Unstable Oxygen Supply and Glaucoma

Instabile Sauerstoffversorgung und Glaukom

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- Flammer syndrome

Abstract

The pathogenesis of the glaucomatous optic neuropathy (GON) is an ongoing bone of contention. While the role of intraocular pressure (IOP) is well known, it is also clear that a variety of other factors, particularly those of a vascular nature, are involved as well. In contrast to other eye diseases, it is an unstable oxygen supply, as opposed to chronic hypoxia, that contributes to GON. The major cause of fluctuations in the local oxygen tension is an unstable ocular blood flow (OBF). OBF, in turn, fluctuates if the IOP spikes, blood pressure drops, or OBF autoregulation is defective. The main reason for disturbed autoregulation is a primary vascular dysregulation (PVD), particularly in the context of the so-called Flammer syndrome. Unstable oxygen tension leads to local oxidative stress with many detrimental effects, such as the activation of glial cells, which alters their morphology and gene expression. As a consequence, the local concentrations of nitric oxide and the metalloproteinases increase. The metalloproteinases digest extracellular matrix and thereby contribute to tissue remodelling. The short-lived nitric oxide easily diffuses into the neighbouring neuronal axons, allowing a fusion with the superoxide anion and thereby generating the cell-damaging peroxynitrite. Both this tissue remodelling and damage of the axons contribute to the development and progression of GON.

Zusammenfassung

Die Pathogenese der glaukomatösen Optikusneuropathie (GON) wird noch immer kontrovers diskutiert. Die Bedeutung des Augeninnendrucks für die Entstehung von GON ist unbestritten. Es ist aber ebenso unbestritten, dass auch andere Faktoren, insbesondere vaskuläre Faktoren, eine Rolle spielen. Entgegen älterer Auffassung ist es aber weniger die Hypoxie als vielmehr die instabile Sauerstoffversorgung, v. a. bedingt durch Schwankungen in der Augendurchblutung, die zur GON beiträgt. Schwankungen der Augendurchblutung wiederum gibt es bei Augendruckspitzen, Blutdruckabfällen und einer gestörten Autoregulation der okularen Zirkulation. Eine häufige Ursache der gestörten Autoregulation ist die vaskuläre Dysregulation im Rahmen eines Flammer-Syndroms. Eine schwankende Sauerstoffkonzentration führt zu lokalem oxidativen Stress. Im Auge betrifft das vor allem die Mitochondrien des Sehnervenkopfes. Denn dort haben wir nicht nur besonders viele Mitochondrien wegen der nicht myelinisierten Axone, sondern auch besonders häufig Schwankungen der Durchblutung. Die hypoxieresistenten Astrozyten werden aber sowohl durch mechanische Belastung wie auch durch oxidativen Stress aktiviert. Dadurch ändern sie nicht nur ihre Morphologie, sondern auch ihre Genexpression, was u. a. zur vermehrten Bildung von Stickstoffmonoxid und Metalloproteinasen führt. Letztere verdauen die extrazelluläre Matrix und tragen so zum Umbau des Sehnervenkopfs bei. Stickstoffmonoxid wiederum kann aus den Astrozyten in die neuronalen Axone diffundieren, wo es mit dem Hyperoxid-Anion fusioniert und dadurch das zelltoxische Peroxynitrit bildet. All diese Mechanismen tragen wesentlich zur Entstehung und Progression der GON bei.

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Abbreviations

GON: glaucomatous optic neuropathy
IOP: intraocular pressure
OBF: ocular blood flow
ONH: optic nerve head
NO: nitric oxide
ET-1: endothelin-1
PVD: primary vascular dysregulation
SVD: secondary vascular dysregulation
ROS: reactive oxygen species
MMPs: metalloproteinases

Introduction

The pathogenesis of glaucomatous optic neuropathy (GON) is an ongoing point of contention. It is well known that elevated intraocular pressure (IOP) contributes to glaucoma damage. However, a variety of other systemic and ocular factors, particularly those of a vascular nature, are also involved.

