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Review article

Functional dichotomy: glutathione and vitamin E in homeostasis relevant to primary open-angle glaucoma

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Primary open-angle glaucoma (POAG) is a complex chronic neurological disease that can result in blindness. The goal of understanding the aetiology of POAG is to be able to target effective treatment to individuals who will eventually go blind without it. Epidemiological studies of POAG have not specifically addressed the possibility that nutrition may play a role in the development of POAG. A handful of papers have considered that nutrition may have an impact on POAG patients. POAG is not believed to be a 'vitamin-deficiency disease'. The concept of 'vitamin-deficiency diseases' and the recommended daily allowances have not kept pace with the growing understanding of the cellular and molecular functions of vitamins and other micronutrients. The aetiology of POAG remains a mystery. Discoveries in cell physiology can be assimilated from the literature and applied to known homeostatic mechanisms of the eye. In this way the possible roles of nutritional components involved in the aetiology of POAG can be described. The mechanisms may be subject to many influences in ways that have yet to be defined. Two distinct changes in the trabecular meshwork can be identified: trabecular meshwork changes that cause intra-ocular pressure to increase and trabecular meshwork changes that are directly correlated to optic nerve atrophy. Compelling evidence suggests that collagen trabecular meshwork extracellular matrix (ECM) remodelling is correlated to increased intraocular pressure in POAG. Elastin trabecular meshwork ECM remodelling is correlated to POAG optic nerve atrophy. There appear to be two different pathways of ECM remodelling and apoptosis induction in POAG. The pathway for collagen remodelling and apoptosis induction seems to be exogenously influenced by water-soluble antioxidants, for example, glutathione. The pathway for elastin remodelling and apoptosis induction seems to be influenced by endogenous lipid-soluble antioxidants, for example, vitamin E. Roles can be defined for antioxidants in the two different pathways of ECM remodelling and apoptosis induction. This suggests that antioxidants are important in maintaining cellular homeostasis relevant to the aetiology of POAG.

Primary open-angle glaucoma: Glutathione: Vitamin E

Primary open-angle glaucoma (POAG) is a complex chronic neurological disease that can result in blindness. Treatment is directed to lowering intra-ocular pressure (IOP) medically or surgically. Two recent studies have shown that evidence of POAG neurological damage can be delayed in ocular hypertensive patients whose IOP is medically reduced. These studies demonstrated that pressure lowering is helpful in preventing disease progression for some, but not for others. They also show that some ocular hypertensive patients do not develop neurological deficits when IOP is untreated (Lichter *et al.* 2001; Kass *et al.* 2002).

The goal of understanding the aetiology of POAG is to be able to target effective treatment to individuals who will eventually go blind without it. Several categories of study contribute to that effort: clinical, epidemiological, genetic, and physiological. The fruits of these studies have been the identification of POAG risk factors and establishing the complexity of the disease. The aetiology of POAG remains a mystery.

Epidemiological studies of POAG have not specifically addressed the possibility that nutrition may play a role in the development of POAG. A handful of papers have considered that nutrition may have an impact on POAG patients (Ernyei, 1966; Asregadoo, 1979; Bunin *et al.* 1992, 1993; Kurysheva *et al.* 1996; Popova & Kuz'minov, 1996; Cellini *et al.* 1998; Makashova *et al.* 1999). POAG is not believed to be a 'vitamin-deficiency disease'. 'The concept of 'vitamin deficiency diseases' and the recommended daily allowances have not kept pace with the growing understanding of

Abbreviations: ECM, extracellular matrix; HSP, heat-shock protein; IOP, intra-ocular pressure; MMP, matrix metalloproteins; ON, optic nerve; PEX, pseudoexfoliation; POAG, primary open-angle glaucoma; ROS, reactive oxygen species; SD, sheath-derived; TGF, transforming growth factor; TIMP, tissue inhibitors of matrix metalloproteins; TM, trabecular meshwork.

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the cellular and molecular functions of vitamins and other micronutrients.' (Challem, 1999).

Recent discoveries in cell physiology can be applied to known homeostatic mechanisms of the eye. In this manner the possible roles of nutritional components in the aetiology of POAG can be described. This does not define the aetiology; rather, it strongly suggests aspects of the aetiology that could contribute to the disease. Describing these mechanisms of action in isolation does not imply that they function independently. The mechanisms may be subject to many influences in ways that have yet to be defined (genetic and blood flow among the possible influences).

The accepted belief has been that POAG optic nerve (ON) damage is caused by IOP that is 'too high'. IOP is not correlated to ON atrophy. ON atrophy in POAG is directly correlated to trabecular meshwork (TM) elastin extracellular matrix (ECM) remodelling (Lutjen-Drecoll, 1999). This suggests that whatever is causing the increased IOP in POAG is not what is causing the ON atrophy.

Two distinct changes in the TM can be identified: TM changes that cause IOP to increase; TM changes that are directly correlated to ON atrophy. Collagen TM ECM remodelling seems to be correlated to increased POAG IOP. Elastin TM ECM remodelling is correlated to POAG ON atrophy. There appear to be two pathways of ECM remodelling and apoptosis induction in POAG. The pathway for collagen remodelling and apoptosis induction seems to be exogenously influenced by water-soluble antioxidants, for example, glutathione. The pathway for elastin remodelling and apoptosis induction seems to be influenced by endogenous lipid-soluble antioxidants, for example, vitamin E.

Oxidative stress

Reactive oxygen species

Oxidative destruction is caused by reactive oxygen species (ROS) known as oxidants (for example, lipid peroxides such as H_2O_2) and free radicals (for example, superoxide, hydroxyl radical) (Rose *et al.* 1998). They can be introduced to the cell exogenously from the extracellular fluid or produced endogenously by the cell itself. Their source in the eye is sunlight, mitochondria respiration, and intra- and extracellular metabolic reactions (Roth, 1997). The aqueous humour contains photochemically generated ROS, and lipid peroxidation products that flow through the TM, acting on the ECM or directly upon cellular membranes themselves (Babizhayev & Bunin, 1989; Babizhayev & Costa, 1994; Green, 1995; Rose *et al.* 1998). Lipid peroxidation products have been found in significantly higher concentrations in the aqueous humour and trabecular tissue of glaucoma patients compared with control subjects (Babizhayev & Bunin, 1989; Kurysheva *et al.* 1996).

Low-density lipids, which are oxidised in lipid peroxidation, are taken up by trabecular cells in the greatest capacity of any anterior segment ocular cells (Chang *et al.* 1991). Lipid peroxidation is a process where pro-oxidant compounds, such as ROS, react with PUFA of biological membranes. This oxidative modification of lipids causes changes in the physical and chemical properties of the membranes (Kourie, 1998; Vendemiale *et al.* 1999).

There are many ways that ROS can affect cells. ROS initiate many metabolic cascades with a wide variety of downstream effects. Lipid peroxides may affect membrane signal transduction (Girotti, 1998; Keller & Mattson, 1998; Kamata & Hirata, 1999) and ion exchange (Kourie, 1998). Gene regulation may be affected dramatically by photochemically generated ROS: 'early response genes' (c-fos, c-jun, c-myc); matrix metalloproteinases (MMP); adhesion molecules; cytokines. It has been implied that gene expression through protein tyrosine kinase and nuclear transcription factors are regulated by ROS (Ryter & Tyrrell, 1998). Oxidants enhance the expression of heat-shock factor, the upstream transcription factor for promoter elements of heat-shock genes (Calabrese *et al.* 2000).

Antioxidants

Two complimentary antioxidant systems participate in the maintenance of ECM remodelling and apoptotic cascades. Water-soluble antioxidants (glutathione and vitamin C) scavenge ROS in fluid outside the cell, and within the cell. As exogenous scavengers they help to prevent the introduction of excessive ROS from outside the cell. Within the cell and its organelles they scavenge ROS produced endogenously (Cardoso *et al.* 1998). The fat-soluble antioxidant vitamin E prevents endogenous mitochondrial production of ROS (Southam *et al.* 1991).

Three defence strategies are used to protect cells from oxidative damage: neutralisation of free radicals, metabolism of free radicals by enzymes, and/or repair of macromolecular damage. This is accomplished by water-soluble antioxidants (for example, ascorbic acid, cysteine, glutathione), lipid-soluble antioxidants (for example, tocopherols and retinols), specific enzymes (for example, superoxide dismutase, glutathione peroxidase), metal-binding proteins (transferrin, etc) (Babizhayev & Costa, 1994; Rose *et al.* 1998) and flavonoids (genistein, diazine, glycyrrhizin, etc) (Kapiotis *et al.* 1997; Wang *et al.* 1998). The most important antioxidants for the TM are water-soluble ascorbic acid and glutathione. A high level of ascorbic acid is necessary to maintain oxidative balance in the aqueous humour. The aqueous humour ascorbic acid: plasma ascorbic acid ratio is 35:1. Glutathione protects anterior segment tissues from low levels of H_2O_2 (Costarides *et al.* 1991).

Aqueous humour antioxidant activity in patients with POAG is reduced as the disease progresses (Bunin *et al.* 1992; Kurysheva *et al.* 1996; Makashova *et al.* 1999). As the stages of glaucoma advance, lacrimal fluid antioxidant levels decline at a faster rate and increase at a slower rate (when antioxidant therapy is administered) compared with blood plasma levels (Bunin *et al.* 1992; Kurysheva *et al.* 1996; Makashova *et al.* 1999).

