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Review

Glaucomatous outflow pathway and oxidative stress☆

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Abstract

Oxidative free radicals and reactive oxygen species (ROS) are able to affect the cellularity of the human trabecular meshwork (HTM). These findings suggest that intraocular pressure increase, which characterises most glaucomas, is related to oxidative degenerative processes affecting the HTM and specifically its endothelial cells. Much evidence indicates that in this region ROS play a fundamental pathogenic role by reducing local antioxidant activities, inducing outflow resistance and exacerbating the activities of superoxide dismutase and glutathione peroxidase in glaucomatous eyes. Furthermore, hydrogen peroxide induces rearrangement of HTM cells and compromises their integrity. Glaucomatous subjects might have a genetic predisposition rendering them more susceptible to ROS-induced damage. A fairly significant correlation between oxidative DNA damage in the HTM and intraocular pressure increase and visual field defects in glaucomatous patients has been demonstrated. Thus, oxidative stress may play a significant role during glaucoma course initially damaging HTM cells, then contributing to the alteration of the homeostasis between NO and endothelins, and finally through its possible involvement in ganglional cell death. On the whole, these findings support the hypothesis that oxidative damage is an important step in the pathogenesis of primary open-angle glaucoma, and might be a relevant target for both prevention and therapy.

Keywords: oxidative stress; free radicals; glaucoma; primary open-angle glaucoma; glaucoma pathogenesis; oxidative DNA damage

1. Introduction

Glaucoma is an optic neuropathy, characterised by the progressive degeneration of retinal ganglion cells and visual field damage, representing the final stage caused by a number of different conditions affecting the eye. Although increased intraocular pressure (IOP) is probably the most important risk factor for primary open-angle glaucoma, several concomitant factors, i.e. the increasing of glutamate levels (Shen et al., 2004), the alteration of nitric oxide (NO) metabolism (Galassi et al., 2004), vascular factors (Chung et al., 1999) and oxidative damage caused by reactive oxygen species (ROS) overproduction may significantly contribute to the neurodegeneration (Meldrum and Garthwaite, 1990).

IOP lowering improves visual field preservation. All recent randomised controlled trials on IOP-lowering treatment for glaucoma have reached this conclusion, though large inter-individual variations in retinal ganglion cell (RGRGC) survival remain unexplained (Collaborative Normal-Tension Glaucoma Study Group, 1998; Lichter et al., 2001; The Advanced Glaucoma Intervention Study, 2000).

IOP elevation and visual field damage have been shown to be proportional to the DNA oxidative damage found in the human trabecular meshwork (Saccà et al., 2005). This finding provides a basis for the role of oxidative stress in the pathogenesis of glaucoma and provides new insight into the molecular mechanisms involved.

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2. Outflow pathway

The conventional outflow pathway consists of trabecular lamellae covered with human trabecular meshwork (HTM) cells, in front of a resistor consisting of juxtacanalicular HTM cells and the inner wall of Schlemm's canal. The outermost juxtacanalicular or cribriform region has no collagenous beams, but rather several cell layers which some authors claim to be immersed in loose extracellular material/matrix (Tian et al., 2000).

The main resistance to the aqueous humour outflow is located in the TM directly underneath the inner wall of Schlemm's canal (Maepea and Bill, 1992). Ultrastructural changes in glaucomatous trabecular meshworks are similar to, but much more intense than, those observed in the normal trabecular meshwork in the elderly (Potau et al., 2000a). These changes include the thickening of basal membranes and trabecular beams, their enlargement or collapse, partial loss of endothelial cells and the accumulation of materials such as pigment granules and calcium precipitates (Potau et al., 2000b), central nucleus changes such as an increase in electrodense plaques and collagen, and loss of endothelial cells (Lutjen-Drecoll, 2005). Furthermore, outflow resistance increases with age (Becker, 1979).

Thus, the increase in oxidative DNA damage in the cellular component of the trabecular meshwork could directly affect regulation of the extracellular matrix structure and the associated regulation of intraocular pressure, leading to the clinical onset of glaucoma (Knepper et al., 1996a; Lutjen Drecoll and Rohen, 1996). Indeed, TM endothelial cells drive a mechanism that controls the permeability of the TM by the endothelial cells lining the lumen of Schlemm's canal. These endothelial cells release vasoactive cytokines and other factors able to increase the permeability of the endothelial barrier of the Schlemm's canal. Alterations in these cytokines may not allow sufficient flow through Schlemm's canal and, consequently, the IOP may rise to abnormal levels (Alvarado et al., 2005).

This interpretation of glaucoma physiopathology is in agreement with the view that increased intraocular pressure (IOP) is secondary to a decline in trabecular meshwork cellularity (Alvarado, 1984a,b). Lutjen-Drecoll (2005) has recently claimed that "common factors are involved in the pathogenesis of both the TM and the optic nerve changes". The common denominator involved in the cellular alterations of the TM structure and optic nerve damage is on one hand the oxidative stress, and on the other the vascular damage described in both glaucoma (Flammer, 1994) and ageing.