Chronic but stable hypoxia, such as that which is associated for example with anaemia or atherosclerosis, is surprisingly well tolerated by the eyes. An unstable oxygen supply, however, has a major impact on cells, particularly in the optic nerve head (ONH). In this review, we discuss the conditions leading to an unstable oxygen supply as well as the mechanisms by which fluctuating oxygen tension damages the ONH and therefore contributes to the pathogenesis of GON.

Unstable Oxygen Supply

The oxygen supply to the eyes is temporarily decreased if the oxygen tension in the blood is decreased for a limited period of time; this particularly occurs in patients with sleep apnoea. Oxygen supply to the eye, however, is also temporarily decreased if the blood flow to the eye drops for a moment. This can occur if the IOP rises or the blood pressure drops beyond the capacity of the autoregulation of ocular blood flow (OBF). However, even if the IOP and blood pressure fluctuate within their normal ranges, this can still lead to OBF instability if the autoregulation itself is disturbed. This occurs particularly in subjects with the so-called primary vascular dysregulation syndrome. To understand the essence of vascular dysregulation, we first discuss some basic aspects of vascular regulation, particularly the regulation of OBF.

Regulation of Blood Flow in General

Blood circulation transports a large variety of molecules, including oxygen, cells, such as leukocytes, and heat. The regulation of blood flow is necessary to adapt to changing internal and external conditions. The overall blood flow is regulated by the *cardiac output*, which is mainly controlled by the autonomic nervous system and circulating hormones. The distribution of this cardiac output to the different organs is regulated by the *local resistance to flow*. This local resistance is mainly (but not only) regulated by the vascular endothelial cells, which release vasoactive molecules. The most important molecules are nitric oxide (NO), which induces vasodilatation, and endothelin-1 (ET-1), which is a strong vasoconstrictor [1].

Regulation of OBF

The regulation of OBF compensates for varying perfusion pressure (autoregulation), adapts to retinal activity (neurovascular coupling), and keeps the back of the eye at a constant temperature (thermoregulation).

The eye has different vascular beds, which are regulated differently. All ocular vessels are under the local control of the vascular endothelial cells. Additionally, the retinal vessels are influenced by the activity of the neural und glial cells (neurovascular coupling), and the choroidal vessels are influenced by both the autonomic nervous system and circulating hormones. The vessels of the ONH are influenced by both the activity of the neural und glial cells and circulating hormones. In a healthy retina, circulating hormones, such as vasoconstrictors, ET-1, or angiotensin II, have no direct access to smooth muscle cells and pericytes of the vessels due to the blood-retina barrier. The situation is different in the choroid, where these hormones can escape through fenestrations of the capillaries and directly access smooth muscle cells. These molecules can also diffuse from the choroid into the ONH [1, 2]. As a consequence, circulating vasoactive molecules such as ET-1 or angiotensin II have little effect on retinal circulation; however, they have a major effect on choroid and ONH circulation. In a number of diseases, such as for example giant cell arteritis [3], the ET-1 level is increased in the circulating blood, resulting in a reduction of choroidal and ONH blood flow.

Autoregulation and Instability of OBF

In the literature, autoregulation is not consistently defined. In this context, we define autoregulation as the ability of an organ to keep the blood flow independent of perfusion pressure. However, autoregulation has limits: Major drops or rises of perfusion pressure can exceed the capacity of autoregulation. While morphological alterations of the vessels (such as in atherosclerosis) reduce OBF, they normally do not contribute to the instability of OBF. Major clinical conditions leading to OBF instability include IOP spikes, blood pressure drops, and disturbed autoregulation. A particular cause of disturbed autoregulation is primary vascular dysregulation (PVD).