Heat-shock protein

Heat-shock proteins (HSP) are produced in response to stress from ROS or heat (Iwaki *et al.* 1993; Kukreja *et al.* 1994;

Mallouk *et al.* 1999). When antioxidants do not prevent oxidative stress there is an increased level of HSP expression (Gorman *et al.* 1999; Calabrese *et al.* 2000). According to Rokutan (Polla *et al.* 1996; Rokutan *et al.* 1998), 'There is growing evidence that HSPs play an essential role in protecting cells against oxidative injury.' HSP induction has been observed in ischaemia–reperfusion, neurodegenerative diseases, and with elevated excitatory amino acids such as glutamate (Rokutan *et al.* 1998). 'Protective effects of HSP against oxygen radical-induced cellular damage may be targeted to any of the following: membrane lipid peroxidation, proteins, DNA, and mitochondria.' Polla *et al.* (1996) suggested that mitochondria are selective targets for the protective effects of heat shock against oxidative injury. They showed that overproduction of HSP prevented H_2O_2-induced cell death. Lipid peroxidation of the mitochondrial membrane was prevented by HSP (Polla *et al.* 1996).

Another role of HSP seems to involve a direct interference with apoptotic processes via the mitochondria pathway of caspase-dependent cell death. Apoptosis induced by mitochondria can be inhibited by HSP (Bruey *et al.* 2000; Li *et al.* 2000*a*; Saleh *et al.* 2000).

HSP may be evidence of oxidative stress in glaucomatous eyes. Compared with normal eyes, glaucomatous eyes have increased concentrations of HSP in both the lamina cribrosa and TM (Tamm *et al.* 1996; Lutjen-Drecoll *et al.* 1998; Tezel *et al.* 2000). H_2O_2 activates both trabecular cells and astrocytes to produce HSP (Tamm *et al.* 1996; Lee *et al.* 2001; Takuma *et al.* 2002).

Extracellular matrix remodelling and apoptosis

In POAG, activated trabecular cells and astrocytes in the lamina cribrosa create two distinct changes in their respective tissues. Collagen and elastin remodelling occur in the TM (Tengroth & Ammitzboll, 1984; Tengroth *et al.* 1985; Gottanka *et al.* 1997*b*) and ON lamina cribrosa (Tengroth & Ammitzboll, 1984; Umihira *et al.* 1994). The ECM remodelling in these tissues appears to be the result primarily of either exogenous or endogenous oxidative stress.

Exogenous and endogenous oxidative stress

The pathophysiology of POAG involves two distinct processes at work in the TM and lamina cribrosa. One process is apoptosis (Quigley, 1995; Matsuo & Matsuo, 1997; Sibayan *et al.* 1998; Agarwal *et al.* 1999; Levin, 1999; Love, 1999; Carmody & Cotter, 2000; Grierson *et al.* 2000; Hong *et al.* 2001; Loewen *et al.* 2001; Gu *et al.* 2002; Hamard *et al.* 2002; Wentz-Hunter *et al.* 2002). The other process is ECM remodelling (Tengroth & Ammitzboll, 1984; Hernandez *et al.* 1990; Hernandez, 1992; Hernandez & Ye, 1993; Rohen *et al.* 1993; Umihira *et al.* 1994). Oxidative stress can cause either (Tanaka *et al.* 1993; Slater *et al.* 1995; Bachem *et al.* 1999; Zhou *et al.* 1999; Li *et al.* 2000*b*; Izzotti, 2003). Apoptosis is redox sensitive (Susin *et al.* 1998; Nomura *et al.* 1999; Sugano *et al.* 1999). It can be initiated by exogenous H_2O_2 or H_2O_2 produced endogenously by mitochondria (Richter *et al.* 1995; Slater *et al.* 1995; Hirsch *et al.* 1997*a*,*b*; Hortelano & Bosca, 1997; Macho *et al.* 1997; Marchetti *et al.* 1997; Petit *et al.* 1997; Susin *et al.* 1997*a*,*b*).

Ageing and extracellular matrix changes

The prevalence of glaucoma increases with ageing. The TM and lamina cribrosa change with ageing and glaucoma (Alvarado *et al.* 1984; Babizhayev & Brodskaya, 1989; Hernandez *et al.* 1989; Hirano *et al.* 1991; Tripathi *et al.* 1994*a*; Albon *et al.* 1995; Okisaka *et al.* 1997; Agarwal *et al.* 1999; Hayreh *et al.* 1999). These ageing changes are similar in other tissues. The ageing process has been identified by the relative rates of biosynthesis of ECM components and post-synthetic modifications of matrix macromolecules. Interaction between ECM macromolecules and the cells that secrete them is continuous. According to Robert (1998), 'This 'program' of matrix biosynthesis changes progressively with age and reflects the modifications of cell phenotype in ageing tissues.' Increasingly, oxidative imbalance has been shown to initiate an adverse impact on this process in age-related chronic disease (Joseph *et al.* 1996; Poli & Parola, 1997; Papadopoulos *et al.* 1998; Wei, 1998; Christen, 2000; Esposito *et al.* 2000).

Trabecular meshwork and intra-ocular pressure

IOP is the result of all factors affecting the flow of aqueous humour in the eye. Aqueous humour homeostasis depends upon production by the ciliary body, adjacent tissue metabolism and outflow. Outflow is affected by oxidative destruction and/or metabolic irregularities that cause change in the TM and uveoscleral pathway (Babizhayev & Bunin, 1989; Gonzalez-Avila *et al.* 1995; Green, 1995).

Apoptosis

Aqueous humour flows from the anterior chamber through the inner TM layer and through the intermediate, then outer layer into Schlemm's canal. In POAG, TM cell loss corresponds to the flow of aqueous humour: 65 % of inner-layer trabecular cells die in POAG, while the outer (juxtacanalicular) layer has the same cell count as in normal eyes (Alvarado *et al.* 1984). Remaining trabecular cells of the inner layer are hypertrophic, but appear normal in the outer layer.

Extracellular matrix remodelling

TM ECM remodelling corresponds to the flow of aqueous humour in POAG. Innermost TM layers accumulate the most abnormal collagen (Tengroth *et al.* 1985). Subendothelial basement membrane thickening usually begins on the side of trabecular beams that face the aqueous humour flow (Quigley & Addicks, 1980). Collagen-associated amino acids found in POAG eyes are different from those found in normal eyes (Tengroth & Ammitzboll, 1984). Reducing collagen within the uveoscleral pathway by prostaglandin F-2 α (latanoprost) has been associated with IOP reduction (Lindsey *et al.* 1996, 1997*a*,*b*; Ocklind, 1998; Sagara *et al.* 1999).

Trabecular meshwork fibrotic processes common to other tissues suggest that the same processes occur at different rates in different tissues

TM ECM remodelling in POAG is similar to other tissues that are fibrotic (see Table 1). In atherosclerosis, hepatic, nephrotic and pulmonary fibrosis ECM-producing cells play a central role in their pathogenesis. According to Poli & Parola (1997), 'Molecular mechanisms of interaction among these cells... have much more in common than is superficially apparent.' The common mechanisms must therefore have metabolic initiators and facilitators that vary between individuals and between tissues. Trabecular cells are endothelial-like cells similar to the fibrogenic cells in these disorders. They have capacities of phagocytosis, and the ability to produce matrix-degrading enzymes, ECM elements and transforming growth factor (TGF)-β (Tripathi *et al.* 1994*a*,*b*; Yue, 1996).

TGF-β is increased in the aqueous humour of POAG eyes (Tripathi *et al.* 1994*b*). TGF-β has been shown to be fibrogenic in trabecular cells (Alexander *et al.* 1998). This fibrogenic cytokine also plays a key role in other fibrotic conditions such as atherosclerotic, pulmonary and hepatic fibrogenesis (Galera *et al.* 1992; Kahari *et al.* 1992; Katchman *et al.* 1994; Eickelberg *et al.* 1999; Garcia-Trevijano *et al.* 1999). A direct correlation between oxidative stress and TGF-β expression has been demonstrated (Poli & Parola, 1997). It has also been shown that ROS impair TM cell adhesion to the ECM (Zhou *et al.* 1999). This may rearrange the cytoskeleton structure. Cellular signalling pathways that regulate the cell can be affected by changes in the cytoskeleton structure (Oren *et al.* 1999; Tian *et al.* 2000). These findings suggest that ROS may be related to fibrotic TM changes in POAG.

Collagen remodelling

ROS may be related to outflow obstruction induced by collagen deposition in the TM. Collagen synthesis has been increased and collagen degradation reduced by human POAG aqueous humour (but not from normal controls, congenital or neovascular glaucoma). The element responsible has not been identified (Gonzalez-Avila *et al.* 1995). H_2O_2 is an identified element of aqueous humour that has reduced outflow facility by 33 % in calf TM when the balancing antioxidant, glutathione, was depleted (Kahn *et al.* 1983).

Trabecular cells have been identified with collagen synthesis (Yun *et al.* 1989; Tripathi *et al.* 1994*a*) and apoptosis (Agarwal *et al.* 1999). Mediators of human trabecular cell apoptosis have been up regulated in human endothelial cells by H_2O_2 (Suhara *et al.* 1998; Agarwal *et al.* 1999). At least one type of ROS, oxidised low-density lipids, can initiate both collagen synthesis and apoptosis (Jimi *et al.* 1994; Napoli *et al.* 2000). Bachem *et al.* (1999) have suggested that oxidative imbalance causes a biphasic response. They found that lower concentrations of oxidised low-density lipids have stimulated ECM remodelling, while higher concentrations have stimulated apoptosis in human coronary smooth muscle cells (Bachem *et al.* 1999).

