THE ROLE OF OXIDATIVE STRESS AND VASCULAR INSUFFICIENCY IN PRIMARY OPEN ANGLE GLAUCOMA

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Abstract

Primary Open Angle Glaucoma (POAG) is a chronic, irreversible optic neuropathy leading to the progressive death of retinal ganglion cells, clinically observed as silent visual field loss along with a decrease in colour and contrast sensitivity. Multiple pathogenic theories have been issued and some of them have proven their involvement in disease development: mechanical damage due to increased intraocular pressure, variable susceptibility of the optic nerve, mutation in specific nuclear genes, increased glutamate levels, alteration in nitric oxide (NO) metabolism, changes in the mitochondrial genome, vascular disturbances, and toxic effects and oxidative damage caused by reactive oxygen species [1].

The aim of this article is to highlight the pathogenic role of vascular disturbances and reactive oxygen species in POAG with the further possibilities for prevention and gene therapy.

Keywords: primary open angle glaucoma; genetic risk factors; oxidative stress; glutathion; nitric oxide.

Primary Open Angle Glaucoma (POAG) is described as a multifactorial optic neuropathy with a chronically progressive course marked by specific structural changes of the optic nerve head and associated progressive and irreversible visual field loss. Glaucoma is the second most common cause of blindness worldwide [1] and POAG is by far, the most common form of glaucoma.

The prevention, early detection and accurate management of POAG have been the subject of continuous research worldwide. Newly developed imaging techniques like confocal scanning laser ophthalmoscopy (eg, HRT III), scanning laser polarimetry (eg, GDX) or optical coherence tomography (eg, Stratus OCT) can be used to document the status of the optic nerve and the thickness of the nerve fiber layer and they can be used to detect changes over time. Although their value in diagnosis and monitoring is still investigated, they are currently used in association with clinical evaluation, intraocular pressure (IOP) measurement and visual field testing for the early detection and further follow-up of glaucoma patients.

Until recently, elevated IOP was considered the main risk factor for glaucoma and as a result, therapy mainly aimed at lowering IOP values. However, it should be noted that, only one third to half of all glaucoma patients show elevated IOP in the initial stages. Furthermore, while some of these patients benefit from the currently available anti-glaucoma therapies and result in a positive outcome, others may experience progressive neuronal loss in spite of successful lowering their IOP levels.

Consequently, while elevated IOP is a major risk factor (perhaps even the most important singular factor) in relation to glaucoma neuropathy, it is by no means the only factor responsible for glaucoma neuropathy. Extensive investigations have revealed multiple additional risk factors such as mutations in specific nuclear genes, increased glutamate levels, alteration in nitric oxide (NO) metabolism, changes in the mitochondrial genome, vascular disturbances, and toxic effects and oxidative damage caused by reactive oxygen species [1].

Manuscript received: 12.07.2014
Recieved in revised form: 28.08.2014
Accepted: 15.08.2014
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Genetic risk factors

Linkage analysis has led to multiple chromosomal loci being linked to POAG, 15 being designated as GLCIA to GLCIO by, but mutations have been identified only in the case of a limited number of genes, namely myocilin (MYOC), optoneurin (OPTN), WD repeat domain 36 (WDR36) and neurotrophin -4 (NTF-4) for adult and juvenile POAG, and CYP1B1 and LTB4 in congenital glaucoma. While the genes identified above *exhibit* POAG-associated mutations, they also show high allelic heterogeneity among different populations [2,3]. At the same time their overall involvement in disease pathogenesis is unclear, as related to their mutation spectra, and case-control based association studies based on these genes showed their limited involvement. Considering the complex mechanisms involved in the pathogenesis, POAG could be attributed to multiple genes with varying amplitude of effects.

Positive associations with migraine [4] and other peripheral vascular abnormalities point towards the involvement of vascular insufficiency in glaucoma development. In POAG patients, ocular blood flow is reduced to all globe areas: optic nerve head, choroid, retina and retrobulbar vessels.

Hence, **primary injury** could be attributed to higher than tolerated IOP and local vascular disturbance. Impaired optic nerve circulation makes the optic nerve more susceptible to IOP insult, thus leading to 'axoplasmic flow obstruction within the retinal ganglion cells at the lamina cribrosa and changes in the laminar glial and connective tissue' [5]. **A second attack** due to glutamate or glycine cytotoxic effects and of nitric oxide oxidative damage, would result in the irreversible death of retinal ganglion cells. At the same time oxidative stress can lead to the early impairment of trabecular meshwork (TM) cells, obstructing aqueous humor outflow and thus and causing higher than tolerated IOP values. Oxidative damage is known to accumulate in degenerating TM [1], especially in the elderly.

Oxidative Stress and Glaucoma

Oxidative damage plays a well known part in the pathogenesis of various chronic-degenerative diseases like cancer or atherosclerosis but also cataract formation, macular degeneration [5,6] and, more recently, glaucoma [7]. Over 20 years ago, Alvarado [8,9] considered that excess free radicals were responsible for the progressive loss of trabecular meshwork cells encountered in POAG patients. Moreover, the non-proliferating nature of these cells further enhances the apoptotic and degenerative process triggered by oxidative damage. Consequently, IOP values increase and visual field defects progress [10].

