Oxidative stress in primary open-angle glaucoma

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1. ABSTRACT

Glaucoma is the second leading cause of blindness worldwide and is still a mysterium. Despite some risk factors are known, like individually elevated intraocular pressure, myopia, age, genetic factors, as well as vascular risk factors and smoking, the exact mechanism developing a glaucomatous optic neuropathy are still unknown. In the pathogenesis of glaucoma oxidative stress seems to play an important role. The mitochondria have an abnormal DNS and the antioxidative capacity is reduced. In addition, in the anterior segment, e.g. trabecular meshwork as well as in the posterior pole glaucoma patients show an increased oxidative stress. Therefore oxidative stress should be considered in therapeutic approaches to glaucoma patients.

2. INTRODUCTION

Primary open-angle glaucoma (POAG) is described by the European Glaucoma Society as an optic neuropathy with characteristic visual field loss and papillary abnormalities, whose origin is regarded as a multifactorial phenomenon. In addition to individually elevated intra-ocular pressure and excessive diurnal pressure fluctuations, genetic factors, increasing age, myopia, vascular risk factors, and smoking are other risk factors for the development of a glaucomatous optic neuropathy. However, the precise pathogenetic mechanisms which lead to this socio-economically important condition are still largely unknown, although some insight into the complex biochemical processes involved has been gained in the past 20 years. For this

reason, the main focus has been on the cellular mechanisms triggered by artificially increasing intra-ocular pressure, to enable the effects of elevated intra-ocular pressure on the structures of the eye to be investigated more precisely in an experimental manner. This has made it possible to show that the structures of both the anterior and posterior chambers of the eye react to elevated intra-ocular pressures, resulting in major biochemical changes. One of the most important intermediate stages occurring between the elevation of intra-ocular pressure and the resulting biochemical changes in the eye is oxidative stress. This is generally understood to mean an imbalance between the formation of free radicals / reactive oxygen species and/or a reduction in the protective systems that buffer the free radicals (1). The cells are thereby flooded with free radicals, and direct and indirect cellular damage occurs, which may lead to organ transformation with a corresponding loss of function. Due to increasing knowledge of the functions of the cell, especially of the mitochondrion, the definition of oxidative stress has recently changed, in that it is understood to be mainly a disturbance of mitochondrial redox signal transmission and redox control (2). Due to these biochemical processes, structural changes also occur on the cell surface, which may lead to immunological reactions, such as auto-immune reactions for example. In addition, oxidative stress causes transformation processes in the retinal ganglion cells and nerve fibres, which result in accelerated apoptosis of the cells.

To summarise, it is useful to have a good knowledge of the processes relating to oxidative stress in glaucoma, because oxidative stress plays an important role as mediator in the subsequent reaction chains, as a general form of biochemical reaction, and this may give rise to new therapeutic options. We will structure our presentation of the current state of knowledge of oxidative stress in POAG by describing the experimental observations and clinical findings for the anterior and posterior chambers of the eye separately.

3. OXIDATIVE STRESS AND THE ANTERIOR CHAMBER

3.1. General

The human eye is exposed to powerful photo-oxidative stress during the day and therefore needs a correctly-functioning buffer system in order to scavenge the free radicals and reactive oxygen species (ROS) constantly being formed. Hydrogen peroxide (H_2O_2) (3) and superoxide anions, for example, have been found in the aqueous humour. In the anterior chamber of the eye, a particular additional feature is increased stress on the structures due to metabolic end-products which are released into the aqueous humour by the avascular structures of the cornea and lens. The metabolites result from the process of mitochondrial respiration and other metabolic processes and may exhibit the properties of free radicals. This is why an effective protective system consisting of several individual components is necessary. For example:

3.1.1. The aqueous humour acts as a stream of fluid that flushes out free radicals

The aqueous humour, of which approximately 80% is actively secreted and is subject to dynamic

neurohumoral secretion control, is formed at the rate of 3.1 µL/minute of aqueous humour in the morning, whilst the output at night is 2.3 µL/minute and drops to 1.6 µL/minute during sleep (4). This ensures that the aqueous humour is replaced approximately every 1½ - 2 hours at the times when light stress is greatest, and therefore free radicals, cell debris and metabolites are flushed from the eye.

