factors have to be considered: the conventional outflow pathway via trabecular meshwork with resistance of 3–4 mmHg/μl/min and the episcleral vein pressure of 8–10 mmHg [106–108] results in IOP of about 15.5 ± 2.57 mmHg in normals [109–111].

Because the outflow through the trabecular meshwork (TM) is dependent on resistance of the trabecular meshwork, the factors, influencing resistance in TM, are of special interest in IOP.

4. Trabecular meshwork

In patients with POAG, the trabecular meshwork is characterized by an increased outflow resistance leading to an elevated IOP. It is assumed that the increased outflow resistance is attributed to various morphological and biochemical alterations of the TM of glaucomatous eyes. So have electron microscopic and immunohistochemical studies of TM cells demonstrated an increased accumulation of extracellular matrix, the so-called plaque material, in the juxtacanalicular region of the TM [112, 113]. An increased accumulation of fibronectin was found in the TM of glaucomatous donor eyes as compared to the TM of control eyes [114]. In addition, TM cells showed an increased cell loss as compared to age-matched control TM cells [115]. Later, Liton et al. have also demonstrated an accelerated senescence of TM in comparison with age-matched control donors [116].

The reasons for these changes are still not clear. One factor, which may play an important role in the pathogenesis of POAG, is oxidative stress [117]. Patients with POAG showed mutations in the mitochondrial genome and a reduced mitochondrial respiratory activity compared to control groups [118]. Also the antioxidative capacity in the aqueous humor of patients with POAG was found to be reduced by more than 50% compared to non-glaucomatous eyes [119]. Furthermore, an increased level of 8-hydroxy-2′-deoxyguanosine, a molecular biomarker for oxidative damage, was detected in TM samples collected from glaucoma patients undergoing standard filtration surgery [10]. The oxidative damage in these cells correlated with the visual field defects of these operated glaucoma patients [10]. Besides that, oxidative stress can also

induce inflammation of the TM cells [8] and alter their mobility [120], which leads to contractile dysfunction and damage of the TM cells. Thus, these studies suggest that oxidative stress may be involved in the pathogenesis of POAG.

The human TM is a highly sensitive tissue to oxidative stress, since it is equipped with few antioxidant defense mechanisms [121, 122]. *In vitro* studies showed that increased levels of hydrogen peroxide stimulated the migration of TM cells, which lead to trabecular thickening and enlargement or collapse [123]. Furthermore, hydrogen peroxide could also cause endothelial cell loss and thus a disruption and collapse of the TM [124]. It is assumed that TM cell loss is caused by oxidative stress-induced apoptosis via inflammation, mitochondrial damage, hypoxia and endothelial dysfunction [125]. We could demonstrate that oxidative stress in form of hydrogen peroxide was able to induce cell death, extracellular matrix production, and accelerated senescence in cultured human TM cells. Based on these studies, it was postulated that oxidative stress may play a role in cell death, release of inflammatory markers, extracellular matrix accumulation, advanced senescence and disarrangement of the cytoskeleton of TM cells. These oxidative stress-induced TM changes may be responsible for a reduced outflow facility and thus an increased IOP.

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