Notions in the Context of Vascular Dysregulation

Vasospasms have long been known in the field of medicine and were described in the retina more than one hundred years ago. It was observed that subjects suffering from spasms in one organ often had simultaneous or sequential spasms in other organs; e.g., in the retina and in the nail fold capillaries. Therefore, the term *vasospastic syndrome* [4] was introduced. As the technology for observing and quantifying blood flow progressed, it became evident that vasospasms were just the tip of the iceberg. The condition behind it encompasses both inappropriate vasoconstrictions and insufficient, but also boosted, vasodilation of arteries, veins, and capillaries. In respect to the eye, this can manifest as, for example, disturbed autoregulation or insufficient neurovascular response. To describe this more general vascular condition, the term *vascular dysregulation* was introduced [5]. Vascular dysregulation can lead to both overperfusion and underperfusion of a particular supply territory. To not confuse this condition (most probably based on a genetic background)

with dysregulation induced by other diseases, the term *primary vascular dysregulation* (PVD) was introduced to distinguish it from *secondary vascular dysregulation* (SVD) [6]. Like spasms, general dysregulation often occurs in many organs of the same individual, which led to the introduction of the term *PVD syndrome* [7]. The PVD syndrome is often associated with other vascular signs and symptoms, such as low blood pressure, cold extremities, or barrier dysfunction (which in the eye can lead to, for example, optic disc haemorrhages), as well as with nonvascular signs and symptoms, such as increased sensitivity or altered sleep behaviour. To embrace the entire condition, we introduced the term *Flammer syndrome* [8].

Flammer Syndrome

The Flammer syndrome [7–9] can be found in a subgroup of mostly healthy subjects with a cluster of symptoms and signs normally not indicative for diseases. It refers to a predisposition to react differently to a number of stimuli, such as coldness [4] or physical or emotional stress. The most prominent sign is the dysregulation of vessels. However, the syndrome encompasses a number of additional vascular and nonvascular signs and symptoms [10] (see **Table 1** and **Fig. 1**).

The most common sign are *cold extremities* with an increased response to coldness [4]. These subjects often have cold hands and/or feet, both subjectively and objectively. The extremities can be already cold in normal environmental temperatures, but they particularly respond with vasoconstriction to coldness or psychological or mechanical stress.

However, the Flammer syndrome should not be confused with Raynaud's disease. Raynaud's disease is rarer and severer. Patients with Raynaud's disease also respond to coldness and psychological stress, but with excessive blood flow reduction. This leads to a severe hypoxia of the superficial skin which turns white and blue. When the blood flow returns, the skin turns red and throbs or tingles. The loss of blood flow can cause sores or tissue death. This condition may also cause nails to become brittle with longitudinal ridges.

In contrast, the blood flow disturbance in Flammer syndrome is less acute, less severe, and less localized. It can sometimes lead to pale extremities. However, it leads neither to white extremities nor to trophic changes of the extremities. And the Flammer syndrome comprises many additional vascular and nonvascular symptoms and signs not related to Raynaud's disease [7].

The second most common sign is *low blood pressure* [11], especially when the subjects are young. Blood pressure can be generally low or can drop when subjects stand up (orthostatic hypotension) or when they are sleeping (nocturnal over-dipping). The major cause for systemic hypotension in these subjects might be a loss of sodium due to reduced reabsorption of sodium in the proximal tubule of the kidneys as result of the activation of the ET-1/prostaglandin-E2 axis. As these subjects age, their blood pressure can normalize or even increase.

Table 1 Potential signs and symptoms of PVD in the context of Flammer syndrome.

Cold hands and/or feet	+++
Low blood pressure	+++
Low body weight	++
Reduced feeling of thirst	++
Long sleep onset time	++
Increased sensitivity, e.g.:	++
▸ increased response to certain drugs	
▸ increased smell sensation	
▸ increased pain sensation	
▸ increased high altitude sensitivity	
▸ increased meteorosensitivity	
▸ increased vibration sensitivity	
Tendency toward perfectionism	++
Migraines and headaches	+
Tinnitus	+
Reversible skin blotches (red and white)	(+)

These subjects often take *longer to fall asleep* [12]. Indeed, body temperature and sleep are interrelated. Sleep is typically initiated when the central body temperature declines and heat loss from the extremities is maximal. Therefore, one can fall asleep only after the extremities are warmed up, and this often takes longer in subjects with Flammer syndrome.