3.1.2. Water-soluble antioxidants

The composition of the aqueous humour can be specifically influenced. This applies in particular to ascorbic acid, which is actively transported into the aqueous humour via a Na^+/ascorbate cotransporter (5). Thus very high vitamin C concentrations can be attained in aqueous humour as compared to those in plasma (6). Ascorbic acid (AA) is one of the water-soluble essential nutrients and consequently it must be ingested in food. Its physiological importance lies in collagen biosynthesis, in the breakdown of cholesterol and in the synthesis of neurotransmitters. It is an effective antioxidant, regenerates vitamin E, detoxifies toxic metabolites and stimulates the immune system. In the aqueous humour, AA scavenges 75-85% of free radicals (7) and is therefore the most significant free radical scavenger. In addition, the administration of AA induces a lowering of intra-ocular pressure (8, 9, 10). The action mechanism of AA is still under debate. On the one hand, an osmotic effect has been hypothesised (11), and, on the other, AA has been found to improve trabecular outflow (12), which may be due to stimulation of hyaluronic acid in the trabecular meshwork (TMW) (13). In addition, AA can dose-dependently influence the metabolism of TMW cells (14).

Further water-soluble antioxidative substances in the aqueous humour include L-tyrosine (15) and glutathione (16). Glutathione has been shown to buffer hydrogen peroxide (H_2O_2) in the anterior chamber of the eye (17).

3.1.3. Fat-soluble antioxidants

Vitamin E (tocopherol) is a fat-soluble essential nutrient and is regarded as the most important lipophilic chain-breaking antioxidant in the human body. Following oral administration, elevated levels of glutathione have been detected in plasma and also in the aqueous humour in man (18), with the consequential hypothesis that vitamin E has an indirect stimulant effect on the antioxidant potential of the aqueous humour.

3.1.4. Specific enzymes

Both catalase (19) and superoxide dismutase (20) have been found in the epithelium and endothelium of the cornea, in the lens, particularly in the epithelium of the lens, in the iris and in the ciliary body, as well as in the TMW (21). There are indications of the presence of glutathione in the iris, ciliary body (22) and TMW (23).

3.2. Age-related changes

As the secretion of aqueous humour is an active transport process, it too is subject to age-related changes. It is believed that approximately 20% less aqueous humour is formed by the time the 80th year of life is reached, as

compared with a 20-year old (24). On the one hand, this means that the time free radicals and metabolic end-products are retained in the aqueous humour is prolonged, and on the other, that the activity of active transport mechanisms declines. This is why the ascorbic acid concentration of the aqueous humour also declines with increasing age (25). The consequence is diminished buffering of free radicals in the aqueous humour, and this is further intensified by the decline in performance of the antioxidative enzyme systems. As far as the TMW is concerned, for example, an age-related decrease in superoxide dismutase has been demonstrated (26). Due to these processes, a preponderance of free radicals occurs and leads, among other things, to accelerated cataract development (27), and also transformation processes in the TMW (28, 29), which result in turn in a slight increase in intra-ocular pressure in advanced age (30).

3.3. In primary open-angle glaucoma

Independently of the age-related changes in the anterior chamber of the eye, protection from free radicals is markedly reduced in primary open-angle glaucoma (POAG), and this leads to pronounced oxidative stress with all its consequences. The aetiology has not yet been completely elucidated. It was shown in a pioneering study that patients with POAG generally have mitochondrial abnormalities (31), which weaken the supply of energy to cells and can lead to morphological and structural cellular changes. Independently of this, the total reactive antioxidant potential in the aqueous humour of glaucoma patients is 64% lower than in cataract patients in the same age group (32). This can be attributed to reduced ascorbic acid (11) and glutathione (33) concentrations. In consequence, superoxide dismutase, on the one hand, is reactively up-regulated (32), and, on the other, lipid peroxidation increases (34, 35). This leads to damage to cell membranes which can significantly influence the physical and chemical properties of the membranes (36). As the TMW has receptors for low-density lipoproteins (LDL) (37), direct impairment of the oxidised LDL on the TMW is to be expected. An additional factor is that oxidised LDL can activate plasminogen activator inhibitor-1 (PAI-1) via autocrine activation of growth factor TGF-β (38), which is produced in the ciliary body (39) and is elevated in the aqueous humour in POAG (40). Interestingly, cultured astrocytes from the optic disk also produce PAI-1 under the effect of TGF-β (41). As a result, the extracellular matrix of the cribriform plate and TMW may be activated and the viscosity of the aqueous humours increased, and this may make it more difficult for the aqueous humour to drain.