Matrix metalloproteinases and collagen remodelling

Collagen synthesis is regulated by MMP and tissue inhibitors of matrix metalloproteinases (TIMP). MMP are Zn-dependent endopeptidases. They degrade ECM collagen and proteoglycans. Increased MMP activity decreases collagen deposition. TIMP reduce the capacity of MMP to degrade extracellular collagen (Brew *et al.* 2000). In a human aqueous humour outflow model, aqueous humour outflow facility was increased by stimulating MMP activity. Adding TIMP reduced the flow rate by decreasing endogenous MMP activity (Epstein *et al.* 1990; Pasquale *et al.* 1993; Lindsey *et al.* 1996; Bradley *et al.* 1998).

Ciliary body collagen and aqueous humour

The ciliary body produces aqueous humour. Collagen ECM remodelling of the ciliary body is important in glaucoma because it may affect aqueous humour content. In POAG patients, adjacent to the ciliary body, rough collagen fibre bundles accumulate in the anterior iris border and collagen increases in the adventitial sheath of iris vessels (Okamura & Lutjen-Drecoll, 1973).

TGF-β and TIMP have been identified with aqueous humour-producing ocular sites. TGF-β and TIMP are found in higher concentrations in the ciliary body compared with other anterior eye structures. In one study of the human eye, TGF-β was located in stroma proximal to the ciliary processes, in the ciliary processes, muscles of the ciliary body and stroma adjacent to pigmented epithelium of the pars plana, but not in the TM, corneal stroma, corneal endothelium, iris or ciliary epithelium (Pasquale *et al.* 1993). In human eyes, TIMP-1 and -2 were found in the ciliary body, and to a lesser degree in the TM (el-Shabrawi *et al.* 2000).

The POAG aqueous humour contains lipid peroxides (Babizhayev & Bunin, 1989), TIMP (Gonzalez-Avila *et al.* 1995) and TGF-β (Tripathi *et al.* 1994*b*) in higher concentrations than in normal eyes. A similar situation has been found in the kidney. When fed an atherogenic diet, experimental rats developed increased levels of lipid peroxides, collagen and TIMP-1. Antioxidant therapy (vitamin E and probucol) significantly reduced kidney collagen and TIMP-1 (Eddy, 1998).

Elastin remodelling

Sheath-derived plaque material. Elastin remodelling in POAG differs from collagen. Ciliary muscle elastic tissue extends into the outer TM layer, helping to form a delicate network of elastic-like fibres known as the 'cribriform plexus'. Fibroblasts of the ciliary muscle are continuous with the trabecular lamellae. In the area of the cribriform plexus, large amounts of elastic-like sheath-derived (SD) plaque materials are formed in low-tension glaucoma and POAG (Lutjen-Drecoll & Rohen, 1996).

Elastin and intra-ocular pressure. Elastin deposition often does not happen in POAG patients. There is no correlation between SD plaque formation and IOP. However, SD plaque formation is proportional to ON cell loss (Gottanka *et al.* 1997*b*; Lutjen-Drecoll, 1999).

Table 1. Fibrotic tissue changes: extracellular matrix components and cytokines

Fibrotic changes	Optic nerve in POAG	Trabecular meshwork in POAG	Nephrotic fibrosis	Atherosclerotic fibrosis	Hepatic fibrosis	Pulmonary fibrosis
Bcl-2	↓ Nickells (1999)	–	↓ Chevalier *et al.* (2000)	↓ Napoli *et al.* (2000)	↓ Gong *et al.* (1998)	–
Collagen I	↑ Morrison *et al.* (1990); Hernandez & Ye (1993)	↑ Tengroth & Ammitzboll (1984)	↑ Eddy (1996, 1998)	↑ Shekhonin *et al.* (1985, 1987)	↑ Desmouliere *et al.* (1997); Poli & Parola (1997)	↑ Raghu *et al.* (1985); Poli & Parola (1997)
Elastin	↑ Pena *et al.* (1998, 2001)	↑ Umihira *et al.* (1994)	–	–	↑ Desmouliere *et al.* (1997)	↑ Hoff *et al.* (1999)*
Fas–Fas ligand		Agarwal *et al.* (1999)*	↑ Razzaque *et al.* (1999); Ying *et al.* (2000)	↑ Napoli *et al.* (2000); Schneider *et al.* (2000)	↑ Luo *et al.* (1997)	↑ Kuwano *et al.* (1999*a*,*b*, 2000)
Heat-shock protein	↑ Tezel *et al.* (2000)	↑ Lutjen-Drecoll *et al.* (1998)	↑ Razzaque *et al.* (1998)	↑ Rocnik *et al.* (2000)	↑ Masuda *et al.* (1994)	↑ Razzaque *et al.* (1998)
Lipid peroxidation products	–	↑ Babizhayev & Costa (1994)	↑ Eddy, (1996, 1998)	↑ Hoff *et al.* (1975)	↑ Poli & Parola (1997)	↑ Poli & Parola (1997)
Tenascin	↑ Pena *et al.* (1999)		↑ Nakatsuji *et al.* (1998)	↑ Fukumoto *et al.* (1998); Wallner *et al.* (1999)	↑ Richter *et al.* (1998)	↑ Kaarteenaho-Wiik *et al.* (1998)
Transforming growth factor β-1	–	↑ Tripathi *et al.* (1994*a*,*b*)	↑ Eddy, (1996, 1998)	Fukumoto *et al.* (1998)*	↑ Poli & Parola (1997)	↑ Poli & Parola (1997)
TIMP-1	–	↑ Alexander *et al.* (1991, 1998); Gonzalez-Avila *et al.* (1995); Alexander *et al.* (1998)	↑ Eddy (1996, 1998)	Fabunmi *et al.* (1998)*	↑ Desmouliere *et al.* (1997)	↑ Kikuchi *et al.* (1995); Eickelberg *et al.* (1999); Yazawa *et al.* (2000)

POAG, primary open-angle glaucoma; TIMP, tissue inhibitor of matrix metalloproteins.
↓, the extracellular component or cytokine is decreased in the fibrotic condition of the tissue in the column; ↑, the extracellular component or cytokine is increased in the fibrotic condition of the tissue in the column.
*Lack of unambiguous observations.

Elastin formation. Increases in SD plaque material may be related to changes in elastic tissues, according to Umihara *et al.* (1994). Elastic fibres are formed in developing tissues by the combination of elastin and microfibrils (Robb *et al.* 1999). Some elastogenic cells can be reactivated to synthesise some elastic fibre components. When this happens the elastic fibres are usually aberrant and may disrupt or impair the normal function of the tissue. ROS may be the stimulus that leads to the production and degradation of elastin molecules in at least one form of elastosis leading to the accumulation of fragmented elastic fibres (Hayashi *et al.* 1998; Robb *et al.* 1999).

Stimulated trabecular and smooth muscle cells can produce elastin. The other elastic fibre component, microfibril, is expressed by ciliary epithelium. Ciliary epithelium does not produce the elastin precursor tropoelastin (Robb *et al.* 1999; Robert, 1999). In POAG and low-tension glaucoma, plaque material is found in areas that contain elastin: the cribriform layer, between anterior tips of ciliary muscle and surrounding vessel walls of the iris (not the iris itself, which is without elastin).

Trabecular meshwork morphology

Cell types in trabecular meshwork

Morphologically different cell types exist in POAG TM. This may be due solely to differences in activation of the same cell population (Lutjen-Drecoll, 1999). Differences in HSP staining and ECM remodelling suggest differences in cell activation. POAG, low-tension glaucoma, and pseudoexfoliation (PEX) glaucoma have different patterns of HSP staining and ECM remodelling (see Fig. 1) (Alvarado *et al.* 1984; Lutjen-Drecoll *et al.* 1986*a*,*b*, 1998; Umihira *et al.* 1994; Ritch *et al.* 1996; Gottanka *et al.* 1997*a*,*b*; Lutjen-Drecoll, 1999; Tezel *et al.* 2000).

Exogenous cell activation v. endogenous cell activation

In POAG, trabecular cell activation yields extensive inner-layer collagen deposition. Exogenous trabecular cell activation in POAG is suggested by the presence of excessive cell activators in the aqueous humour (H_2O_2 and TGF-β), higher TIMP levels, reduced antioxidant levels (glutathione) in the aqueous humour, a pattern of TM collagen ECM remodelling corresponding to the direction of aqueous humour flow, cell loss corresponding to the direction of aqueous humour flow, and a lack of HSP staining. In contrast, endogenous activation in low-tension and PEX glaucoma is suggested by HSP staining of all layers of the TM and ECM remodelling and cell activation, which lacks correlation to aqueous humour flow.

Cell activation and morphology

HSP synthesis is a response to endogenous cell activation. HSP are protective of mitochondria (Polla *et al.* 1996; Rokutan *et al.* 1998). All TM layers stain for HSP in low-tension glaucoma. In POAG less than one half of all TM stain in all layers for HSP (Lutjen-Drecoll *et al.* 1998). This suggests that endogenous cell activation predominates in low-tension glaucoma, but not in POAG.