3. Oxidative stress

Under physiological conditions, there is a state of equilibrium between the endogenous production of free radicals and their neutralisation by antioxidant defence mechanisms. When the production of radicals exceeds the organism's neutralizing capacity ("scavenging" activity), damage ensues; this condition is known as oxidative stress.

More than 90% of the oxygen is consumed by mitochondria in aerobic living organisms. Under normal physiological conditions, about 1–5% of the oxygen consumed by mitochondria is converted to reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide (H_2O_2), and hydroxyl radicals (Trounce et al., 1989). Mitochondrial respiratory function declines with age (Yen et al., 1989) and this increases the production of ROS and free radicals in mitochondria. Consequently, ROS production essentially depends on mitochondrial function and on the levels of antioxidant defences (Camougrand and Rigoulet, 2001).

Free radicals are neutralized both by a range of enzymes, such as superoxide dismutase, glutathione peroxidase or catalase, and by numerous molecules that are either endogenously produced, such as glutathione, or dietary assumed, such as flavonoids, vitamins C and E and others. These molecules have the capacity to capture free radicals as they are able to accept the unpaired electron and then to "pass it on". The most well-known free-radical chain reaction is lipid peroxidation, that is to say free-radical attack to lipid membranes. The main targets of these reactions are proteins, cell membranes, and DNA, particularly mitochondria DNA. Indeed, mitochondrial DNA is less protected than nuclear DNA (Balansky et al., 1996), and is therefore more sensitive to free radical attack (De Grey, 1997) (Fig. 1). This entire mechanism is believed to underlie the etio-pathogenesis of degenerative diseases (Fig. 2).

Peroxidation phenomena are counteracted by a range of antioxidants compounds inhibiting free radicals formation. These compounds include water-soluble antioxidants (e.g. ascorbic acid, cysteine, glutathione), lipid-soluble antioxidants (e.g. tocopherols and retinols), and enzymes such as superoxide dismutase (SOD), which catalyses the transformation of free radicals into hydrogen peroxide. Although hydrogen peroxide is also an active oxygen compound, it can be further transformed into oxygen and water by catalase (Fig. 3). Several metal-binding proteins (transferrin, etc.) (Babizhayev and Costa, 1994; Rose et al., 1998) and flavonoids (genistein, diazine, glycyrrhizin, etc.) (Kapiotis et al., 1997; Wang et al., 1998) are also antioxidants.

Metal-mediated formation of free radicals causes various modifications of DNA bases, enhances lipid peroxidation, and alters calcium and sulfhydryl homeostasis. Lipid peroxides, formed by the attack of radicals on polyunsaturated fatty acid residues of phospholipids, can further react with redox metals, finally producing mutagenic exocyclic DNA adducts (Valko et al., 2005) and 8-hydroxy-2′-deoxyguanosine (8-OH-dG), which is a typical indicator of oxidative DNA damage.

Oxidative modifications and mutations of mitochondrial DNA (mtDNA) occur with great ease, and the extent of such alterations of mitochondrial DNA increases exponentially with age. Oxidative modification in mtDNA is much more extensive than that in nuclear DNA (Ames et al., 1993; Yakes and van Houten, 1997). Age-related alterations in the respiratory enzymes not only decrease ATP synthesis but also enhance the production of ROS through increasing electron leakage in the respiratory chain. With the accumulation of genetic defects in mechanisms of mitochondrial energy production, the issue of neuronal susceptibility to damage as a function of ageing

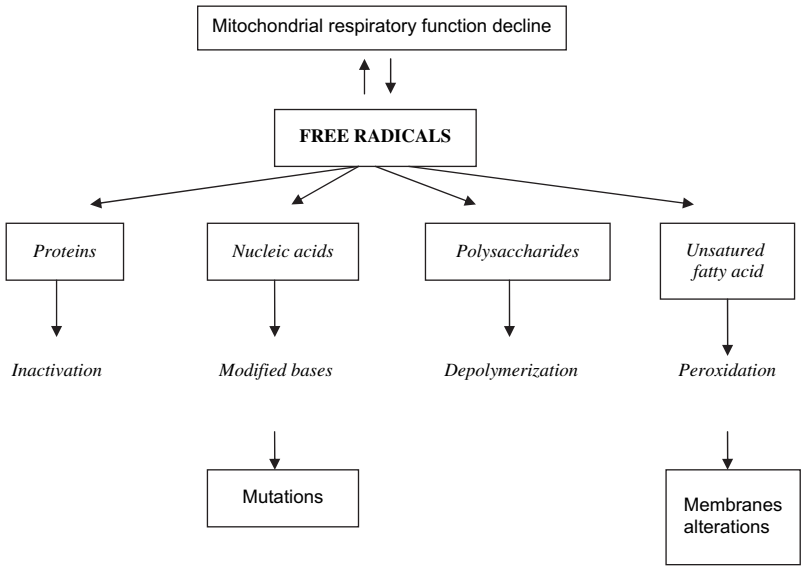

Fig. 1. Oxygen free radicals dangerous effects in living cells: a wide spectrum of alterations in aged individuals and senescent cells are similar and are correlated to cellular response to sublethal dose of oxidative stress. These alterations and responses include: decline in mitochondrial respiratory function; increase in the rate of production of reactive oxygen species; accumulation of mitochondrial DNA mutations; increase in the levels of oxidative damage to DNA, protein, and lipids; and decrease in the capacities of degradation of oxidatively damaged proteins and other macromolecules (Lee and Wei, 2001).

