

Targeted preventive measures and advanced approaches in personalised treatment of glaucoma neuropathy

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Received: 16 March 2010 / Accepted: 6 May 2010 / Published online: 10 June 2010
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Abstract Glaucoma is a major cause of vision loss worldwide with nearly 8 million people bilaterally blind from the disease. This number is estimated to increase over the next 10 years. The key to preventing blindness from glaucoma is effective diagnosis and treatment. The classical glaucoma treatment focuses on intraocular pressure (IOP) reduction. Better knowledge of the pathogenesis has opened up additional therapeutical approaches often called non-IOP lowering treatment. Whilst most of these new avenues of treatment are still in the experimental phase, others are already used by some physicians. These new therapeutic approaches allow a more personalised patient treatment. Non-IOP lowering treatment includes improvements of ocular blood flow, particularly blood flow regulation. This can be achieved by improving the regulation of ocular blood flow (improving autoregulation) by drugs such as carbonic anhydrase inhibitors, magnesium or calcium channel blockers. It can also be improved by decreasing blood pressure over-dips. Blood pressure can be increased by an increase in salt intake or in rare cases by treatment with fludrocortisone. Experimentally, glaucomatous optic neuropathy can be prevented by inhibition of astrocyte activation, either by blockage of epidermal growth factor receptor or by counteracting Endothelin. Glaucomatous optic neuropathy can also be prevented by nitric oxide-2 synthase inhibition. Suppression of matrix metalloproteinase-9 inhibits apoptosis of retinal ganglion

cells and tissue remodelling. Upregulation of heat shock proteins protects the retinal ganglion cells and the optic nerve head. Reduction of oxidative stress especially at the level of mitochondria also seems to be protective. This can be achieved by ginkgo, dark chocolate, polyphenolic flavonoids occurring in tea, coffee or red wine and anthocyanosides found in bilberries as well as by ubiquinone and melatonin. This review describes the individual mechanisms which may be targeted by non-IOP lowering treatment based on our pathogenic scheme.

Keywords Preventive · Personalised · Glaucomatous optic neuropathy · Vascular regulation · Oxidative stress

Introduction

For the past century glaucoma has been considered a disease for which diagnosis and treatment was focussed mainly on intraocular pressure. Large studies such as ‘The Ocular Hypertension Treatment Study’ or ‘The European Glaucoma Prevention Study’ recognised ocular hypertension as the most important factor for the development of primary open angle glaucoma. Because elevated intraocular pressure (IOP) was associated with the development of glaucoma, and reducing IOP, reduced the risk of visual field progression, IOP was considered a good surrogate for glaucoma treatment. The focus on IOP as the only risk factor, however, left several questions unanswered: Why do the majority of people with increased IOP not develop glaucomatous optic neuropathy (GON) (Fig. 1)? On the other hand, why do we see an increasing number of patients acquiring GON who have an IOP in the normal range? Why does reduction of IOP, whilst on the average