Morphological differences caused by differences in cell activation may explain ECM remodelling differences in POAG, low-tension glaucoma and PEX glaucoma. ECM remodelling in PEX glaucoma (PEX material) is directly correlated to IOP and to ON axon loss (Gottanka *et al.* 1997*b*). The correlation between PEX deposition, IOP and ON axon loss in PEX glaucoma contrasts with the absence of correlation between IOP and axon loss in POAG.

Elastic tissue: microfibrils increase intra-ocular pressure; elastin does not increase intra-ocular pressure

PEX material comprises microfibrils related to elastic tissue in the subendothelial region. There is no increase in SD plaque formation. Similarly, fine fibrils distinct from SD plaques accumulate below the inner-wall endothelium of Schlemm's canal in corticosteroid-induced glaucoma. Of the extracellular material, 90 % is composed of this fine fibrillar material in corticosteroid glaucoma, but only 30 to 36 % in normal or POAG eyes (Lutjen-Drecoll, 1999). It appears that fibrils associated with elastic tissue are distinct from SD plaque formation. They may be related to outflow obstruction in PEX, corticosteroid-induced, and juvenile glaucoma. The production of fibrils and SD-plaque deposits in glaucoma suggests endogenous cell activation, which produces either of two distinct elastic fibre components: microfibrils or elastin. These findings suggest different ECM components associated with outflow obstruction in POAG, PEX and corticosteroid-induced glaucoma (see Table 2).

Conclusion

Increased IOP in POAG is not correlated to ON axon loss. On the other hand, ECM elastin remodelling is correlated to ON axon loss in POAG (Gottanka *et al.* 1997*b*). This same elastin remodelling is found in low-tension glaucoma. The correlations between endogenously initiated TM ECM remodelling and ON axon loss suggest similar cell activation in the TM and ON.

Optic nerve

Elucidating physiological interactions at the ON lamina cribrosa can present common factors responsible for glaucomatous optic atrophy and TM SD plaque formation.

Extracellular matrix remodelling

Elastic-like tissue change associated with SD plaque formation in the TM may be incidental to increased IOP in POAG; but POAG elastin changes in the TM are not irrelevant to ON nerve fibre loss. Curled elastin is found more often in areas of the nerve head with nerve fibre loss, and not in zones without loss of nerve bundles (Quigley *et al.* 1994). (Elastosis also occurs in the lamina cribrosa, but not in other areas of the ON in PEX glaucoma

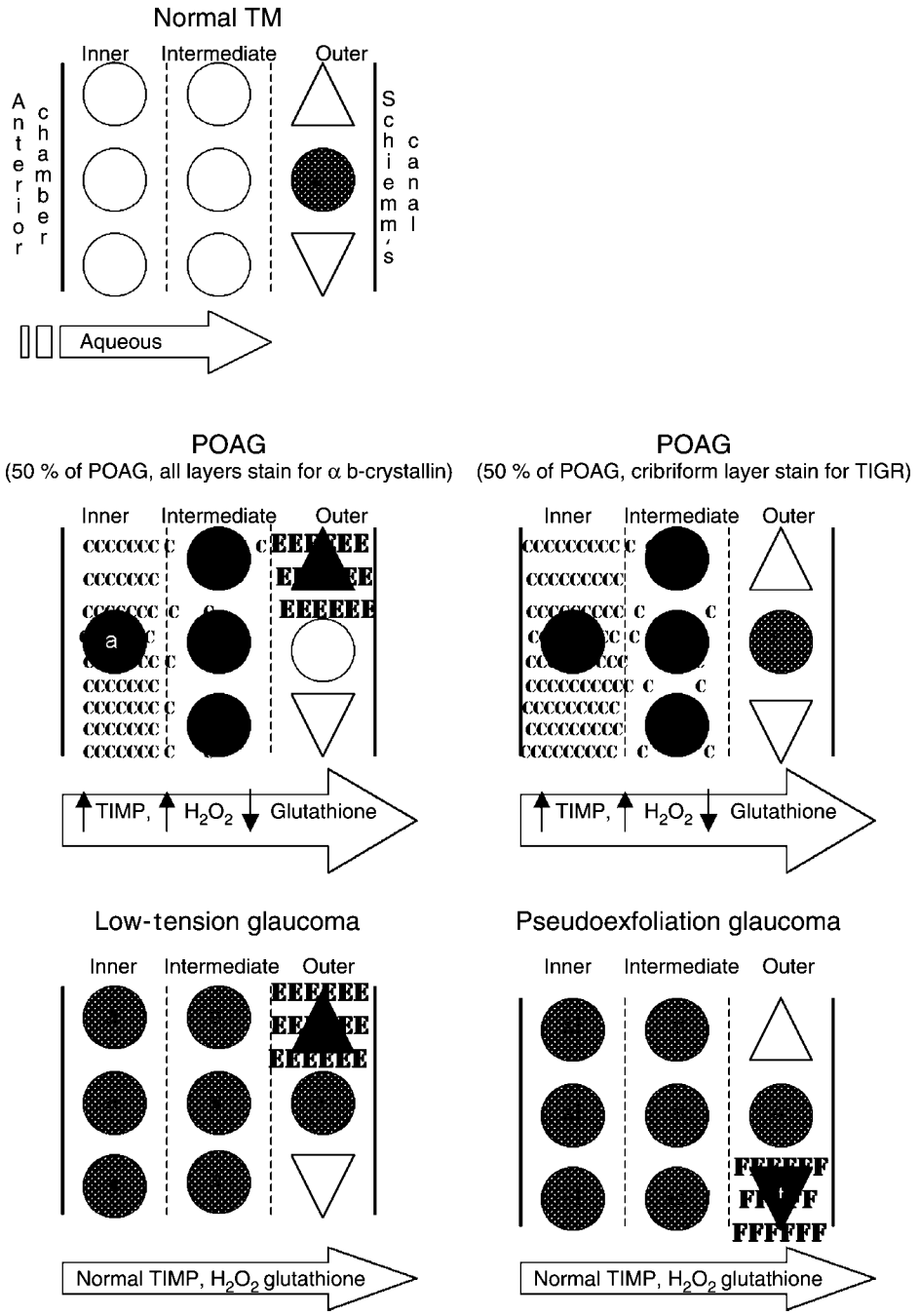

Fig. 1. Trabecular meshwork (TM) matrix schematic: cell morphology. Trabecular cells change in TM layers in different types of glaucoma. The TM layers are distinguished by different amounts of trabecular cell losses, different activities of cell activation, different staining by α b-crystallin or trabecular meshwork-induced glucocorticoid response protein (TIGR), and different extracellular matrix (ECM) remodelling. Reduced glutathione, increased H_2O_2, and increased tissue inhibitors of matrix metalloproteins (TIMP) are features of primary open-angle glaucoma (POAG) aqueous humour. ECM materials: C, collagen; E, elastin; F, fibrillin. Heat-shock proteins: a, α b-crystallin; t, TIGR. Cell types: (○), normal trabecular cell; (▨), activated, pre-secreting cell; (▩), highly activated, secreting cell (collagen secretor); (●), highly activated, apoptotic; (Δ), elastin-secreting cell; (▼), fibrillin-secreting cell.

(Netland *et al.* 1995).) Elastin changes along with collagen changes are found in the glaucomatous cup (see Table 1).

Glaucomatous elastin and collagen changes have not been found in ON atrophy due to transection. The implication is that the lamina cribrosa biochemical alteration may be a selective response to POAG progression rather than a response to ON atrophy (Morrison *et al.* 1990; Pena *et al.* 2001).

Optic nerve anatomy and physiology

Optic nerve anatomy. According to Cohen (1981), 'The axons of the retinal ganglion cells sweep across the inner face of the retina, just below the surface mosaic of expanded foot processes of the glial cells of Muller, and head toward the nerve head, where they turn sharply, penetrate the retina, and leave the eye.' Axons that form the ON

originate in the ganglion layer of the retina. At the optic disk, ON axons are covered and segregated only by astrocytes, which are fibrous glial cells with fine protoplasmic processes extending in all directions. Beginning at the lamina cribrosa, nerve bundles are enclosed in connective tissue septa. In the orbit and ON canal the ON is covered by meningeal sheets continuous with those of the brain. The ON has the same glial and blood-supply organisation as brain white matter. Glia play an important role in the nutrition of the axons (Hogan *et al.* 1971; Cohen, 1981). Blood supply in the prelaminar layer is from branches of the short posterior ciliary arteries and Zinn's circle, while the choroidal vessels contribute only a few branches. Prelaminar and laminar layers have the narrowest capillaries, making the lamina cribrosa the most sensitive part of the ON to ischaemia (Zhao & Li, 1987).

Reduced vascular capacity at the lamina cribrosa. A reduced vascular capacity makes the prelaminar and laminar ON the most vulnerable to metabolic compromise. Under normal conditions the vascular supply to this area is capable of maintaining homeostasis. Fibrotic changes in the glaucomatous ON lamina cribrosa are evidence of chronically compromised metabolism. In the core of the cribriform plates of the glaucomatous lamina cribrosa, granular masses of elastin appear, and elastic fibres become increasingly disorganised with the progression of the disease (Hernandez *et al.* 1990). Little or no elastin synthesis occurs in the adult ON. During glaucomatous optic neuropathy, astroglial cells synthesise tropoelastin. The synthesised tropoelastin is without defect, so impairment in elastic fibre formation probably occurs in the extracellular compartment during fibre assembly (Pena *et al.* 1996).