becomes important (Parihar and Brewer, 2006). The variation in the individual and regional predisposition to degenerative diseases and cancer may result from the interaction of modern dietary caloric intake and ancient mitochondrial genetic polymorphisms (Wallace, 2005).

3.1. Defences against free radicals and the eye

Both vitamin C and glutathione operate in fluid outside the cell and within the cell (Cardoso et al., 1998), whereas vitamin E prevents endogenous mitochondrial production of ROS (Southam et al., 1991). This may be important in maintaining cellular homeostasis, which is relevant to the etiology of POAG (Veach, 2004). Indeed, vitamin E prevents apoptosis during hypoxia and oxygen reperfusion (Tagami et al., 1999), and it may reduce apoptosis by means other than antioxidation (Barroso et al., 1997; Osborne et al., 1998; Lizard et al., 2000).

Ascorbic acid is thought to be a primary substrate in ocular protection because of its high concentration in the eye. Within the cell, vitamin C helps to protect membrane lipids from peroxidation by recycling vitamin E (May, 1999). It is present at high concentrations in vitreous humour (Hanashima and Namiki, 1999), cornea (Brubaker et al., 2000) and tear film (Dreyer and Rose, 1993). One of ascorbate's presumed functions is to protect the lens and retina from the damaging effects of ultraviolet radiation (Ringvold et al., 2000). In addition, it exerts a filter-like function against UV radiation in both the central corneal epithelium and aqueous humour (Giblin et al., 1984) and reacts with O_2 to form H_2O_2. The ratio

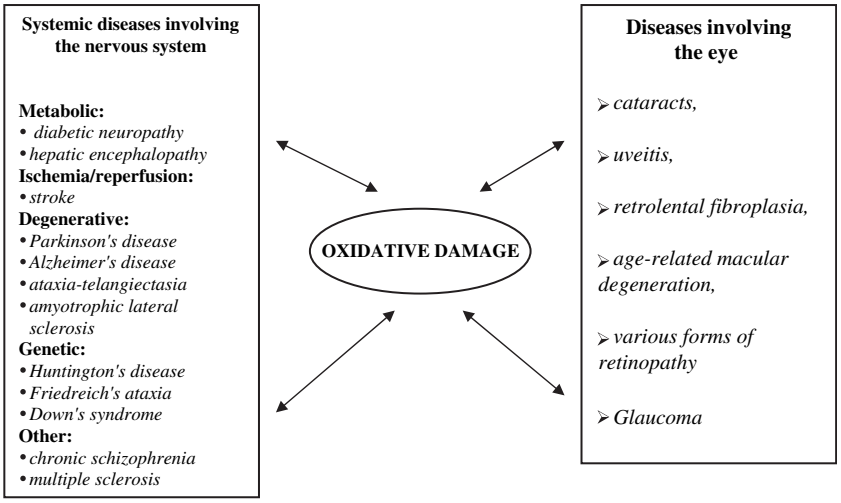

Fig. 2. Ocular and systemic diseases due to the oxidative damage.