These subjects have a *reduced feeling of thirst* [13]. They, however, normally drink enough, as they know they need to do so. The reduced feeling of thirst may be due to an increased plasma level of ET-1, which suppresses the centre of thirst in the brain via the prostaglandin-E2 axis.

Based on clinical experience, these subjects are generally *more sensitive*. They have increased sensitivity to certain drugs (e.g., calcium channel blockers and systemic beta blockers). This might partly be explained by the different expressions of certain ATP binding cassette (ABC) transporter proteins [14]. They have also increased pain sensation, increased sense of smell [15], and increased sensitivity to high altitude.

They often suffer from *tinnitus* and sometimes even from sudden (mostly reversible) hearing loss [16]. Under psychological stress, an increased heterogeneity of skin perfusion (reversible red and white skin blotches) is observed in some subjects.

The syndrome occurs more often in females than males, in subjects who are slim more than in those who are obese [17], in academics more than in blue-collar workers, and in Asians more than in Caucasians. In addition, a typical trait of these individuals is a tendency toward *perfectionism*.

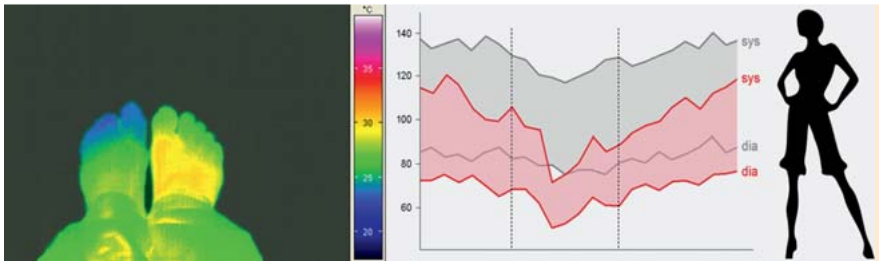

Fig. 1 Leading symptoms and signs of Flammer syndrome: cold extremities and low blood pressure. *Left:* Thermography picture of the feet of a PVD subject with asymmetric temperatures (from [7], with permission). *Middle:* Outcome of blood pressure monitoring in a subject with PVD (red) and without PVD (grey) (from [10], with permission). PVD occurs particularly often in slim women (*right*).

In terms of ocular perfusion, PVD – and therefore also Flammer syndrome – is often associated with reduced autoregulation [18], increased retinal venous pressure, stiffer retinal vessels [19], reduced neurovascular coupling [20], optic disc haemorrhages [21] and a correlation between OBF and finger blood flow [22]. These subjects have also slightly increased ET-1 plasma levels and altered gene expression in circulating lymphocytes. Interestingly, all of these characteristics (see **Table 2**) occur in both healthy subjects with Flammer syndrome and glaucoma patients progressing despite a normal IOP.

Aside from glaucoma [23], PVD is also related to other eye diseases, such as retinal arterial and vein occlusions [24], anterior ischemic optic neuropathy [25], Susac syndrome [26], retinitis pigmentosa [27], optic nerve compartment syndrome, central serous chorioretinopathy, and Leber hereditary optic neuropathy [7]. For more details, please refer to recent major reviews [7,9].

Secondary Vascular Dysregulation

If vascular dysregulation is secondary to a disease that affects the corresponding organ or remote organs, it is called secondary vascular dysregulation. One of the most important intermediators is ET-1. While under physiological conditions ET-1 is mainly produced by vascular endothelial cells, other cells can start to produce ET-1 if they experience stressful conditions (particularly in inflammatory diseases). This leads (among other changes) to an increased concentration of ET-1 in the circulating blood. The ET-1 plasma level is increased, for example, in multiple sclerosis, optic neuritis, rheumatoid arthritis, giant cell arteritis, fibromyalgia, and retinal vein occlusion [7].