Oxidative imbalance at the lamina cribrosa. Degradation of the ECM may lead to axonal loss (Hernandez & Pena, 1997; Hernandez, 2000). Reduced vascular capacity at the lamina cribrosa suggests that something that is not removed, or something that is not provided, in the ECM leads to astrocyte stimulation and the impairment of elastic fibre assembly. Evidence that ROS stimulate astrocyte secretions (Tanaka *et al.* 1999) and degrade tropoelastin (Hayashi *et al.* 1998) suggests that POAG lamina cribrosa ECM remodelling may be due to increased ROS.

The presence of HSP suggests oxidative imbalance. Astrocytes in the laminar region of the optic disk are the most highly stained astrocytes for HSP 27. Retinal ganglion cells farthest from blood vessels stain more intensely for HSP 27 than those nearest to the blood supply (Tezel *et al.* 2000). Compromised vasculature and increased HSP demonstrate more opportunity and evidence of ROS imbalance at the ON lamina cribrosa than at other parts of the ON. This can explain why the locus of ON dystrophy is in the lamina cribrosa.

Optic nerve atrophy

ON damage in POAG has been explained by direct mechanical effect or compromised vasculature (ischaemia) (Dreyer, 1998). As the effects of ischaemia are elucidated it appears that ischaemia may be the primary factor in

Table 2. Glaucoma (GLC) extracellular matrix (ECM) remodelling*

GLC type	IOP	Collagen	SD plaque	Fibril
POAG (2·8% of population; Kozobolis *et al.* 2000)	Increased	Increased	Strong correlation to ON axon loss. No correlation to IOP	Weak correlation to ON axon loss
Low-tension GLC (9·67% of POAG patients; Kozobolis *et al.* 2000)	Normal or low	Normal	Increased	
PEX GLC (25·80% of POAG patients; Kozobolis *et al.* 2000)	Increased	-	Normal	PEX material correlated to ON loss and correlated to IOP
	Increased	-	Normal	Increased
Corticosteroid-induced GLC	Increased	Not increased	Increased	Similar to corticosteroid-induced GLC. Cribriform thicker than POAG
Juvenile GLC				

IOP, intra-ocular pressure; SD, sheath-derived; POAG, primary open-angle glaucoma; ON, optic nerve; PEX, pseudoexfoliation.
*ECM remodelling and IOP in different types of GLC are compared. Values from one study are used to show GLC prevalence relative to the other types of GLC. The prevalence of corticosteroid and juvenile GLC is so low that their representations as proportions of GLC cases are not reported in the literature.

POAG, with the mechanical effects of IOP exacerbating the condition. Optic atrophy, ischaemia and ROS are related. POAG ON atrophy is due principally to apoptosis (Nickells & Zack, 1996; McKinnon, 1997; Okisaka *et al.* 1997). ROS have been shown to cause apoptosis (Giardino *et al.* 1998; Carmody & Cotter, 2000; Li *et al.* 2000*b*). According to Bonne *et al.* (1998), 'ROS generated under ischemia and at reperfusion can transiently disturb neurones or kill them either by necrosis or apoptosis, depending on their concentration. They (ROS) can also be responsible for excitatory amino acid (glutamate) release and are secondarily produced by these neurotransmitters in a vicious cycle.'

Glutamate

Dystrophic by-product. Even in POAG patients with well-controlled IOP, glutamate levels in the vitreous humour are sufficiently elevated to kill retinal ganglion cells. It is uncertain whether glutamate plays a primary or secondary role in glaucomatous damage. The source of glutamate is undetermined (Dreyer, 1998). Most of the glutamate released during ischaemia is of cytosolic origin (O'Regan *et al.* 1997). Osborne *et al.* (1999) state that 'it seems unlikely that factors (for example, glutamate) are released from dying or dead ganglion cells to cause the death of the remaining ganglion cells.' Other studies seem to suggest that increased glutamate levels are a dystrophic by-product. Before cell death, intra-ocular glutamate levels have been shown to increase in partially crushed rat optic neurones (Yoles & Schwartz, 1998). In ischaemic optic neuropathy, axonal injury is independent of glutamate (Fern & Ransom, 1997).

Glutamate from mitochondrial reactive oxygen species. Ischaemia may cause excessive ROS production by mitochondria, leading to high levels of extracellular glutamate and high levels of intracellular Ca. According to Dreyer (1998), 'Destabilisation of calcium homeostasis within the neurone could be due to ROS damage to mitochondria that results in a prolonged energy deficit. A sustained increase in intracellular calcium can also lead to the activation of many calcium-sensitive enzymes such as endonucleases and proteases, which can themselves lead to cell death.' Mitochondrial shifts of redox and membrane potentials are linked directly to excitotoxicity (Schinder *et al.* 1996).

Non-synaptic glutamate. In central nervous system white matter (such as the ON) anoxic injury is mediated by cellular mechanisms that do not involve synapses (Waxman *et al.* 1993). Although there are no synapses at the lamina cribrosa, the astrocytes and ON axons can influence each other through a wide range of glial–neural interactions (Ransom & Orkand, 1996). ON axons can release glutamate into the extracellular space and to astrocytes along ON axons (Ransom & Orkand, 1996). The increased permeability of damaged retinal cells could also increase the intracellular enzymes responsible for glutamate synthesis (Dreyer, 1998). At the lamina cribrosa, astrocytes form the barrier between the vitreous humour and ON axons. It is possible that ROS produced by mitochondria within the cell initiate glutamate release by lamina cribrosa ON axons to the extracellular space. The extracellular glutamate increase would be further exacerbated by ROS blockage of glutamate absorption. This would present an opportunity to raise vitreous humour glutamate levels in glaucoma.

Mitochondria concentration at lamina cribrosa is affected by ATP demands

Mitochondria produce energy in the form of ATP. ATP is needed by the cell to maintain ionic balance through active transport. Ionic pumps, which are essential for the transmission of action potentials along cell membranes, use nearly 50 % of ATP produced by mitochondria. At the lamina cribrosa, the axons of ganglions lack myelin sheaths. A generalised depolarisation along the ON cell membrane takes place as the action potential is conducted through the lamina cribrosa. To the posterior, membrane depolarisation occurs only at myelin sheath junctions known as the nodes of Ranvier. Therefore, energy (ATP) demands to restore ionic balance following conduction are greater at the lamina cribrosa compared with the myelinated portion posterior to the lamina cribrosa (Andrews *et al.* 1999). Evidence suggests that increased energy demands cause increased concentrations of mitochondria (Lee & Wei, 2000).

Mitochondria produce reactive oxygen species and consume oxygen

The major producers of ROS in the cell are mitochondria, converting up to 5 % of oxygen consumed by mitochondria to ROS (Chow *et al.* 1999). Mitochondria consume 85 % of all oxygen used by the cell. Normal ON accumulate higher concentrations of mitochondria in the lamina cribrosa compared with the rest of the axon. POAG ON axons accumulate a higher concentration of mitochondria than normal ON axons (Hollander *et al.* 1995). Organelle accumulation and impairment of axonal transport may result from vitamin E deficiency. '...The ganglion cell layer and optic nerve are exquisitely sensitive to the disruption of mitochondrial biogenesis.' (Southam *et al.* 1991; Howell, 1997*b*) As the number of mitochondria increases more oxygen is consumed and more ROS are produced. In the lamina cribrosa this occurs where circulatory capacity is most likely to result in ischaemia.

Large optic nerve axons in primary open-angle glaucoma *v.* small optic nerve axons in optic nerve ischaemia

'Increasing evidence supports the concept that impairment of mitochondrial energy metabolism may underlie the pathology of most important neurodegenerative disorders.' (Calabrese *et al.* 2000; Lee & Wei, 2000) Contrasts between the progression of ON atrophy in POAG and ON atrophy in Leber's and ON ischaemia may be examples of a physiological dichotomy that originates at the mitochondria.

Apoptosis v. *necrosis*

Severe ATP depletion leads to necrosis. Necrosis does not occur when only a fraction of normal ATP is present. If ATP is not entirely exhausted apoptosis develops when ATP is depleted (McConkey, 1998; Lemasters *et al.* 1999). Apoptosis is responsible for POAG ON atrophy. POAG vision loss occurs gradually over 10 years, the optic cup undergoes ECM remodelling, and large ON axons are the first to be apoptotic (Dreyer *et al.* 1994; Vickers *et al.* 1995; Cai & Jones, 1999).

Metabolic challenge: large v. small axons

Reactions of large axons and small axons differ in response to metabolic challenge. Large axons recover more slowly from Ca imbalance (Stys & Lesiuk, 1996). ROS induce mitochondrial Ca imbalance (Richter, 1993). The greater concentration of mitochondria in large (*v*. small) axons could produce more ROS in large axons and be responsible for the slower Ca recovery of large axons. Glutamate is more toxic to large axons than to small (McKinnon, 1997; Sucher *et al.* 1997). Glutamate injury can be mediated by mitochondria (Lemasters *et al.* 1999). ROS can be responsible for glutamate release (Bonne *et al.* 1998). The greater glutamate toxicity to large axons compared with small could also be explained by higher concentrations of ROS producing mitochondria in large axons *v*. small. This may be exacerbated by ROS inhibition of glutamate uptake in astroglial cells (Bonne *et al.* 1998).