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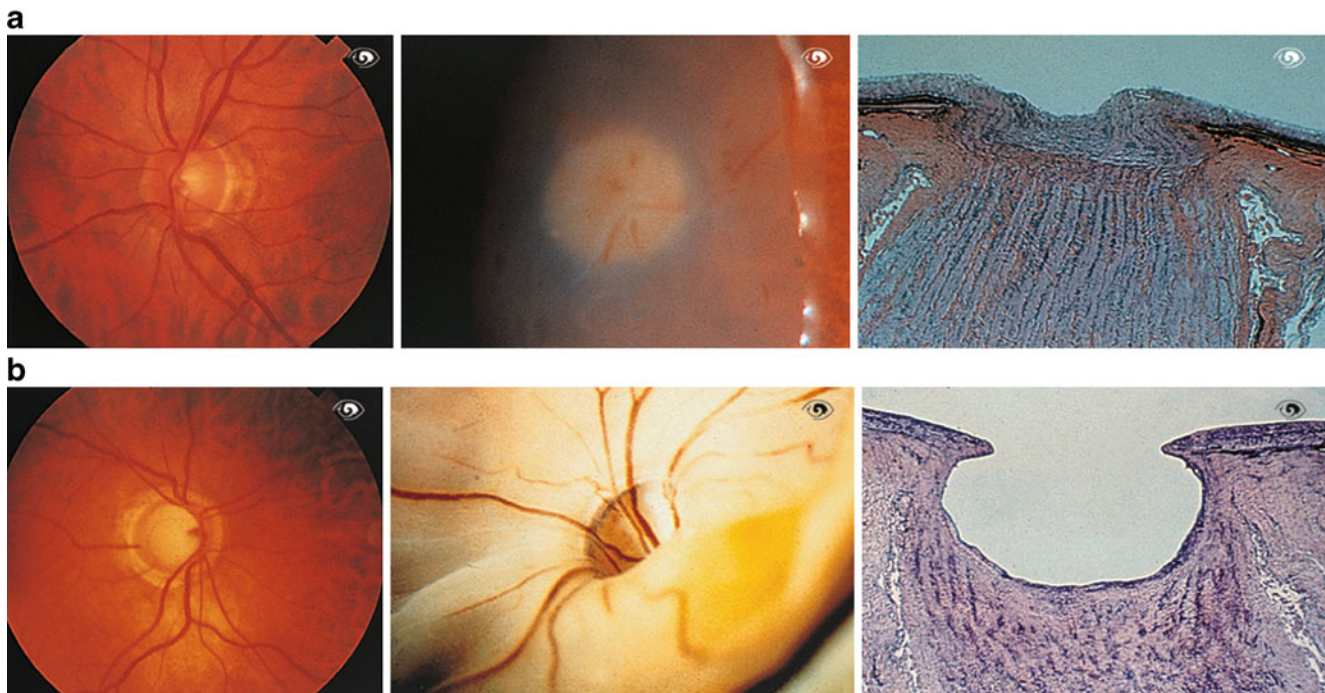


Fig. 1 Normal optic disc. **a** Normal optic disc in a healthy person (*left*), in the eye of a cadaver (*middle*), and a histological section (*right*). **b** Glaucomatous atrophy of the optic disc. The eye of the patient is shown on the left, of a cadaver in the middle and a histological section in the right

improving prognosis, not stop progression in all patients? And why do some patients need a very low IOP, indeed sometimes an even unphysiological low IOP to stop progression of this disease? These questions can be answered when considering additional risk factors such as systemic hypotension or vascular dysregulation. The elucidation of these additional factors has led to the investigation of non-IOP lowering treatment. Whether such treatment will be an adjunctive to the conventional IOP-lowering treatment (e.g. in patients with primary open angle glaucoma (POAG)) or whether it shall be used by itself (e.g. in patients with normal-tension glaucoma (NTG)) remains to be seen.

Some IOP lowering glaucoma medications have additional effects. For example carbonic anhydrase inhibitors improve regulation of ocular perfusion. This review, however, will focus solely on drugs that do not reduce IOP. Furthermore, we will discuss prevention of GON and only marginally deal with the prevention of IOP increase.

For more details on the pathogenesis and risk factors in glaucoma we refer to the article by Flammer and Mozaffarieh [1]. Fact remains that glaucoma is a multifactorial disease in which the different risk factors known may finally damage through the same or similar pathomechanisms. In order to visualise the individual mechanisms that may be targeted by treatment we have recapitulated the figure of the pathogenic scheme by Flammer and Mozaffarieh (Fig. 2) [1]. The section numbers below correspond to the numbers in Fig. 1.

Therapeutic targets

1. Inhibition of epidermal growth factor receptor (EGFR) prevents the activation of astrocytes and improves ocular blood flow
- 1A. Combat of mechanical stress prevents the activation of astrocytes

The activation of the astrocytes in the optic nerve head (ONH) and retina plays an essential role in the pathogenesis of GON [2–4]. Both mechanical and ischemic stress can

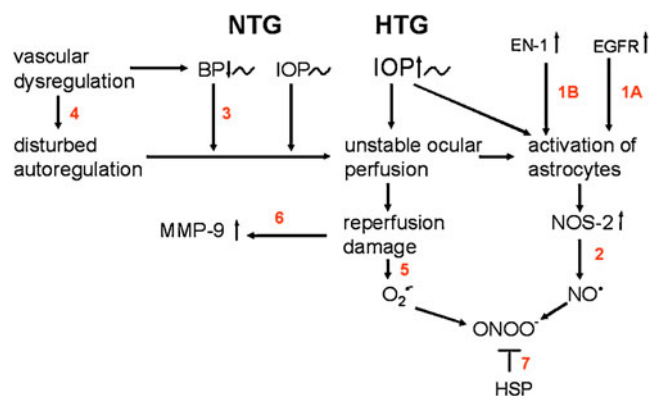


Fig. 2 The pathogenic scheme of GON. The pathogenic scheme by Flammer and Mozaffarieh [1], depicts the individual mechanisms that may be targeted by non-IOP lowering treatment. The numbers in red correspond to the section numbers in the manuscript