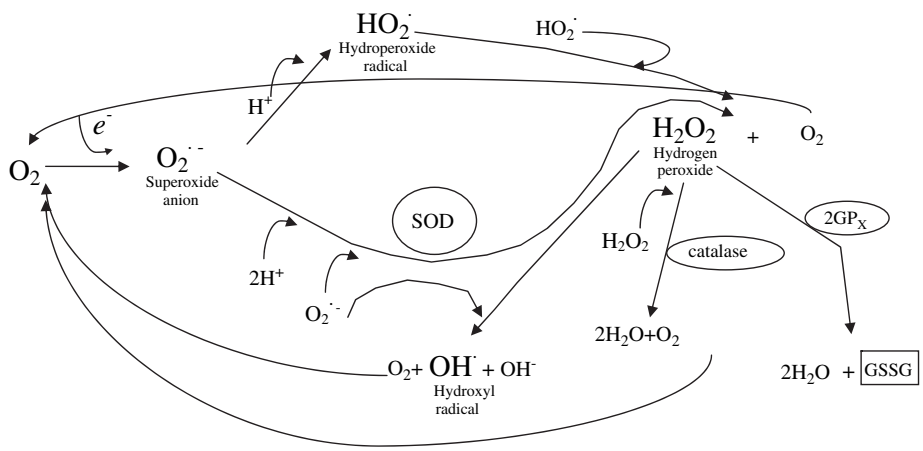

Fig. 3. Biochemical pathways involved in the generation of reactive oxygen species (ROS) and in their scavenging.The reduction of O_2 (e^-) leads to the formation of the *superoxide anion* ($O_2^{\bullet-}$) in the cell. $O_2^{\bullet-}$can react with H^+ to produce the *hydroperoxide radical* and then *hydrogen peroxide* (H_2O_2). The reaction between H_2O_2 and $O_2^{\bullet-}$ generates the *hydroxyl radical* ($OH^{\bullet}$). The enzyme *superoxide dismutase* (SOD) catalyses the reaction of two $O_2^{\bullet-}$ to form H_2O_2. As H_2O_2 is detrimental to cell integrity, *catalase* catalyses its dismutation to O_2 and H_2O and *peroxidases* catalyse its reduction to H_2O. *Glutathion-peroxidase* (GP_X) reduces reactive oxygen species by oxidising *glutathion*.

between the amount of ascorbic acid in the aqueous humour and that in the plasma is 15:1 (Becker, 1957). The direct correlation between the concentrations of ascorbic acid and H_2O_2 in aqueous humour suggests that ascorbic acid is the primary source of H_2O_2 in this fluid (Giblin et al., 1984), although it has been claimed that H_2O_2 levels can be overestimated (Garcia-Castineiras et al., 1992). A high level of ascorbic acid is necessary to maintain oxidative balance in the aqueous humour, while vitamin E deficiency increases H_2O_2 levels (Chow et al., 1999).

Unfortunately, these processes are not able to eliminate free radicals completely, and, if oxidative stress is very severe, it may ultimately cause cell death (Martindale and Holbrook, 2002).

Reduced glutathione (GSH) is the main intracellular endogenous antioxidant produced, and is synthesised in two sequential reactions catalysed by gamma-glutamylcysteine synthetase, a GSH1 gene product, and glutathione synthetase, a GSH2 gene product (Sugiyama et al., 2000). Glutathione participates directly in the neutralisation of free radicals and reactive oxygen species, and maintains exogenous antioxidants such as vitamins C and E in their reduced (active) forms. The GSH redox system is believed to protect ocular tissues from the damage induced by low H_2O_2 concentrations, whereas catalase is thought to protect ocular tissues from the damage induced by higher H_2O_2 concentrations (Costarides et al., 1991; Riley, 1990). A decline of catalase activity with age has been observed in the iris and in the corneal endothelium of rabbits (Riley, 1990). Both glutathione and ascorbate have been detected in aqueous humour (Richer and Rose, 1998). These antioxidants seem to play a particularly important role in glaucomatous disease. Indeed, patients with glaucoma exhibit low levels of circulating glutathione, suggesting a general compromise of antioxidative defences (Gherghel et al., 2005). Moreover, genetic polymorphisms have been detected for GSH transferase isoenzyme, and the *GSTM1*-null genotype has been found to be significantly more common in patients with primary open-angle glaucoma than in controls (Izzotti et al., 2003).

On the basis of the currently available literature, free radicals seem to play an important role in the pathogenesis of ocular diseases (Fig. 2). Indeed, reactive oxygen species (ROS) are a cause of cataract (Shen et al., 2004; Marsili et al., 2004), are implicated in age-related macular degeneration (Beatty et al., 2000; Totan et al., 2001; Yildirim et al., 2004), and also may play a significant role in glaucoma pathophysiology (Ferreira et al., 2004; Izzotti et al., 2003; Saccà et al., 2005).

3.2. Oxidative stress and the trabecular meshwork

The concept that the eye outflow system is a passive filter is outdated. Indeed, it has now been established that the structures through which the aqueous humour leaves the anterior chamber are biologically active, and that influence the transit of fluids (Fig. 4). These tissues may be the targets of physical or pharmacological manipulation. TM cells have the capacity to migrate (Zhou et al., 1999), become more committed to phagocytosis with age (Gabelt and Kaufman, 2005), produce extracellular matrix and participate in the modulation of TM composition and functions (Yue, 1996).

Glaucomatous human TM cells synthesise hyaluronic acid at a lower rate than normal cells. Ascorbic acid is reported to stimulate increased hyaluronic acid synthesis in glaucomatous trabecular cells compared with normal human trabecular cells (Schachtschabel and Binninger, 1993). TM extracellular matrix production may be mediated by vitamin C (Epstein et al., 1990; Sawaguchi et al., 1992).