Small axons: optic nerve ischaemia

Atrophy of small axons precedes that of large axons in ON ischaemia and Leber's optic atrophy (Sadun *et al.* 2000). Necrosis is responsible for ON atrophy in ON ischaemia and Leber's disease (Howell, 1997*b*; Zamzami *et al.* 1997). In both of these diseases vision loss is rapid, small axons are affected first and there is swelling of the nerve head (Fern & Ransom, 1997; Howell, 1997*b*). Ischaemic ON atrophy patients in their seventies experience sudden loss of central vision when vision loss is not total. Ischaemic optic atrophy results from the loss of blood flow, and therefore oxygen supply. This causes a dramatic loss of ATP production (Lemasters *et al.* 1999; Osborne *et al.* 1999).

Small axons: Leber's optic atrophy

In Leber's optic atrophy, carriers of the Leber's gene are afflicted with the sudden onset of dense bilateral central scotomas in their second decade of life. The hereditary dysfunction of mitochondrial energy production (ATP) is thought to be responsible for Leber's optic atrophy. Optic neuropathy in families with the Leber's gene shows incomplete penetrance. About 50 % of males and 10 % of females in large European Leber's families become afflicted. Secondary aetiological factors, which may be non-genetic, are suspected. Heavy alcohol and/or tobacco use increase the risk of optic neuropathy in Leber's family members (Howell, 1997*a*).

Conclusion: large optic nerve axons, more mitochondria

It has been suggested that the reason central visual fields are affected first in Leber's optic atrophy is because 'parafoveal ganglion cells may be 'poorer' in mitochondrial content' (Howell, 1997*a*). In Leber's and ON atrophy, large axons may have enough mitochondria to delay necrosis by producing enough ATP. On the other hand, richer concentrations of mitochondria in large axons may deplete oxygen supplies in large axons before small axons when low-grade ischaemia is chronic. This may increase the concentration of ROS from mitochondria in large axons compared with small (Chandel *et al.* 1998). The result would be a depletion of antioxidant stores in larger axons before smaller axons. This could lead to large axon apoptosis in POAG before small axon apoptosis (see Fig. 2).

Reactive oxygen species: cause and effect in primary open-angle glaucoma

Two probable causes of increased ROS at the lamina cribrosa of the POAG ON have been identified: ischaemia–reperfusion and increased mitochondria. The presence of ROS effects is additional evidence of ROS involvement. Specific effects of ROS have been identified in POAG, the retina and the ON. In a review, Bonne *et al.* (1998) enumerated ROS effects in the retina. These were: (1) ionic imbalance; (2) inhibition of glutamate re-uptake by astroglial cells; (3) inhibition of the Na-dependent glutamate transporter; (4) inhibition of glutamate synthase

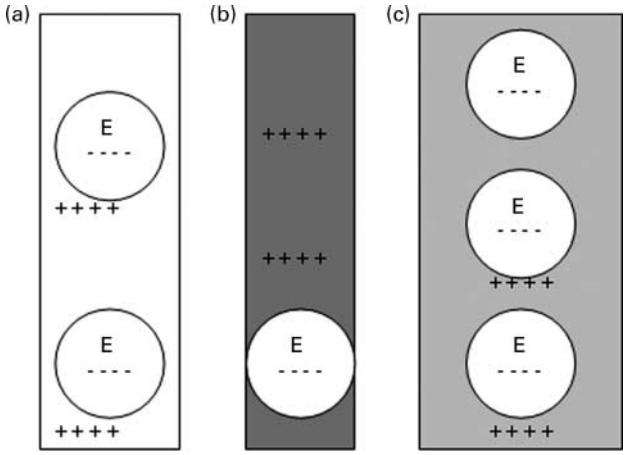

Fig. 2. Schematic representation of cellular necrotic and apoptotic responses to changes in ATP and oxidative balance (reactive oxygen species; ROS). Energy units, ROS units and antioxidant units are conceptual representations only; actual measurements of these units are not currently available. Cell viability for this exercise requires two units of energy and a larger number of antioxidants than ROS to prevent ROS damage. In a normal-sized axon (a), ROS units and antioxidant units are equal. Two mitochondria produce two units of energy needed for cell viability. In a smaller axon (b), one mitochondrion does not produce enough ATP to prevent necrosis. Antioxidant units outnumber ROS produced by mitochondria. In a larger axon (c), three mitochondria produce enough ATP for cell viability, but produce ROS in excess of antioxidant capacity and this leads to apoptosis. (□), Normally functioning axon; (■), necrotic axon; (▒), apoptotic axon; (○), mitochondria; E, energy unit (ATP); + , antioxidant; -, ROS.

(which metabolises glutamate); (5) induction of protein synthesis; (6) induction of apoptosis; (7) induction of HSP; (8) induction of catalase; (9) induction of vascular endothelial growth factor; (10) induction of vascular permeability factor. Effects of ROS in the ON include: ionic imbalance; inhibition of glutamate uptake by astrocytes; apoptosis and protein synthesis (Haun *et al.* 1992; Waxman *et al.* 1993; Hori *et al.* 1994, 1996; Sucher *et al.* 1997; Suzuki *et al.* 1997; Behzadian *et al.* 1998; Blanc *et al.* 1998). Specific effects of ROS that have been found in the POAG ON are apoptosis (Okisaka *et al.* 1997), expression of neural cell adhesion molecule (Ricard *et al.* 1999), expression of HSP (Tezel *et al.* 2000), and enhanced expression of tenascin (Pena *et al.* 1999). The presence of known causes of ROS and known effects of ROS in POAG ON suggests that oxidative imbalance plays a role in the development of optic neuropathy of POAG.

Nutrition

Vitamin C, vitamin E and glutathione are the principal antioxidants in metabolism relevant to glaucoma. The actions of these antioxidants, the pathology of glaucoma, and oxidative homeostasis converge. Relative to one another these antioxidants function with independence, interdependence and redundancy. Vitamin C and glutathione scavenge ROS from intracellular and extracellular fluid. Vitamin E interrupts chain reactions initiated in cell and organelle membranes by ROS. These antioxidants also have additional roles affecting cell viability.

Glutathione

Glutathione is an hydrophilic enzyme that inhibits lipid peroxidation (Lii *et al.* 1998). Lipid peroxidation by-products, H_2O_2 and other peroxides are removed by the glutathione redox system (Cardoso *et al.* 1998; Ferreira *et al.* 1999). In mitochondria, glutathione is the only defence against H_2O_2 (Fernandez-Checa *et al.* 1998). A biphasic pattern of glutathione depletion has been demonstrated: there is rapid disappearance of cytosolic glutathione without comparable mitochondrial decreases (Chen *et al.* 1999). Glutathione is synthesised only in the cytosol from extracellular glutathione degradation products transported to the cytosol (Meister, 1995). From there it is transported to mitochondria. Transport mechanisms preserve mitochondrial glutathione even when cytosolic glutathione is depleted. Little efflux of glutathione from mitochondria occurs when cytosolic levels are low. On the other hand, 'Administration of glutathione esters to glutathione-depleted animals produces substantial increase in the mitochondrial glutathione level without comparable increase of the cytosolic glutathione level.' (Meister, 1995).

Trabecular meshwork heat-shock protein staining and glutathione. Within the cell, glutathione transport mechanisms preserve mitochondrial glutathione even when cytosolic glutathione is depleted. Due to this preservation of mitochondrial oxidative balance, secondary defence mechanisms, such as HSP, may not be activated. In the inner-layer TM less than 50 % of POAG eyes stain for HSP, yet there is 66 % trabecular cell loss in POAG eyes. The mitochondria may possibly take more glutathione from the cytosol to balance oxidative stress. This would prevent endogenous cell activation and HS staining, but exacerbate exogenous cell activation.

Glutathione and collagen deposition in trabecular meshwork. In POAG decreased aqueous humour glutathione reduces the amount of glutathione degradation products available for transport into trabecular cell cytosol. According to Li *et al.* (2000*b*), 'The hydrogen peroxide down-regulation of glutathione may be more important for apoptosis than hydrogen peroxide induction of lipid peroxidation; and the hydrogen peroxide induced changes in redox status of the cell may be among the original events which lead up to other biochemical changes.' 'Other biochemical changes' of inner-layer TM in POAG include significant collagen synthesis. Collagen gene induction is enhanced when glutathione is reduced in the presence of H_2O_2 in one type of fibrogenic cell (hepatic stellate cells). Lipid peroxidation does not seem to be a factor in this pro-fibrogenic effect (Nieto *et al.* 1999; Varela-Rey *et al.* 1999). These findings suggest that reduced aqueous humour glutathione and increased H_2O_2 may contribute to collagen deposition in POAG TM.

Glutamate: a glutathione precursor in the optic nerve. The demand for glutathione at the ON lamina cribrosa increases if the increased POAG lamina cribrosa mitochondria concentration causes increased H_2O_2 production. These conditions conspire to reduce the availability of glutathione. Glutathione is transported to nerves after it has been synthesised in the cytosol of glial cells (Schutte & Werner, 1998). Glutathione synthesis in the cytosol of glial cells is limited by a glutathione precursor, glutamate (Huster *et al.* 2000). ROS block the uptake of glutamate (Muller *et al.* 1998). While sub-toxic above-normal glutamate levels can increase neurone resistance to toxicity, excessive glutamate can be toxic (Schwartz & Yoles, 2000). Excessive extracellular glutamate also blocks the uptake of another glutathione precursor, cysteine (Wilson, 1997). H_2O_2 causes the release of glutamate from neurones (O'Regan *et al.* 1997). The efflux of glutathione precursors from the cytosol can result from vitamin E depletion (Pascoe *et al.* 1987) (see Fig. 3).