Vascular Insufficiency and Endothelial Disfunction

Although vascular disturbance is characteristic for POAG, it is unclear whether these ocular blood flow deficits are triggers or direct consequences of the glaucomatous process. In either case, vascular disturbance induces ischemia and reperfusion, thus enhancing oxidative damage. The vascular function is mostly controlled by the molecules released by the vascular endothelium, dilators such as nitric oxide (NO) and prostacylin I_2 (PGI_2) and constrictors, such as endothelin-1 (ET-1). Their equilibrium is essential for vascular homeostasis. However, endothelial dysfunction refers not only to a shift towards reduced vasodilatation, but also an increased proinflammatory and prothrombotic state. Nitric Oxide Synthetase (NOS) produces NO by oxidation of L-arginine. The inducible form of this enzyme, iNOS/NOS-2 is known to generate excessive NO amounts under certain conditions such as cytokine exposure and high pressure. NO is a free radical of moderate activity, but excessive levels enter the cell giving birth to highly reactive free radicals such as peroxinitrite free radicals which in turn trigger massive cell destruction. POAG patients exhibit high NO levels in the aqueous humor, while NOS-2 is significantly increased in the astrocytes and microglia at the optic nerve head [10]. The genetic association between NOS-2 and POAG has already been observed [11]. The toxic effects of NO on retinal ganglion cells were confirmed by the neuroprotective effects of Aminoguanidine, a specific NOS-2 inhibitor in rats with chronically elevated IOP [12]. At the same time, NO elicits glutamate release, thus enhancing cell damage [13]. ET-1, one of the most potent vasoconstrictors, has also been proved to play a role in glaucoma including reduction of ocular blood flow. Vasoconstrictive retinal effects are mediated mainly through ET-1 type A receptors (ET_A) [14]. In addition, research is now being conducted for several polymorphisms in the ET_A receptor and ET-1 gene.

Oxidative Stress defense

In spite of effective antioxidant mechanisms present within the ocular tissue like gluthatione, superoxide-dismutase-catalase system or ascorbic acid, glaucomatous patients experience a decreased antioxidant potential while being exposed to increased oxidative stress damage [10]. Consecutive endothelial dysfunction alters NO and ET-1 production and balance and further amplifies cellular damage. Accumulated free radicals are known to damage DNA structure of the trabecular meshwork, cell adhesion and increase outflow resistance [10]. At the same time, the optic nerve head and proximal optical pathways are prone to similar DNA alterations. Human studies have revealed deficient drainage of aqueous humor following hydrogen peroxide exposure [15]. Once triggered, the trabecular degeneration process leads to increased intraocular pressure and its consequences, namely, extracellular matrix changes, reactive oxygen species release, axoplasmic flow obstruction and retinal ganglion cells death.

Gluthatione S Transferases (GST) are a class of

enzymes meant to protect the eye from oxidative strees exposure. The GST isoenzymes expressed in human tissues comprise the alpha, mu, pi, theta, kappa, sigma, zeta and omega gene families. They are important phase II metabolizing enzymes involved not only in the detoxification of exogenous xenobiotics but also in the inactivation of endogenous end products formed during oxidative stress.

Major polymorphisms were identified in the classes GSTM1 and GSTT1 [5]. Five Mu-class genes (GSTM1-GSTM5) were described on chromosome 1. Polymorphisms identified in the GSTM1 class include **GSTM1*0**, **GSTM1*A** and **GSTM1*B**. **GSTM1*0** is a deleted allela and homozygotes (GSTM1 null genotyppe) totally lack the enzyme. **GSTM1*A** and **GSTM1*B** exhibit similar enzymatic activity and differ by a single base. The GSTT1 gene located on chromosome 22 has two alleles: a functional, wild allele (**GSTT1*1**) and a null allele (**GSTT1*0**). The null allela exhibits a deficient enzimatic activity. These polymorphisms (null genotypes) have been associated with a higher risk of developing cancer, cardiovascular disease, or cataract. However, their study as possible risk factors for POAG has led to controversial results.

In spite of the 'protective role' attributed to GST enzymes, Juronen et al [16] observed an increased risk of POAG (OR=1.83) in patients GSTM1 positive (wild type allele) with the risk being further enhanced when smoking was present. No relevant association was found for GSTT1. Findings of the clinical research conducted by Unal et al in Turkey [17] and Rasool et al [18] in Egypt supported the assertion that a GSTM1- positive genotype is a risk factor. Although GST enzymes are detoxifying enzymes, they also elicit toxic compounds formation and activation which can trigger the degenerative trabecular process in POAG. Further evidence for the involvement of GSTM1 positive genotype comes from autoimmunity studies. Yang et al [19] found GST antigen (Mu class) with corresponding high GST antibodies to prevail in POAG patients as compared to controls. GSTM1 null carriers express less GST Mu-class enzymes as compared to GSTM1 positive individuals. Hence, GSTM1 positive genotype could induce autoantibody against GST, which is associated with a higher POAG risk.

However, the previously mentioned results are not consistent with all the published data. Jansson et al [20] found no evidence of GSTM1 positive genotype association with POAG.

Moreover, Izottie et al [21,22] reported the GSTM1 null genotype as POAG risk factor in the Italian population. Similar results were reported in China [23] Brazil [24] and Turkey [25]. These results are inconsistent with the previously mentioned studies which associated POAG with GSTM1-positive genotype [16,17,18,19]. GSTT1 study results are limited and most of them point towards a lack of association [17,25].

The different results may reflect the differences in ethnic, genetic and environmental background of the studied populations. Moreover, disparities among study design and methodology could be responsible for the different outcomes.

Further studies of greater source and using standardized methodology are required to elucidate the GSTM1 and GSTT1 implication in POAG.

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