Alvarado has found that the most severe alterations in the cellular component or in the entire trabecular meshwork, during POAG or ageing (Table 1), occur in the inner layers near to the anterior chamber (Alvarado, 1984a,b). The toxic substances present in aqueous humour could in some way

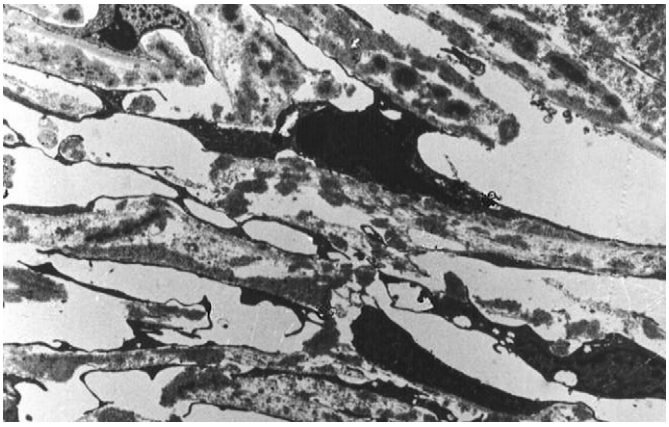

Fig. 4. Human trabecular meshwork (HTM) sample analysed by transmission (X1200) electron microscopy. In glaucomatous patients, HTM oxidative DNA damage level appears to be appreciably high (Izzotti et al., 2003; Saccà et al., 2005).

contribute to the appearance of the pathogenetic alterations affecting the trabecular meshwork. HTM cells are in contact with relatively high concentrations of H_2O_2, and exposure to H_2O_2 is reported to have no effect on outflow in normal eyes, while causing a 33% decrease in outflow in GSH-depleted eyes. When mitochondrial glutathione and vitamin E levels are reduced to 20% of the normal level, lipid peroxidation occurs (Augustin et al., 1997). Therefore, GSH probably does not participate directly in regulating the outflow of aqueous humour but is able to protect the HTM against H_2O_2-induced oxidative damage that would otherwise lead to a decrease in outflow facility (Kahn et al., 1983). Insufficient glutathione combined with exogenous H_2O_2 may induce collagen matrix remodelling

Table 1
HTM changes in ageing and glaucoma onset (data reviewed from Gabelt and Kaufman, 2005; Hogg et al., 2000; Tamm et al., 1996)

Decreased	Increased
Aqueous flow	Ocular rigidity
Depth and volume of anterior chamber	Turnover rate of anterior chamber aqueous
HTM cells	Incidence of cells detaching from trabeculate
mRNA for various matrix	Fusion between adjacent trabeculae
Metalloproteinases	Pigmentation of TM cells
Actin filaments	Phagocytosis capacity of TM cells
Contractile protein	Fibrillar material within trabeculum ciliare
Cytoskeletal network	Inter-muscular spaces at muscle tips
Thickness of the cribriform meshwork	Diameter of the elastic fibres in cribriform meshwork
Dimensions of Schlemm's canal	Accumulating sheath material (SD-plaques)
Inner wall pore density	Alteration of microfibrils
Total amount of GAGs in the HTM	Association of myocilin with microfibril-associated elements
Hyaluronic acid and keratansulfate	Extracellular material in the subendothelial region of Schlemm's canal
	Chondroitin sulphate
	Oxidative DNA damage in HTM

and trabecular cell apoptosis independently of mitochondria. Although human meshwork cells can proliferate in culture, their capacity for replication in situ appears limited. Moreover, it is known that the decline of HTM cellularity is linearly related to age (Alvarado et al., 1981, 1984a,b). It has been calculated that at 20 years of age the estimated cell number for the whole meshwork is 763,000 (Grierson and Howes, 1987) and that the number decreases to 403,000 by the age of 80 years, with a loss rate of 6000 per year (Grierson et al., 1982). The mechanism of cell loss and the environmental factors contributing to it are not yet known. However, this phenomenon may be brought about by cell death caused by noxious insult, such as free radical attack (Yan et al., 1991; Padgaonkar et al., 1994).

Cell loss specifically affects the filtering area (Alvarado et al., 1981). With age, resistance increases and alterations of the extracellular matrix in the juxtacanalicular region occur (Tian et al., 2000). The loss or altered functionality of HTM cells has been suggested to be the result of an increase in oxidative stress (De La Paz and Epstein, 1996). Outflow resistance increases in the presence of high levels of H_2O_2 (Kahn et al., 1983). Moreover, the specific activity of superoxide dismutase demonstrates an age-dependent decline in normal HTM collected from cadavers (De La Paz and Epstein, 1996). In addition, the effect of H_2O_2 on the adhesion of HTM cells to extracellular matrix proteins results in a rearrangement of cytoskeletal structures that may lead to HTM disruption (Zhou et al., 1999). Oxidative stress can also influence biological reactions of HTM cells (Barksdale Sbhuyan and Podos, 1986; Tamm et al., 1996) and may contribute to the changes observed in ageing and in primary open-angle glaucoma (Tamm et al., 1996), such as trabecular thickening and trabecular fusion (Hogg et al., 2000) (Table 1).