Although it reduces OBF, SVD does not normally interfere with autoregulation and therefore does not lead to OBF instability. This is in contrast to PVD and explains why PVD, but not SVD, is a risk factor for GON.

Blood Flow Instability and Reperfusion Damage

Chronic but stable hypoxia leads, via an increase of hypoxia-inducible factor 1-alpha (HIF-1-alpha), to an increased expression of genes such as VEGF, ET-1, or erythropoietin. These mechanisms have been well studied, for example, in the context of wet age-related macular degeneration. In terms of ONH, stable hypoxia can result in ONH atrophy, but not in glaucomatous excavation.

The effects of reversible hypoxia are completely different (**Fig. 2**). *Unstable hypoxia* leads to major local *oxidative stress*, particularly in the mitochondria. While hypoxia slightly increases the number of the oxygen free radicals, they are boosted during reperfusion. This is why the resulting damage is called *reperfusion injury* [28]. During restoration of circulation, the oxygen concentration increases again. In this period, free oxygen radicals (particularly superoxide anion $O_2^{\bullet -}$) are formed, leading to oxidative damage and thereby also inducing mild inflammation. During reperfusion the free radicals are particularly produced within the mitochondria. The reason why ONH is most often involved is the fact that OBF is particularly unstable in the ONH and the mitochondria there are crowded due to the lack of myelin sheaths. Such reperfusion injuries in glaucoma patients are very mild but occur repeatedly. For this reason we discuss here oxidative stress in some more detail.

Table 2 Signs common to both healthy subjects with PVD and glaucoma patients progressing despite a normal IOP.

Reduced OBF autoregulation
Stiffer retinal vessels
Reduced neurovascular coupling
Increased retinal venous pressure
Optic disc haemorrhages
Correlation between OBF and finger blood flow
Altered gene expression in circulating lymphocytes
Increased oxidative stress
Slightly increased ET-1 plasma level

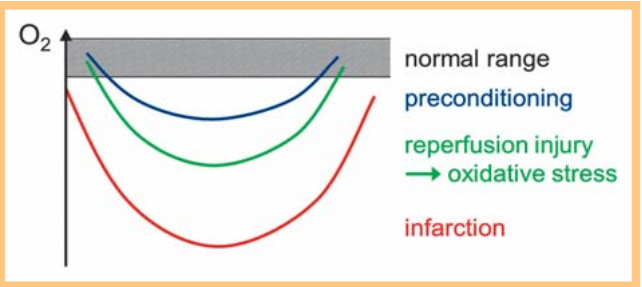

Fig. 2 The effects of reversible hypoxia depend on intensity and duration of oxygen reduction (from [1], with permission).

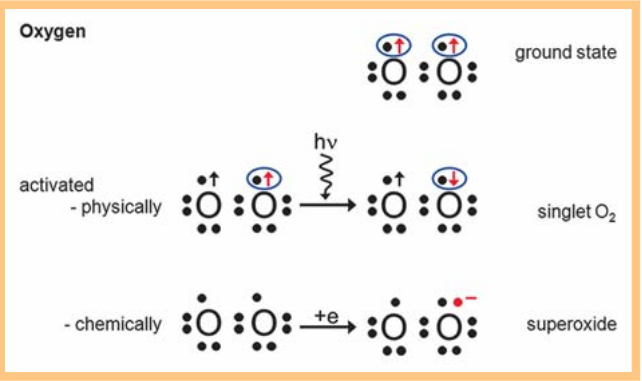

Fig. 3 Activation of oxygen. Under normal conditions, oxygen is inert (ground stage oxygen). It can, however, be activated either physically (via excitation by light) to form the singlet oxygen with the changed spin of one electron or it can be activated chemically by a reduction reaction (one electron reduction of dioxygen) to form the superoxide anion.