lead to activation of astrocytes. Once activated astrocytes upregulate the production of various molecules, including matrix metalloproteinases (MMP's), nitric oxide synthase-2 (NOS-2), tumor necrosis factor- α (TNF- α), and Endothelin, thereby creating an altered microenvironment leading to tissue remodelling and axonal damage. Mechanical stress leads to stimulation of EGFR which, in turn, leads to activation of astrocytes and thereby to an upregulation of NOS-2. Blockage of EGFR, by a tyrosine kinase inhibitor therefore prevents the activation of astrocytes [5]. Interestingly, such a treatment not only inhibits the activation of astrocytes but also leads to a reduction of loss of retinal ganglion cells. This indirectly indicates that the activation of astrocytes is relevant in GON [2, 3, 6]. Whether this approach will lead to glaucoma treatment in humans can at the moment not be predicted.

1B. Inhibition of the effect of Endothelin-1 improves ocular blood flow

In glaucoma patients plasma concentration of Endothelin-1 (EN-1) is increased [7]. Endothelin not only further reduces optic nerve head blood flow and impairs anterograde and retrograde axoplasmic transport [8, 9], but also activates astrocytes [10]. This is further supported by the fact that patients with a vascular dysregulation more often have activated retinal astrocytes which can be visualised clinically [11, 12]. The effect of Endothelin can be partially blocked by a number of different drugs such as calcium channel blockers (CCB's) including magnesium (a physiological CCB (Fig. 3)), dipyrimadole or Endothelin blockers [13–16]. Whether an inhibition of Endothelin indeed also inhibits the activation of astrocytes has not yet been studied.

2. Inhibition of nitric oxide synthase 2 (NOS-2) decreases damage

Nitric oxide (NO), also known as the “endothelium-derived relaxing factor”, is biosynthesised from arginine and oxygen by various nitric oxide synthase (NOS) enzymes. There are three basic forms of NOS: Neuronal nitric oxide synthase (nNOS or NOS-1), inducible nitric oxide synthase (iNOS or NOS-2) and endothelial nitric oxide synthase (eNOS or NOS-3).

Nitric oxide synthase -2 (NOS-2), leads to a marked production of nitric oxide. NOS-2 can be inhibited by the drug aminoguanidine, a nucleophilic hydrazine compound. Aminoguanidine is an oral insulin stimulant for type 2 diabetes mellitus. It further seems to prevent the formation of advanced glycation end products [17]. In addition, it is a relative specific inhibitor of NOS-2 which is why it was studied in experimental glaucoma. In experimental glauco-

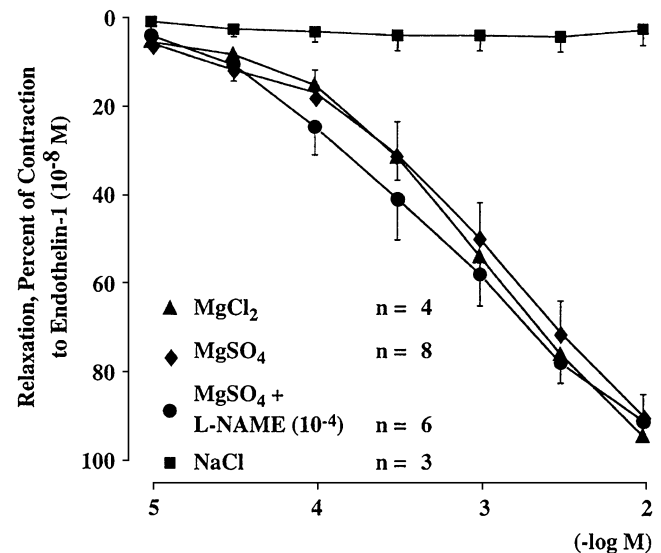


Fig. 3 Magnesium inhibits the effect of endothelin-1 (ET-1). Relaxing effect of increasing concentrations of MgSO₄ and MgCl₂ added to endothelial-precontracted porcine ciliary arteries (10^{-8} M). NaCl, which has the same osmolarity as MgSO₄, did not evoke a relaxation

ma aminoguanidine was capable of preventing the development of GON [18]. Such treatment appears very promising but clinical studies are not yet available.