As far as oxidative reactions in the aqueous humour are concerned, high concentrations of hydrogen peroxide (H_2O_2) occur in POAG. H_2O_2 is stable as an intermediate oxidant. H_2O_2 has been intensively studied in the eye and, via activation of cytosolic calcium, causes the release of noradrenaline, and activation of nitric oxide synthetase (NOS) and arachidonic acid (overview in 42). It has also been shown in perfusion experiments that H_2O_2 increases outflow resistance in the glutathione-impoverished TMW (23). On the one hand, this could be caused by contraction of the TMW cells due to the elevated concentrations of cytosolic calcium, and, on the other, by a

reorganisation of vimentin and actin, reduced adhesion of TMW cells to fibronectin, laminin and Type I and Type IV collagen, and activation of cell nucleus activation factor NF-κB (43, 95). Another point is that the experimental data have been corroborated by clinical investigations. Elevated levels of adhesion molecules and also of messenger RNA, interleukins and NF-κB have been found in TMW specimens from trabeculectomies (44). Oxidative DNA damage in the TMW was also detected (45), which actually correlates positively with the degree of visual field damage and the level of intra-ocular pressure (46). Finally, there is a pronounced transformation of both the structure and function of the TMW, which provokes accelerated ageing and leads to increased outflow resistance (47, 48, 96). The increase in intra-ocular pressure associated with this then leads to further major biochemical changes, which then result in significant problems, especially in the posterior chamber of the eye.

To what extent an iatrogenic influence on oxidative stress in the TMW exists is still unclear at present. The preservative benzalkonium chloride contained in most eye drops results in any case in the release of superoxide anions on the conjunctiva, and these induce in turn apoptosis of conjunctival cells (49). As increased apoptosis due to benzalkonium chloride was also found in the TMW (50), it is quite conceivable that routine treatment with antiglaucoma drugs containing preservatives sustains oxidative stress and in this respect tends to have a deleterious effect on glaucoma.

4. OXIDATIVE STRESS AND THE POSTERIOR CHAMBER OF THE EYE

4.1. General

Like the anterior chamber of the eye, the retina is intensely exposed to light during the day. Short-wavelength blue light in particular can induce the formation of free radicals (51) and in addition can influence ATP synthesis in the mitochondria of retinal ganglion cells, with the consequence that free radicals may be less well scavenged and thus apoptosis may be set in motion (52). In addition, the retina is very susceptible to oxidative stress due to

- it being responsible for the greatest consumption of oxygen in the eye (53),
- the high proportion of polyunsaturated fatty acids in the outer segments of photoreceptors (54),
- the phagocytosis of the retinal pigment epithelium, which results in free radical formation (55), and
- age-related changes in the retina, which intensify oxidative stress due to the formation of catabolites such as lipofuscin and "advanced glycation end-products" (AGEs).

On account of these stresses due to free radicals, there are also numerous buffer systems in the retina. These include

4.1.1. Pigments

The neuromelanin present in the retinal pigment epithelium and the macular pigments lutein and zeaxanthin have antioxidant properties (56, 57).

4.1.2. Hormones

The production of melatonin (58) and its receptors has been demonstrated in the retina (59). Melatonin has antioxidant properties in that it can itself scavenge free radicals. It stimulates antioxidant enzymes and increases mitochondrial oxidative phosphorylation (60).

4.1.3. Water-soluble antioxidants

Glutathione (61) and ascorbic acid are available as antioxidants in the retina (62, 63), and ascorbic acid has a growth-inhibiting effect on the retinal pigment epithelium (64).

4.1.4. Fat-soluble antioxidants

Vitamin E, a fat-soluble antioxidant, has been found in the retina (65).

4.1.5. Specific enzymes

Catalase, glutathione peroxidase and superoxide dismutase have all been found in the retina (19, 29, 66).

4.2. Age-related changes

The proportion of melanin in the retinal pigment epithelium (67) and of lutein and zeaxanthin in the macula (68) fall with increasing age. At the same time, there is an increase in lipofuscin, a pigment formed from the accumulation of molecular aggregates from photo-oxidative processes (69). Lipofuscin itself acts as a photosensitiser, generates free radicals (69) and inhibits antioxidant processes (70). Due to metabolic stress, "advanced glycation end-products" are formed in addition, which can in turn initiate apoptotic processes, cell proliferation and oxidative stress (71, 72, 73). Finally, considerable age-related changes occur in the posterior chamber of the eye, with a decline in free radical buffering power, as a consequence of which oxidative stress on the retina is actually increased further, for example by the formation of drusen, lipofuscin and "advanced glycation end-products". These changes alone explain why physiological sensory capabilities decline with increasing age, because the complex association of cells that is the "retina" is perceptibly disturbed. If there are pre-existing genetic impairments and/or there is severe toxic (smoking) and vascular damage (diabetes mellitus, hypertension, arteriosclerosis), these age-related transformation processes are intensified, and their final stage is age-related macular degeneration.