Oxidative Stress

Under normal conditions, oxygen is inert. It is, however, very reactive if either the spin of one electron is changed (singlet oxygen) or one single electron is added (superoxide) (**Fig. 3**). The term *reactive oxygen species* (ROS) refers to oxygen containing molecules that are highly reactive. Under optimal conditions, the magnitude of ROS formation is balanced by the rate of ROS elimination through the available antioxidants. If the production of ROS exceeds the capacity of its elimination, the amount of ROS increases to a level that leads to oxidative stress.

While glial cells tolerate hypoxia, they respond sensitively to oxidative stress. First, they are activated, and then in the later stage, they disappear.

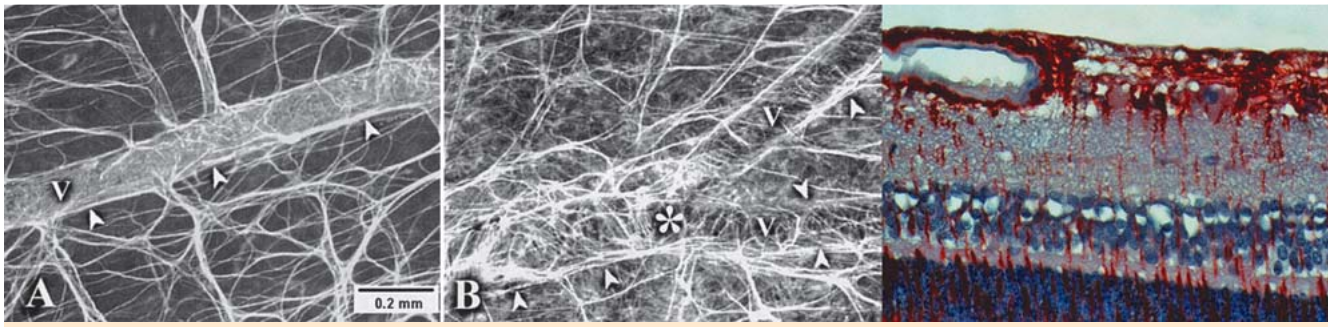

Fig. 4 Activation of glial and Müller cells. The processes of astrocytes connect vessels with neurons. The regular pattern (**A**) is lost when astrocytes are activated (**B**) (from [30], with permission). *Right:* Müller cells in a cross-section of the retina. Activation is made visible by glial fibrillary acid protein (GFAP) staining (brown colour) (from [31], with permission).

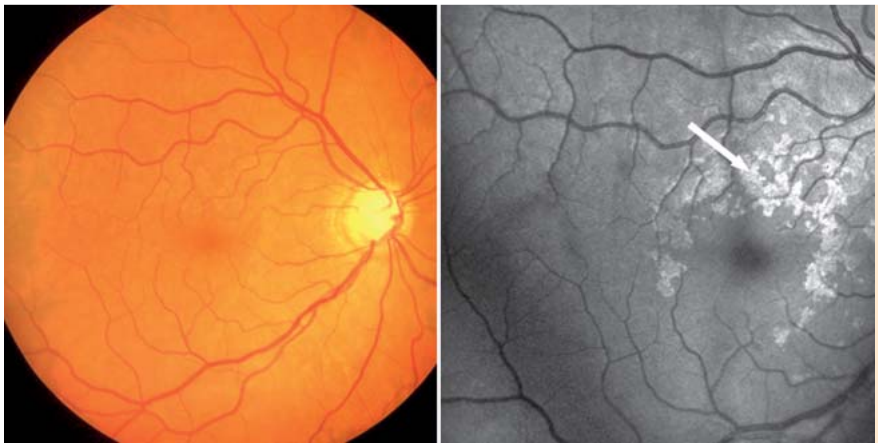

Fig. 5 Clinical correlate of activated astrocytes. The scattered light from the activated astrocytes is barely visible in colour photos (*left*), but is distinctly visible (white arrow) in red-free light (*right*) (from [10], with permission).