Glutathione in the ageing retina. Ageing human retinas have a high inter-individual variability of antioxidant activity. In the peripheral retina (but not macula) there is a tendency for antioxidant activity to decline with age (De La Paz *et al.* 1996). The glutathione content of retinal Muller glial cells declines significantly in ageing guinea-pigs (Paasche *et al.* 1998).

Vitamin E

Vitamin E is a lipid-soluble chain-breaking antioxidant stored in cell membranes and mitochondrial membranes. As Southam *et al.* (1991) state, 'It is able to terminate free radical-generated chain reactions by scavenging peroxyl radicals formed by the action of oxygen-derived free radicals on polyunsaturated fatty acids (PUFA) of membrane phospholipids. It may also protect membrane proteins from oxidation.' The membrane of mitochondria

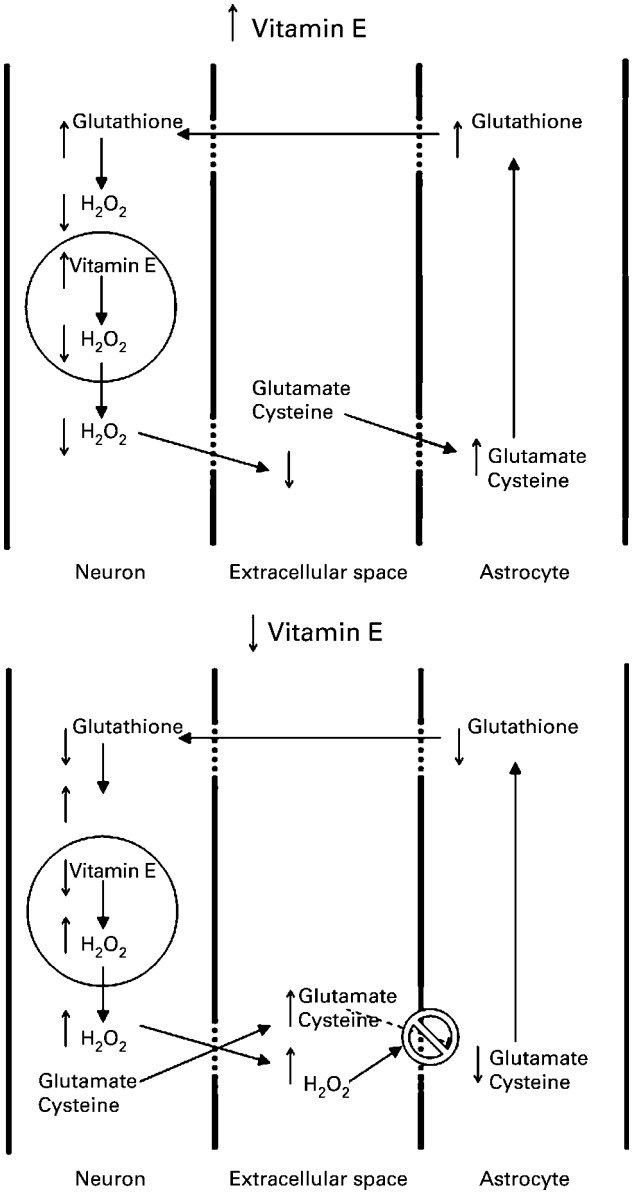

Fig. 3. Schematic representation of glutathione and vitamin E metabolism in astrocytes and neurons. Reactive oxygen species homeostasis in the neuron depends upon the relationship between vitamin E and glutathione that is produced in astrocytes. (○), Mitochondria; (▮), cell walls.

contains a high proportion of PUFA compared with other membranes. Mitochondria also produce ROS as a by-product of oxidative phosphorylation. These factors make the mitochondria more susceptible to damage in vitamin E deficiency (Southam *et al.* 1991). 'Besides serving as an antioxidant, vitamin E has been suggested to be involved in the direct modulation of regulatory proteins, and in the activity of key regulatory enzymes.' (Cardoso *et al.* 1998; Chow *et al.* 1999) Apoptosis during hypoxia and oxygen reperfusion can be prevented by vitamin E (Tagami *et al.* 1999). There is also some evidence to suggest that vitamin E may reduce apoptosis by means other than antioxidation (Barroso *et al.* 1997; Osborne *et al.* 1998; Lizard *et al.* 2000).

Vitamin C

Vitamin C has an adjunct role in maintaining TM and ON homeostasis. It functions together with glutathione to protect mitochondria from oxidative damage (Meister, 1995). Ascorbate increases mitochondrial glutathione in glutathione-deficient animals (Meister, 1995). Within the cell, vitamin C helps to protect membrane lipids from peroxidation by recycling vitamin E (May, 1999). Vitamin C prevents vitamin E oxidation in outer rod segments induced by UV light (Stoyanovsky & Cederbaum, 1996). There may also be an anti-apoptotic function to ascorbic acid (Barroso *et al.* 1997; Osborne *et al.* 1998).

In the eye, vitamin C has been shown to reduce lipid peroxide damage (Augustin *et al.* 1992). All regions of mice brain have demonstrated reduced tert-butyl hydroperoxide-induced lipid peroxidation with prior supplementation of vitamin E and vitamin C (Bano & Parihar, 1997). Glaucomatous human TM cells synthesise hyaluronic acid at a lower rate than normal cells. Ascorbic acid has stimulated increased hyaluronic acid synthesis in glaucomatous trabecular cells compared with normal human trabecular cells (Schachtschabel & Binninger, 1993). TM ECM production may be mediated by vitamin C (Epstein *et al.* 1990; Sawaguchi *et al.* 1992).

Glutathione and vitamin E deficiency

Vitamin E deficiency increases H_2O_2 levels (Chow *et al.* 1999). H_2O_2 is scavenged by glutathione (Fernandez-Checa *et al.* 1998). Glutathione deficiency allows H_2O_2 to rise. High concentrations of H_2O_2 can block the uptake of glutathione precursors, glutamate and cysteine, into astrocytes (Han *et al.* 1997; Sorg *et al.* 1997). Without glutathione precursors, astrocyte glutathione synthesis drops (Anderson, 1998; Huster *et al.* 2000; Pocernich *et al.* 2000). Neural glutathione is derived from astrocytes (Han *et al.* 1997; Schutte & Werner, 1998; Liu *et al.* 1999). Reducing astrocytic glutathione synthesis reduces glutathione levels in neurones for protection from H_2O_2 (Li *et al.* 1998; Schutte & Werner, 1998). An imbalance of this homeostatic cycle can be introduced at any point: vitamin E, glutathione, glutamate or H_2O_2 (see Fig. 3). Vitamin E, glutathione, glutamate and H_2O_2 levels in this cycle are all affected by many factors (Almeida *et al.* 1998; Li *et al.* 1998; Lindenau *et al.* 1998).

Nutrition conclusion

Ciliary body oxidative balance and aqueous humour oxidative balance

The ciliary body may be important to the process of POAG because it generates aqueous humour. Different ocular structures have been shown to require different nutrient levels. This may be due to the different functions of the structures (Khachik *et al.* 1997; Giblin, 2000). There is evidence that the ciliary body plays a greater role in detoxification than other ocular structures. Detoxifying enzymes have been found in higher concentrations in the ciliary body compared with other parts of the eye. Detoxification of ROS during bovine aqueous humour formation has been

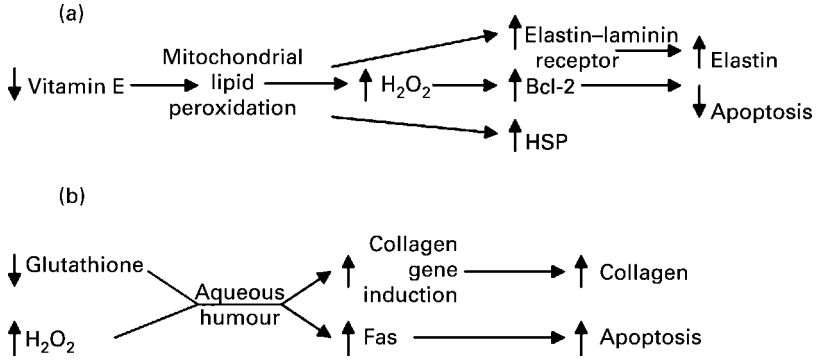

Fig. 4. Dichotomy of cell activation: dichotomy of extracellular matrix remodelling, and apoptosis for (a) elastin, vitamin E in mitochondria and (b) collagen and glutathione in the cell wall. These diagrams represent the dichotomy of cell activation resulting from vitamin E or glutathione deficiency. The components of the metabolic cascades initiated by vitamin E deficiency result in elastin remodelling without apoptosis. The components of the metabolic cascades initiated by glutathione deficiency result in collagen remodelling and apoptosis. HSP, heat-shock protein.

accomplished principally by the ciliary epithelium (Ng *et al.* 1988).

Antioxidant depletion probably occurs more quickly in the ciliary body than in other ocular structures. Animal studies have shown that the ciliary body and iris respond within 1 week to vitamin E deficiency, while retinal tissue vitamin E levels drop more slowly to 10% of controls after 12–15 weeks. After 40 weeks vitamin E levels dropped more significantly (Stephens *et al.* 1988). (Human studies of this type are not very practical because subjects must be withdrawn at regular intervals in order to obtain tissue samples.)