3. Reduction of severe hypotension improves prognosis

Low blood pressure as well as nocturnal over dipping increases the probability of visual field deterioration [19, 20]. We can therefore assume that an increase in blood pressure in patients with hypotension may improve prognosis although interventional studies supporting this view are rare. Treatment of hypotension with vasoconstrictive drugs although increasing blood pressure may further reduce blood flow. Blood pressure, however, can be increased with an increase in salt intake (1–5 g daily) [21]. In severe cases the intake of the low-dosed fludrocortisone, (0.1 mg/2x per week) has been described [22]. Fludrocortisone treatment not only slightly increases blood pressure and reduces nocturnal dips but also improves the regulation of blood flow indirectly [22].

4. Improvement of vascular regulation (autoregulation) stabilises oxygen supply

Vascular dysregulation is a major risk factor for GON [23]. Vascular regulation can be improved by various drugs. Among the IOP lowering drugs, only carbonic anhydrase inhibitors (in particular dorzolamide), have proven to both increase ocular blood flow (OBF) and improve the regulation of OBF [24], thereby decreasing the chance of reperfusion injury [25].

Parallel to an improvement in OBF, an improvement in visual field was observed (Fig. 4). Carbonic anhydrase inhibitors (CAI), like acetazolamide, improve visual fields in glaucoma patients [26–28]. Similar effects have been observed for calcium channel blockers (CCB's). An improvement in OBF and visual function was only observed in patients with a vascular dysregulation [29]. Accordingly, a positive response to OBF on carbon-dioxide breathing predicts the effect of CCB's on OBF [30]. The effect of CCB's on visual field have also been demonstrated in masked double blind studies [31]. Likewise, CCB's decreased the OBF reducing effect of an Endothelin infusion in healthy volunteers [32]. Dipyridamol, a drug often used in the past as a platelet inhibitor, also inhibits the effect of Endothelin [33], and improves OBF [34]. Unfortunately the long-term role of dipyridamole on glaucoma patients has not yet been studied. Among the systemic treatments, magnesium is a weak but harmless drug that inhibits the effect of Endothelin partially [13] and improves OBF [15].

Omega-3-fatty acids (Omega 3-FA's) have a number of different effects including the modulation of intracellular calcium ion release and thereby the stabilisation of circulation [35]. Omega 3-FA's also increase the production of uncoupling proteins and thereby improve ATP independent heat production which is most probably impaired in patients with vascular dysregulation [36, 37].

Cacao beans from the seed of *Theobroma Cacao* contain a subclass of flavonoids, flavan-3-ols, which have been reported to augment endothelial nitric oxide synthase (eNOS), and thereby nitric oxide (NO). This improves endothelium dependent vasorelaxation [38]. Unfortunately the use of cacao beans has not yet been studied in the context of glaucoma.

5. Reduction of oxidative stress improves prognosis

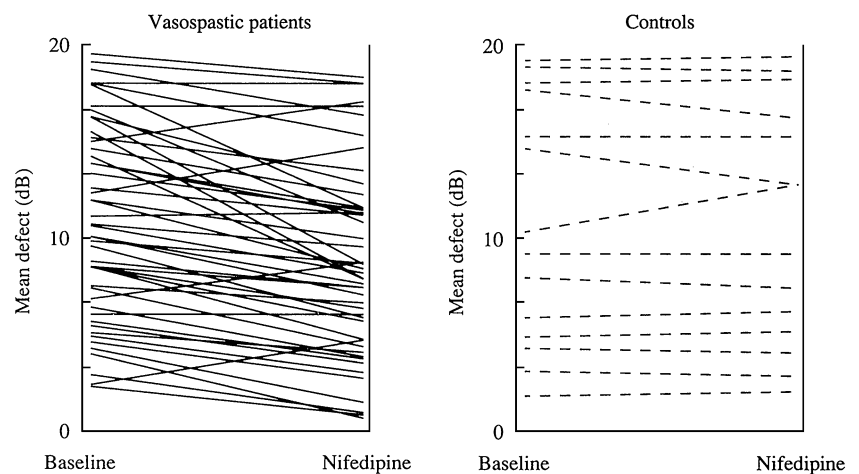
Free radicals are involved in a number of inflammatory and degenerative diseases. Accordingly, oxidative stress is