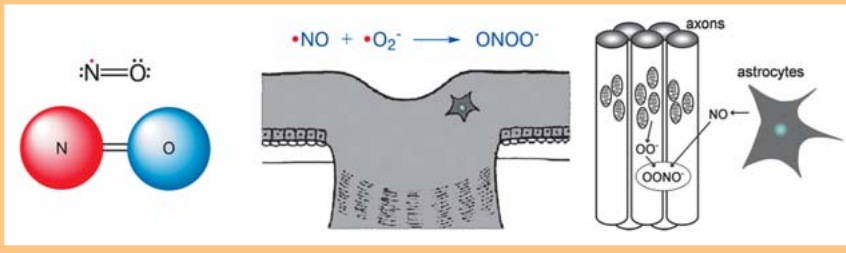

Fig. 6 Formation of highly damaging peroxynitrite ($ONOO^-$). *Left:* Nitric oxide. *Middle:* Nitric oxide reacts with the superoxide anion to form the peroxynitrite in the optic nerve head. *Right:* Corresponding neural axons of the ONH crowded with mitochondria and astrocytes capable of producing NO (from [33], with permission).

The Activation of Glial Cells

In the ONH, the most important glial cells are astrocytes. Indeed, they are activated in both experimental glaucoma and human glaucoma [29]. In the retina, the important glial cells are the astrocytes, which are found in the inner layer of the retina, and the Müller cells, which extend from the inner surface of the retina to the photoreceptor layer. Activated astrocytes change their morphology (**Fig. 4**) [30, 31], which leads to increased light scattering that can be observed clinically as glinting spots (gliosis-like alterations) in a red-free light (**Fig. 5**).

The activation of astrocytes leads to the overproduction of molecules such as NO (**Fig. 6**) and metalloproteinases (MMPs) [32], which both contribute to GON.

Damaging Peroxynitrite

Because of its strong oxidizing properties, peroxynitrite is particularly damaging to cells. It reacts with a wide array of molecules, including DNA and proteins, leading to cell death. Peroxynitrite ($ONOO^-$) is generated when NO fuses with superoxide anion ($O_2^{\bullet -}$) (**Fig. 6**). Therefore, if the concentrations of NO and superoxide anion ($O_2^{\bullet -}$) in a cell increases simultaneously, the probability of cell damage also increases. Activated astrocytes produce excess amounts of NO. It can easily diffuse from the astrocytes into the neuronal axons. If at the same time superoxide anion ($O_2^{\bullet -}$) is overproduced (due to unstable oxygen supply) in the neighbouring mitochondria of the axons, toxic peroxynitrite is formed within the axons (**Fig. 6**). Both the superoxide anion ($O_2^{\bullet -}$) and peroxynitrite cross the cell membrane very poorly; peroxynitrite, however, can diffuse within the axons toward the retina as well

as toward the lateral geniculate ganglion, thereby inducing cell death [33].

Glaucomatous Excavation

On the one hand, glaucomatous excavation implies a loss of tissue elements such as axons, glial cells, and blood vessels [34]. On the other hand, it also implies tissue remodelling, which leads to the bowing and compression of the lamina cribrosa.

In addition to the overproduction of NO, the activation of astrocytes also leads to an overproduction of MMPs. MMPs are enzymes that digest extracellular matrix, leading to disappearance and replacement of certain components of this matrix. This, in turn, leads to tissue remodelling and thereby contributes to ONH excavation, which is the hallmark of glaucoma.

Summary

Local oxidative stress – induced by unstable oxygen supply – contributes to development of GON. An unstable oxygen supply is mainly caused by fluctuation of OBF induced by IOP spikes, blood pressure drops, or disturbed autoregulation. Insufficient autoregulation is often a part of a more general vascular dysregulation occurring particularly in the context of Flammer syndrome.

Conflict of Interest

None

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