Vitamin E deficiency requires a glutathione adjustment to preserve mitochondrial integrity. The uptake of glutathione by the ciliary epithelium mitochondria could cause glutathione reduction in the ciliary epithelium cytosol. This would probably be followed by aqueous humour glutathione reduction.

Trabecular meshwork dystrophy: primary open-angle glaucoma v. *low-tension glaucoma*

Aqueous humour changes (reduced glutathione and increased peroxides) may then cause TM dysfunction (trabecular cell apoptosis, trabecular cell activation and collagen deposition) in POAG. TM ECM remodelling could then lead to increased IOP. (Aqueous humour outflow facility is reduced in the presence of H_2O_2 when glutathione is decreased (Kahn *et al.* 1983).)

In low-tension glaucoma, the inner-layer TM is not similarly dystrophic. Low-tension aqueous humour outflow is not obstructed and IOP is normal. Trabecular cells are not apoptotic in low-tension glaucoma. Collagen deposition is not the predominant form of ECM remodelling in low-tension glaucoma; it is elastin remodelling. This takes place in the outer-layer TM, not inner-layer. TM glutathione levels may be sufficient to prevent trabecular cell apoptosis and collagen ECM remodelling in low-tension glaucoma even when chronic vitamin E deficiency is taking place in the ciliary body, the TM and ON. The result would be elastin deposition and ON atrophy without increased IOP.

Glutathione and vitamin E: interactive dichotomy in trabecular meshwork and optic nerve

Reducing glutathione to 0% and vitamin E to 20% causes lipid peroxidation. Glutathione and vitamin E have different effects on mitochondrial lipid peroxidation. Glutathione levels in mitochondria must be reduced to almost 0% before lipid peroxidation occurs. When mitochondrial vitamin E is reduced to 20% of the normal level, lipid peroxidation occurs (Augustin *et al.* 1997). Depletion of glutathione in the mitochondria depletes glutathione in the cytosol first. The effects of severe glutathione deficiency in the cytosol would probably precede glutathione-induced mitochondrial lipid peroxidation.

Dichotomy in apoptosis initiation and extracellular matrix remodelling: cell activation caused by glutathione deficiency. The dichotomy in cell activation caused by either glutathione deficiency or vitamin E deficiency may lead to a dichotomy in the pathways of apoptosis initiation and ECM remodelling. Insufficient glutathione combined with exogenous H_2O_2 may induce collagen matrix remodelling and trabecular cell apoptosis independently of mitochondria. Collagen gene induction has been demonstrated in the presence of H_2O_2 when glutathione is reduced. Lipid peroxidation does not seem to be a factor in collagen gene induction (Darr *et al.* 1993; Varela-Rey *et al.* 1999). This implies collagen induction independently of mitochondrial wall lipid peroxidation (exogenous activation of collagen ECM remodelling).

Apoptosis induction may also be independent of mitochondria. Fas is an intercellular apoptosis modulator that is expressed on the cell surface. It can stimulate apoptosis in human trabecular cells (Agarwal *et al.* 1999). H_2O_2 induces the up regulation of Fas in human endothelial cells (Suhara *et al.* 1998). H_2O_2 can also affect the cell surface of human trabecular cells (Ozawa *et al.* 1999). These findings suggest that exogenous H_2O_2 may stimulate trabecular cell apoptosis and collagen ECM remodelling without mitochondrial participation.

Dichotomy in apoptosis initiation and extracellular matrix remodelling: cell activation caused by vitamin E deficiency. Mitochondrial lipid peroxidation may activate

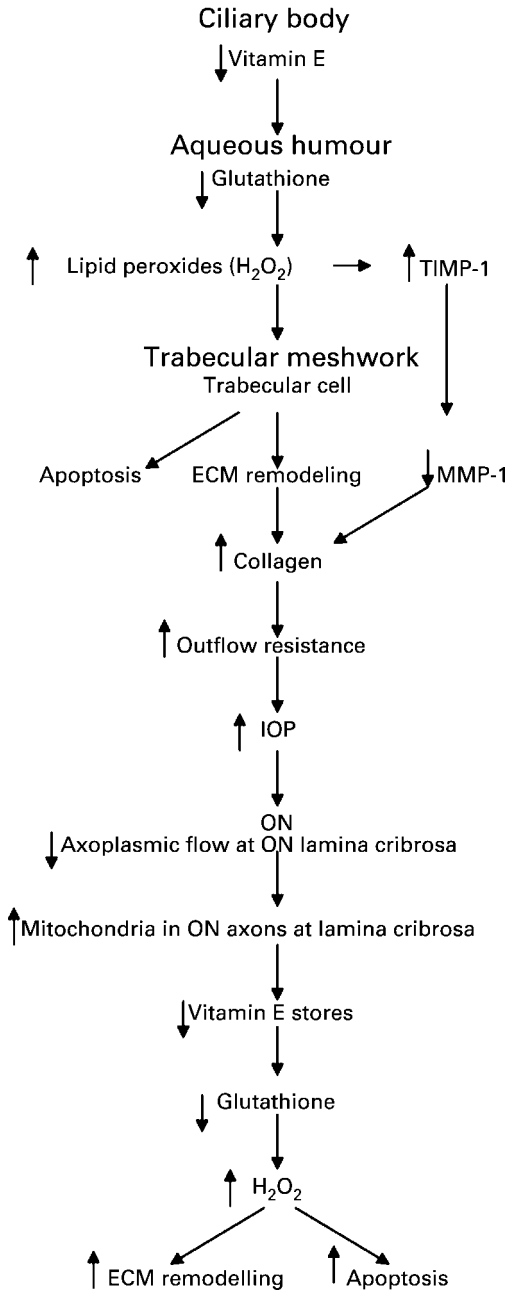

Fig. 5. Primary open-angle glaucoma (POAG) flow chart. Vitamin E reduction in the ciliary body mitochondria may initiate a metabolic cascade that eventually results in optic nerve (ON) apoptosis. There are factors that may affect this cascade at any point. Intervention may also be possible at any point. It is possible that water-soluble antioxidants may be sufficient at the trabecular meshwork, but not at the ON. This would also explain low-tension glaucoma, and ON degeneration despite good intra-ocular pressure (IOP) control in POAG patients. TIMP, tissue inhibitors of matrix metalloproteins; ECM, extracellular matrix; MMP, matrix metalloproteins.

HSP, the elastin–laminin receptor and apoptotic sequences (Slater *et al.* 1995; Satoh *et al.* 1996, 1997; Guenal *et al.* 1997; Danis *et al.* 1998; Susin *et al.* 1998; Cardoso *et al.* 1999; Chaudiere & Ferrari-Iliou, 1999; Dillmann, 1999; Nickells, 1999; Ando *et al.* 2000; Nicholls & Budd, 2000). Concurrent elastin remodelling and HSP staining without apoptosis or collagen ECM remodelling suggests that cell activation is due to mitochondrial lipid peroxidation in low-tension glaucoma (Hogan *et al.* 1971). Bcl-2 is an intracellular apoptosis modulator localised on mitochondrial membranes (Agarwal *et al.* 1999). Bcl-2 has been shown to inhibit H_2O_2-induced apoptosis without affecting ROS production (Satoh *et al.* 1996). It is possible that mitochondrial membrane lipid peroxidation caused by vitamin E deficiency induces HSP, Bcl-2, and stimulates the elastin–laminin receptor.

If this takes place in the TM it could help to explain trabecular cell activation, HSP staining and elastin remodelling in the absence of trabecular cell apoptosis and absence of collagen ECM remodelling in low-tension glaucoma. In low-tension glaucoma, at ON lamina cribrosa Bcl-2 and HSP may interrupt endogenous apoptosis induction. However, this may not prevent apoptosis induction at the cell wall by excessive H_2O_2 if there is insufficient glutathione (see Fig. 4).

Vitamin E deficiency in the ciliary body, trabecular meshwork and optic nerve: the low tension conundrum

Since the ciliary body is probably the earliest ocular structure to experience vitamin E deficiency, oxidative imbalance probably occurs there first. Aqueous humour oxidative imbalance is the probable result. Aqueous humour ROS could then overwhelm the TM as the aqueous humour passes through it (unless glutathione is sufficient). This may cause significant collagen TM ECM remodelling without affecting mitochondrial membranes. The resultant rise in IOP could then mechanically compromise metabolism at the ON lamina cribrosa.

If vitamin E deficiency continues to progress it could eventually affect the mitochondria of the trabecular cells, activating the elastin–laminin receptor. Deficiency of vitamin E at the lamina cribrosa of the ON may be concurrent. As elastin ECM remodelling of the TM and ON lamina cribrosa continues, ON axon atrophy also increases. The dichotomy of cell activation would allow for collagen ECM remodelling without elastin remodelling or vice versa. Of course, both could occur simultaneously (see Fig. 5). This could explain the incidence of HSP staining in only 50 % of POAG TM, and ON atrophy in low-tension glaucoma, and persistent ON atrophy after POAG IOP has been successfully reduced.

Conclusion

In eyes with elevated IOP, antioxidants have synergistic effects, rescuing laboratory rat retinal ganglion cells from atrophy (Buhrmann *et al.* 2000). Direct evidence such as this aids the central thesis that glutathione and vitamin E may play a role in the aetiology of POAG. A dichotomy of cell activation with corresponding pathways of apoptosis initiation and ECM remodelling adds coherence to observations concerning glaucoma.

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