involved in the pathogenesis of GON where free radicals cause a damage to retinal ganglion cells and their axons [39, 40]. In addition, oxidative stress leads to degeneration of trabecular meshwork (TM) [41, 42] and thereby alterations in the aqueous outflow pathway (Fig. 5), leading to increased IOP which, in turn, also damages retinal ganglion cells. The target of oxidative stress relevant in the development of GON are most probably the mitochondria [43]. It is therefore desirable to have a drug protecting the mitochondria, in particular the mitochondria of the optic nerve head [43]. This can unfortunately not be achieved by an increase intake of vitamins such as vitamin C or vitamin E. Only molecules reaching the inner membrane of the mitochondria can be of potential use. Ginkgo contains a number of substances, including polyphenolic flavonoids, that have been proven to protect the mitochondria from oxidative stress and thereby protect the retinal ganglion cells [44–47]. Moreover, ginkgo has been shown to improve visual fields in a long term double masked placebo-controlled study [48]. Efficacy and safety reports have suggested a daily dose of 120 mg to be sufficient and acceptable [49].

There are a number of other naturally occurring substances that could theoretically be beneficial but have not been studied for glaucoma [50–52]. Polyphenolic flavonoids have strong antioxidant capacity due to their free radical scavenging properties. Both green and black tea, are rich sources of flavonoids such as catechin (C), epicatechin (EC), epigallocatechin (EGC) [53, 54]. Coffee also has good antioxidant properties due to polyphenolic compounds. In addition, coffee contains the molecule 3-methyl-1,2-cyclopentanedione (MCP) which has been shown to be a selective scavenger of the peroxynitrite [55]. Other naturally occurring compounds containing polyphenols include dark chocolate [56] and red wine [57].

Anthocyanins, rich in foods such as bilberry, are another class of substances with antioxidant properties. In addition to polyphenolic rings, anthocyanins possess a positively

Fig. 4 Short-term effect of nifedipine. Mean defect of visual field test (Octopus program G1) at baseline and 60 min after intake of 20 mg sustained-release nifedipine in a) improvement in patients with digital vasospasm, and b) no change in patients without digital vasospasm. When treatment was continued for 12 months, the improvement in the group with vasospasm was still detectable (not shown here)



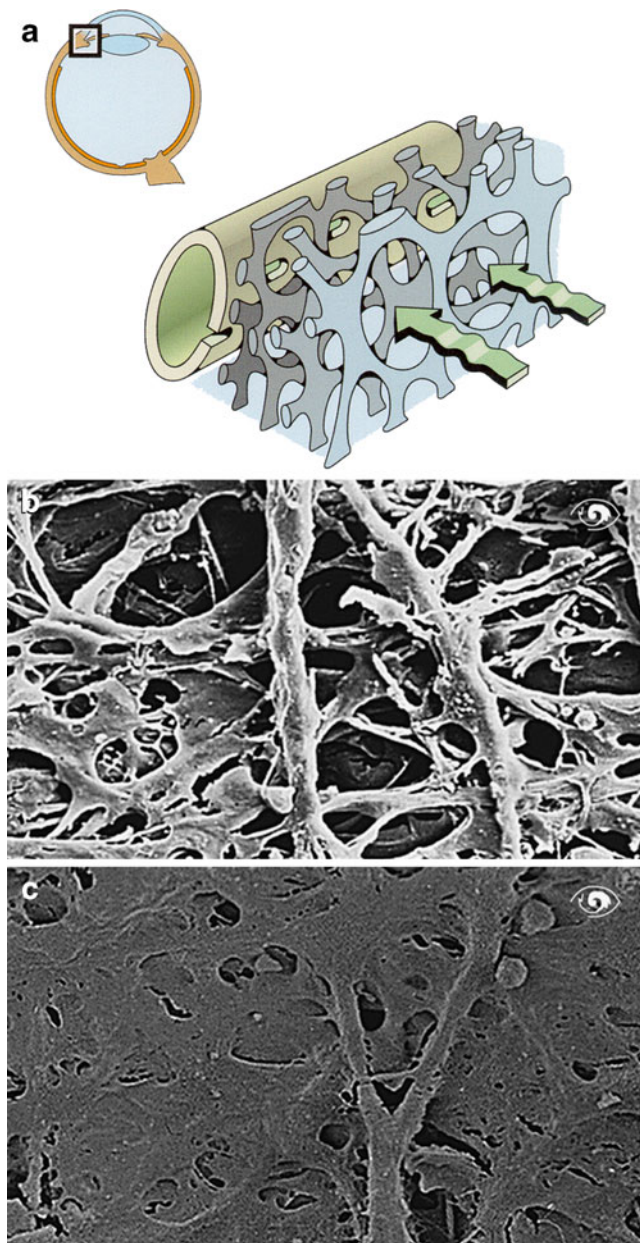


Fig. 5 **a** Drainage of aqueous humor. Aqueous humor (green) drains through the trabecular meshwork into Schlemm's canal. **b** Trabecular meshwork of a healthy individual. **c** Trabecular meshwork of a glaucoma patient

charged oxygen atom in their central ring which enables them to readily scavenge electrons [58].