As far as the optic nerves are concerned, there is a transformation of the collagen structures, provoked mainly by collagen cross-linking. This results in reduced elasticity, and this is also corroborated by the increase in thickness of the septa within the cribriform plate (74, 75, 76).

4.3. In primary open-angle glaucoma

While age-related changes in themselves place a tremendous stress on the retina and optic disk, additional disturbance is especially harmful. In the context of glaucoma, elevated intra-ocular pressure must be particularly mentioned. Under experimental conditions, elevated intra-ocular pressure results in the following in the retina:

- a reduction in superoxide dismutase (77),
- a reduction in catalase (77),
- a reduction in the concentration of melatonin (77), and
- a reactive increase in lipid peroxidation (77)

and the following in the optic disk:

- activation of nitric oxide synthetase-2 in astrocytes (78).

Due to increased intra-ocular pressure and to the changes mentioned above, astrocytes are activated in the cribriform plate (79) and in turn express COX-2 (80), TNF-α (81) and metalloproteinases (82), for example. The chronic inflammatory reaction triggered thereby leads to a transformation of the collagen structures of the cribriform plate, among other things (83). In addition, biochemical disturbance occur. One example of this is the activation of growth factors, such as TGF-β, which has been found at elevated concentration in the aqueous humour of glaucoma patients (84) and is formed among other things by the activated astrocytes (85). For its part, TGF-β increases the extracellular matrix both in the TMW (86) and in the astrocytes of the cribriform plate (87), and contributes to disturbing outflow as well as to the transformation of the cribriform plate. In the context of increased oxidative stress, TGF-β (88) is secreted in addition, thereby further promoting the biochemical transformation processes in the TMW and cribriform plate.

Due to these serious metabolic changes, accelerated metabolic and structural transformation occurs in the retina and optic disk in glaucoma. The transformation also affects the surface structures of membranes and molecules, which can then lead to immunological abnormalities (97). The influence of systemic vascular diseases, which can lead to endothelial dysfunction and to accelerated arteriosclerosis, results in further damage to the already considerably stressed retina and optic disk in POAG (98). The associated ischaemia promotes oxidative stress in the eye and results in a vicious circle.

5. SYSTEMIC MARKERS FOR OXIDATIVE STRESS IN POAG

POAG appears not to be an exclusively ophthalmological condition. We are still only on the threshold of an understanding of this, but one of the most significant studies was that which provided evidence of mitochondrial abnormalities in POAG patients (31), demonstrating a general metabolic disorder in POAG with restricted capability to cope with oxidative stress. This is the rationale for blood tests in patients with POAG. Reduced glutathione levels and elevated levels of malondialdehyde, a reactive catabolite formed in the course of lipid peroxidation (90), have been found in untreated glaucoma patients (89). There is, in addition, disturbance of the vitamin balance, including reduced vitamin B1 levels

(91). This is interesting because vitamin B1 is involved in neuronal glucose metabolism. Under normal conditions, the highest consumption of glucose at any point along the axon of the optic nerve occurs in the disk (92). Accordingly, if too little Vitamin B1 is present in the serum of glaucoma patients, papillary glucose metabolism could be disturbed and neurodegenerative processes promoted. In addition, elevated uric acid and fasting blood glucose concentrations have been found (93).

6. INTENSIFICATION OF OXIDATIVE STRESS IN GLAUCOMA DUE TO SYSTEMIC DISEASE

Patients with POAG frequently have systemic vascular diseases, which can exert a considerable influence on the POAG disease process and promote progression of the glaucomatous optic neuropathy (100, 101). This is mainly because the direct consequences, such as hypoxia, the accumulation of metabolic end-products and free radicals, and the reperfusion syndrome, can exacerbate the biochemical status of the optic nerve. Chronic processes in particular are of significance in this context, because they continuously impact negatively on the oxidative stress and can therefore promote the transformation processes. A classic example of this type of vascular disease is diabetes mellitus, in which endothelial dysfunction occurs and oxidative stress is central to the consequential reactions (102, 103). But endothelial dysfunction and/or increased oxidative stress have been demonstrated in other vascular diseases, such as tinnitus (104) and migraine (105, 106).