Glycosaminoglycans (GAGs) contribute to the outflow of aqueous humour, probably because their concentrations are a significant factor in outflow resistance in POAG, particularly at higher pressures (Knepper et al., 2005). A significant decrease in hyaluronic acid and increase in chondroitin sulphate have been found in the eyes of patients with glaucoma (Knepper et al., 1996a,b; Navajas et al., 2005). In glaucomatous eyes, a decrease in GAG synthesis, particularly hyaluronic acid, has been found (Schachtschabel and Binninger, 1993). Vitamin C is fundamental to the synthesis of glycosaminoglycans (Ronziere et al., 2003). Ascorbate has also been found to reduce the viscosity of hyaluronic acid, thereby increasing outflow through the trabeculum (McCarty, 1998). Moreover, a trial conducted by Virno et al. (1967) on the effect of high-dose vitamin C, first in animals and then in humans, showed that it decreased IOP. Other authors have confirmed this capacity of vitamin C, both orally and topically (Linner, 1969). In any case, the role of vitamins C and E in maintaining glutathione in its reduced form has been established.

The endothelial cells of the trabecular meshwork are subject to oxidative damage (Russell et al., 1989; Zhou et al., 1999; Saccà et al., 2005), and, being immersed in the aqueous humour, they are more easily exposed to this type of damage, both in vivo (Alvarado et al., 1984a,b) and in vitro (Caballero et al., 2003). Izzotti et al. (2006) support the hypothesis that

these cells are responsible for the beginning of the ''glaucomatous cascade'' in primary open-angle glaucoma. Indeed, oxidative stress manifests itself as the occurrence of DNA damage inside HTM cells (Izzotti et al., 2003) (Figs. 1 and 5). Products of lipid peroxidation participate in the destruction of the trabeculae and Schlemm's canal in POAG (Babizhayev and Bunin, 1989). In the aqueous humour of glaucoma patients, there is an inverse relationship between the superoxide dismutase and glutathione peroxidase activities. Therefore, oxidative stress may lead to an induction of antioxidant enzymes and contribute to total reactive antioxidant potential decrease (Ferreira et al., 2004). As a result, free radicals hurt the structures immersed in the aqueous humour, mainly including the endothelial cells of the trabecular meshwork, which can regulate the permeability of the endothelial cells of Schlemm's canal (Alvarado et al., 2005). As long ago as 1987 (Grierson and Howes, 1987), it was thought that many of the alterations observed in the structure of the trabecular meshwork could be attributed to the loss of endothelial cells and to the repair processes of the remaining cells. Indeed, although the trabecular meshwork has a complex organisation and possesses different types of cells, it performs numerous biomolecular functions, all of which contribute to regulating the outflow of aqueous humour (Alvarado et al., 2004), and it is likely that its endothelial cells have a major role in the regulation of aqueous outflow (Alvarado et al., 2005). Indeed, trabecular meshwork pores contribute only to the10% of the aqueous outflow resistance (Sit et al., 1997). It is therefore possible to speculate that the passage across this region is active and not passive (Johnstone, 2004). Indeed, some authors have questioned the real existence of the pores (Johnson et al., 2002).

Hence, oxidative stress is a plausible mechanism for the development of glaucoma, manifesting its effects on the HTM (Izzotti et al., 2003) as an IOP increase (Saccà et al., 2005). Indeed, the trabecular meshwork endothelium is involved in modulating the permeability of the endothelial barrier and the release of endothelins and nitric oxide. These oxidative changes in TM cells result in elevation of the IOP and alteration of the equilibrium between nitric oxide (NO) and endothelins (Haefliger et al., 1999).

In the presence of free radicals, NO produces toxic products by interacting with oxygen, iron and/or copper (Haefliger