Ubiquinone, Coenzyme Q 10, is a coenzyme for the inner mitochondrial enzyme complexes involved in energy production within the cell with strong antioxidant properties. Coenzyme Q10 has been demonstrated to prevent lipid peroxidation and DNA damage induced by oxidative stress [59]. Ubiquinone has been studied well in dermatology but unfortunately studies of its use in glaucoma are lacking and therefore currently limit the use of this agent.

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine, secreted by the pineal gland, which exerts antioxidant properties. Melatonin has been shown to neutralise free radicals [60]. In the retina, melatonin reduces the elevation of cGMP by suppressing nitric oxide synthase (NOS) activity and thereby levels of nitric oxide (NO) indicating a neuroprotective role. In addition, melatonin stimulates a number of antioxidative enzymes [61].

6. Inhibition of metalloproteinase 9 (MMP-9) reduces retinal ganglion loss and tissue remodelling

MMP-2 and MMP-9 are upregulated in astrocytes of glaucoma patients [62]. MMP-9 is also upregulated in the circulating lymphocytes of glaucoma patients [63]. These MMP's, in particular MMP-9, are involved in both retinal ganglion cell loss and in tissue remodelling. MMP-9 can be inhibited pharmacologically by GM6001, also known as Ilomastat (N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide). Studies reveal that inhibition of MMP-9 with GM6001 prevents retinal ganglion cell loss in an animal model [64]. Moreover, MMP-9 knock out mice do not show apoptosis of retinal ganglion cells even when the optic nerve is ligated [65]. MMP-9 most probably plays a role in the pathogenesis of GON.

7. Stimulation of heat shock protein production (HSP) protects proteins

Heat shock proteins (HSP's) are produced by different cells when subjected to stress (e.g. elevated temperatures or oxidative stress). The upregulation of these proteins is a protective mechanism as they act as molecular chaperones protecting the three-dimensional structure of other proteins. In a rat model, pharmacologically induced upregulation of HSP's, by the systemic administration of the compound geranylgeranylacetone (GGA), protected retinal ganglion cells from glaucomatous damage [66]. Whether treatment with this drug or the natural stimulation of HSP's (eg. by sauna baths) is beneficial in humans, needs to be studied.

Concluding remarks

Predictive medicine is expected to be most effective when applied to multifactorial disease such as glaucoma. We still do not know the exact mechanisms that lead to damage of the trabecular meshwork and thereby to an increase in IOP, nor do we know the exact mechanisms leading to GON. Obviously there are a number of factors and mechanisms involved. The different risk factors

known, may finally lead to damage the same or similar pathomechanisms to GON.

Therapeutically, we can either eliminate or mitigate risk factors or target defined pathogenic steps. Risk factors that can be influenced include increased IOP, low blood pressure and vascular dysregulation. Blood pressure dips can be avoided by intake of salt or fludrocortisone [22]. Vascular regulation can be improved locally by carbonic anhydrase inhibitors [24], systemically with magnesium [13, 15] or with low doses of calcium channel blockers [67]. Oxidative stress at the level of mitochondria can be reduced by the intake of ginkgo biloba [44, 45]. Whether such treatment will be an adjunctive to the conventional IOP-lowering treatment (e.g. in patients with POAG) or whether it shall be used by itself (e.g. in patients with NTG) remains to be seen.

Pathogenic steps that can be targeted include activation of astrocytes, upregulation of NOS-2 or MMP's. The very different risk factors finally damage the optic nerve head in a very similar fashion. At the moment any type of clinical or experimental treatment is based on the prevention of GON. A restoration of damaged function is at the moment not possible at all. Already, many of these new treatment strategies have proven to be beneficial in humans [68]. For those remaining, further rigorous investigation is deserved to open up a new therapeutic era in glaucoma.

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