7. THE TRIGGERING OF IMMUNOLOGICAL PROCESSES IN GLAUCOMA DUE TO OXIDATIVE STRESS

Many questions remain unanswered when one considers the pathogenesis of glaucomatous optic neuropathy (95,97). Interestingly, however, many of the events come together to form a common final path and are mediated by certain key proteins. If, as in POAG, mitochondrial activity is primarily diminished (31) and it is adversely affected further under elevated intra-ocular pressure (107), free radicals may be less well buffered, and, at the same time, the cells are not supplied with sufficient energy to counteract this stress. Oxidative stress in the eye affected by glaucoma builds up due to increased fluctuations in diurnal pressures, due to the elevated intra-ocular pressure itself, and, indeed, also due to the underlying systemic vascular diseases and/or due to exogenous factors, such as smoking. As a result – in addition – immunological processes are stimulated. Many of the factors described as influencing the pathogenesis of glaucoma, such as:

1. elevated free radicals and oxidative stress (108), and also smoking (109),
2. short-wavelength light (52),
3. ischaemia/reperfusion (110, 111)
4. surface antigens of bacteria, such as those of *Helicobacter pylori* (112,113), and
5. inflammation mediators, such as IL-1 (114, 115) and TNF-alpha (81),

activate a nucleoprotein, nuclear factor kappa B (NF-κB). The latter is an important transcription factor, which occurs in almost all cell types and tissues. NF-κB is of major importance in the regulation of the immune response, cell proliferation and apoptosis. After activation (116), the following are stimulated:

1. acute-phase response proteins, such as angiotensinogen or complement C3,
2. adhesion molecules, such as VCAM-1, ELAM-1, and ICAM-1, and
3. cytokines, such as IL-1, IL-2, IL-6, IL-8, G-CSF, GM-CSF, TNF-α, TNF-β, and IFN-β.

Due to this up-regulation of a wide variety of factors, biochemical processes are initiated and advance the process of glaucomatous optic neuropathy and lead to numerous reactions. By way of example, it is to be noted that activation of NF-κB leads, firstly, to activation of endothelin-1 (117), which occurs to a greater degree in POAG in both the serum and aqueous humour and, due an adverse effect on outflow resistance, in the trabecular meshwork, and also, due to disturbed perfusion of the optic nerve, plays a significant role in POAG (118). Secondly, it has been shown that hypoxia-inducible factor 1 (HIF-1) is also up-regulated via NF-κB (119). Elevated HIF-1 has been detected in the retina and optic nerve in cases of POAG (120). It has so far been possible to demonstrate up-regulated NF-κB directly in the trabecular meshwork in glaucoma patients (44,121), and also in the ciliary body in an experimental murine glaucoma model (122).

The role of NF-κB in the immunological context has been confirmed. An almost incalculable number of immune reactions occur due to activation of the widest variety of cytokines. For example, cytokines, and also ischaemia, lead to the induction of HLA-DR expression in the astrocytes of the cribriform plate (123). High concentrations of TGF-β, which have been demonstrated in POAG, cause the activation of microglia with corresponding immunocompetence (124). Finally, in the course of POAG, a powerful immune reaction occurs and leads to auto-immune reactions. The pattern of the reactions is partly known, but the individual reactions have yet to be elucidated (106). One of the antibody reactions consists in the formation of antibodies against heat shock proteins, which normally act to protect cells against powerful stresses, such as those that occur in oxidative stress (125). In addition, studies have shown the formation of antibodies against *Helicobacter pylori* (112).

8. SUMMARY

While lowering intra-ocular pressures is the dominant therapeutic option in glaucoma treatment strategy today, and its effectiveness has been proven insofar as a high proportion of glaucoma patients benefit from it, one must not overlook the fact that many patients are not sufficiently helped by lowering intra-ocular pressure alone. For example, in the Early Manifest Glaucoma Trial, the glaucoma had progressed after 6 years' follow-up in 45% of the patients treated (93), and this figure actually rose to

59% after 8 years (100), in spite of lowering intra-ocular pressure by 25% to an average of 15.5 mmHg. This is not to question the value of lowering intra-ocular pressure, but the data relating to POAG presented here show emphatically that the therapeutic strategy should be far more comprehensive, in other words, more holistic. In addition to optimally stabilising the metabolism (blood glucose, blood lipids, thyroid) and blood flow (blood pressure, vasospasticity), orthomolecular therapy should be included as well, to bring oxidative stress under control. Lifestyle strategies are, of course, important to this end as well, such as not smoking, adopting regular eating habits and getting sufficient sleep, for example. These measures require close collaboration between patient, ophthalmologist and general practitioner/internal medical specialist and can only work if the patient is aware of the wider significance of the disease glaucoma.

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