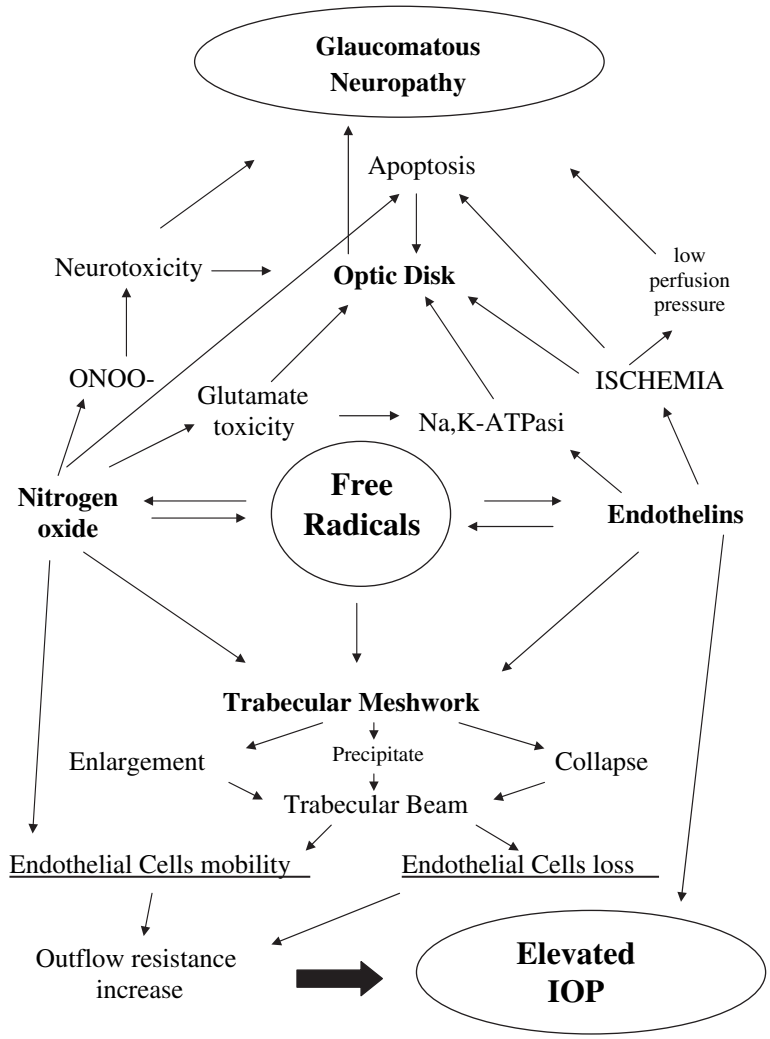

Fig. 5. Intraocular pressure (IOP) increase in glaucoma course is probably linked to oxidative-related degenerative processes affecting HTM and, more specifically, its endothelial cells. Vascular damage and neuronal cell death associated with glaucoma could also have oxidative origin.

et al., 1999), which can aggravate the metabolic conditions of the HTM and alter its mobility (Tamm and Lutjen Drecoll, 1998; Wiederholt, 1998). Moreover, the increased concentration of NO reacting with anion superoxide to form ONOO− (peroxynitrite) or other substances derived from oxidative stress can lead to neurotoxicity (Lipton, 1999). Endothelins can also participate in the regulation of IOP (Haefliger et al., 1999) (Fig. 5) independently of trabecular meshwork mobility (Wiederholt, 1998). Indeed, endothelin levels are more elevated in the aqueous humour of glaucomatous patients than in controls (Noske et al., 1997). Therefore, endothelin-1 seems to be the main effector of glaucomatous ischemia (Orgul et al., 1999). Reduced optic nerve blood flow caused by the exogenous application of endothelin-1 can result in RGC death in the absence of elevated IOP (Chauhan et al., 2004; Orgul et al., 1996). However, the activation of endothelin receptors also results in changes in axonal transport, the proliferation of optic nerve head astrocytes, ischemia and increased production of neurotoxic compounds (Yorio et al., 2002).

Therefore, through the induction of oxidative damage, mechanical and vascular factors working synergistically lead to the same final pathologic consequence (Prasanna et al., 2005).

Oxidative stress therefore seems to play an important role in damaging both the endothelial cells of the trabecular meshwork and the optic nerve head.

4. Genetic elements

Several genes conferring high risk for glaucoma have been identified mainly including myocilin (Stone et al., 1997) and optineurin (Rezaie et al., 2002). However, the contribution of these genes to glaucoma pathogenesis is quite low on an epidemiological basis their mutation occurring only very rarely in the population. In any case, segregation and familial correlation analyses of IOP suggest a polygenetic component with environmental influences (Duggal et al., 2005). In this regard, it is noteworthy that the possibility of a relationship between glaucoma, blepharitis, *Helicobacter pylori* infection and oxidative damage has been raised (Saccà et al., 2006). The myocilin protein was identified in response to steroid treatment and a large increase in its expression was observed. Mutations in this gene are found in 3–5% of adult-onset POAG patients (Wiggs et al., 1998).

Primary congenital glaucoma (PCG) is currently considered a pathogenetic entity different from POAG. A major gene, *CYP1B1*, belonging to the superfamily of cytochromes P450 has been identified. PCG is mostly inherited in an autosomal recessive manner (Francois, 1980). Some studies have reported delayed expression of a *CYP1B1* mutation and co-existence of PCG and POAG in the same pedigree (Soley et al., 2003; Panicker et al., 2002). The *CYP1B1* gene is typically associated with adult glaucoma (Vincent et al., 2002). In the population studied, the frequency of the mutation of the *CYP1B1* gene and its nature vary greatly. Anyway, *CYP1B1* mutation carrier subjects have does not depend directly on IOP elevation. It is possible that expression of *CYP1B1* may lead to the accumulation of a toxic substrate in the neuroretina (Bejjani et al., 2002). It results in greater sensitivity of neural ganglial cells and particularly would lower the threshold necessary for IOP to cause damage to the optic nerve (Melki et al., 2004). It has been demonstrated that human *CYP1B1* can catalyse the hydroxylation of 17-estradiol at positions C-4 and C-2. Allelic variants containing valine at position 432 are characterised by a higher ratio of 4-hydroxyestradiol to 2-hydroxyestradiol production (Shimada et al., 1999). 4-Hydroxyestradiol is carcinogen (Liehr et al., 1986), from here, the possible association of the V432 allele with various cancers like ovarian and prostate ones (Goodman et al., 2001) and for the smoking-related head and neck squamous cell cancer (Ko et al., 2001).

The role of myocilin in the outflow pathways is unclear. This protein has been detected in the extracellular matrix (Ueda et al., 2002), and it is possible that normal myocilin is secreted by the cell, while mutant forms are not secreted (Jacobson et al., 2001). These mutant forms seem to be of great importance, in that they may prevent the processing and secretion of other proteins that are necessary for the normal function of the trabecular outflow pathways (Wiggs, 2005). Moreover, Liu et al. have shown that prolonged expression of Pro370Leu mutant myocilin results in abnormal cell morphology and ultimately in the death of the HTM (Liu and Vollrath, 2004). Recently, mutations of other genes have also been correlated with glaucoma: *OPTN*, which is responsible for coding optineurin (Gong et al., 2004), and a novel gene with multiple G-beta WD40 repeats (*WDR36*), which is highly expressed in a number of ocular and non-ocular tissues (Monemi et al., 2005). However, the exact role of these proteins in the pathogenesis of glaucoma has yet to be determined; indeed, they are extremely rare in the general population (<1–3%).

Other factors are also involved in glaucoma, albeit less directly, and these can, in some way condition the course of the disease. Indeed, glaucoma represents a final common pathway resulting from several different conditions that can affect the eye.

Deletion of the *GSTM1* gene, which has already been shown to be associated with an increased risk of cancers and atherosclerotic lesions, appears to predispose the individual to more severe oxidative DNA damage in TM cells, and this is much more intense in glaucoma patients (Izzotti et al., 2003; Izzotti and Saccà, 2004). The chromosome location of *GSTM1* gene is 1p13.3. The GSTM1 enzyme is one of the major polymorphic forms of the GSTs existing in humans. The GSTs are a family of enzymes responsible for the metabolism of a broad range of xenobiotics and carcinogens. This enzyme catalyses the reaction of glutathione with a wide variety of organic compounds to form thioethers, a reaction that is sometimes a first step in a detoxification process. Deletions in *GSTM1* occur at a frequency of about 15% in human populations. Individuals who have homozygous deletion, i.e., *GSTM1* null, exhibit an absence of enzyme activity. A null allele at the *GSTM1* locus is found in 40–45% of Caucasians. *GSTM1* deficiency may be a risk factor for cancer by providing increased sensitivity to chemical carcinogens. The mechanism

of carcinogenicity may be related to an increased formation of DNA adducts in the presence of the null deletion.

The finding that the *GSTM1*-null genotype is more frequent among glaucoma patients is consistent with these molecular data but warrants confirmation in further epidemiological studies. In a recent study conducted on 153 primary open-angle glaucoma patients and 159 healthy controls, Yildirim et al. (2005) found that the frequency of the *GSTM1*-null genotype among glaucomatous patients was significantly higher than in controls, thus confirming the possible role of the free radicals. These data are in agreement with the finding that expression of the endothelial-leukocyte adhesion molecule (ELAM-1) is consistently detected in TM cells from glaucoma patients but not in controls (Wang et al., 2001). ELAM-1 expression is controlled by interleukin 1 activation autocrine feedback loop through the nuclear transcription factor NFkB, a pathway devoted to protecting cells against oxidative stress (Wang et al., 2001).

Although no direct interaction between decreased scavenging of ROS in *GSTM1* deleted subjects and possible consequences on ELAM-1 expression has been insofar examined, these findings provide parallel evidence that ROS display a pathogenic role in POAG.

5. Conclusions

On the basis of the reported data, it remains to be resolved whether oxidative damage of essential molecules is a principal cause of ocular diseases, although the involvement of oxidative stress has certainly been confirmed in a spectrum of damaging processes. Anyway, oxidative stress plays a role in glaucoma pathogenesis, affecting both the cells of the trabecular meshwork, and the ganglion cells of the retina. This hypothesis is certainly supported by some solid data, but still far away from being definitely proven, indeed, several factors are nowadays known to play a role in these processes, but many more remain to be elucidated. For example, Moreno et al. (2004) suggested that the manipulation of intracellular redox status by means of antioxidants might be a new therapeutic tool for preventing glaucomatous cell death. Unfortunately, eye disease trials are smaller (ranging from 20 to 151 participants) and the time of their treatment and follow up is short (6–24 months). Future research in this area will help to understand the physiopathology of glaucoma and to develop new approaches for its prevention and treatment. In similar studies, particular attention will be focused on the use of chemopreventive and therapeutic drugs displaying antioxidant attitudes. Furthermore, in following years, genetic analysis of polymorphic genes encoding for antioxidant activities could contribute to identify subjects with an increased risk for glaucoma onset.

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