DOI 10.5935/0034-7280.20190017

The influence of nitric oxide on the pathophysiology of glaucomatous neuropathy

A influência do oxido nítrico na fisiopatologia da neuropatia glaucomatosa

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ABSTRACT

Nitric Oxide (NO) is a relaxing endothelium-derived factor and a potent vasodilator that impacts various systems throughout the body. Proven studies of basal ocular blood flow are regulated by NO, being an important regulator of homeostasis, especially within the uveal tissues. The dysfunction of the production associated with glaucoma due to alteration of the optic nerve head associated to the increase of the intraocular pressure by a deficient trabecular meshwork. NO became an attractive molecule for the treatment of glaucoma due to a modulation of the trabecular meshwork, lowering the neuroprotective intra and ocular pressure for a blood surgery in the head of the optic nerve.
Keywords: Nitric oxide; Glaucoma; Perfusion pressure; Trabecular meshwork

RESUMO

O oxido nitrico (NO) é um fator relaxante derivado do endotélio e um potente vasodilatador que impacta em vários sistemas em todo o corpo. Estudos comprovam que o fluxo sanguíneo ocular basal é regulado pelo NO, sendo um importante regulador da homeostase, especialmente dentro dos tecidos uveais. A disfunção da produção de NO seria associado ao glaucoma através da alteração da perfusão da cabeça do nervo óptico associado ao aumento da pressão intraocular devido um sistema de drenagem trabecular deficiente. O NO tornou-se uma molécula atraente para o tratamento do glaucoma devido a possibilidade de modulação da drenagem trabecular, abaixando a pressão intraocular e ação neuroprotetora melhorando a perfusão sanguínea na cabeça do nervo óptico.
Descritores: Oxido nítrico; Glaucoma; Pressão de perfusão; Drenagem trabecular

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The authors declare no conflicts of interests.
Received for publication 17/10/2018 - Accepted for publication 09/12/2018.

Introduction

Nitric oxide (NO) was first discovered in the 1770s by the English chemist Joseph Sacerdote, but was disregarded for medicinal purposes based on the belief that it was an air pollutant.[1]

No nucleated cell has been described so far with the ability to synthesize NO[2] being generated endogenously from L-arginine by a family of oxide synthase (NOS) enzymes and activate the second messenger cyclic guanidine monophosphate (cGMP) that is involved in various homeostatic processes.[3] There are three NOS producing NO in the body, and all are encoded by different genes: NOS1 (neuronal), NOS2 (inducible), and NOS3 (endothelial).[4] This reaction was believed to be the only one to explain syntheses of NO in mammals, although an alternative route known as nitrate (NO_3-) - nitrite (NO_2-) - NO has recently been described.[5]

Within the cell, NO is a free radical with an unpaired electron remaining for a short time (6 to 10 sec) before converting into nitrate (NO_3-) or nitrite (NO_2-).[6] Due to its unique gaseous properties and hydrophobic nature, the intracellular NO generated diffuses through the cell membrane to act rapidly on the target tissues,[7] being a relaxing factor derived from the endothelium and a potent vasodilator impacting on several systems of the body.[8]

Changes such as increased stress or hypoxia by stimulating a membrane receptor on the surface of endothelial cells by an agonist such as acetylcholine leads to increased intracellular calcium that causes NO production and relaxation of smooth muscle, [9,10] playing a key role in the cardiovascular, urogenital, respiratory, gastrointestinal and even immune systems. It also acts on angiogenesis, platelet aggregation, and bone formation.[11]

Indirectly, high NO levels can lead to the production of reactive oxygen species (ROS) and become cytotoxic,[7] which may be pro-inflammatory and also have antimicrobial effects. At low levels due to endothelial dysfunction, they can lead to pathological vasospasm as well as smooth muscle constriction contributing to systemic pathologies such as myocardial infarction, stroke, Raynaud's disease, migraine, pulmonary hypertension, erectile dysfunction, and glaucoma. [12]

Nitric oxide in the eye

All three isoforms of NOS enzyme are expressed in ocular tissues. Due to its short half-life, the measurement of NOS concentration is indirectly identified in tissues to monitor the conversion of L-arginine into L-citrulline [13] and the cGMP concentration.

Plasma cGMP concentration correlates with aqueous humor concentration (AH). In patients with glaucoma, decreased concentrations of NO and cGMP in plasma and AH were found. The lower plasmatic levels of NO indicators in patients with primary open-angle glaucoma (POAG) may reflect an imbalance of the mediators derived from the endothelium.[10] The concentration of NO in the vitreous correlates with the type and severity of glaucoma. [14]

Evidence proves that the presence of NOS in the vascular endothelium of the optic nerve head would be neuroprotective and would promote vasodilation by improving perfusion. [15,16] A proof of this is that after intravenous infusion of NG-nitro-L-arginine (LNMMA), an inhibitor of NOS, there was a reduction of blood flow in the optic nerve head in healthy subjects.[17]

The induction of NO increases the ease of drainage through the trabecular meshwork (TM) and Schlemm's canal (SC) in non-human primates, and may also have effects on the regulation of episcleral blood flow, thus reducing episcleral venous pressure (EVP).[18] Recent studies have described that topic administration of an NO donor, the sodium nitroprusside (SNP), could produce positive or negative effects on EVP based on the dose administered. In this sense, whereas 0.5 mg induced reduction of EVP, a dose of 5 mg produced the opposite effect.[19]

Studies to identify the predominant isoform in the conventional exit pathway have shown that NOS2 (inducible) is the predominant form in TM, probably because of the presence of macrophages, whereas NOS3 (endothelial) is the isoform expressed by SC cells and macrophages found in TM. [13]

In 2009, a study by Ellis et al. in primary cultures of human TM and in an anterior chamber perfusion system in pig eyes evaluated the role of soluble guanylatecyclase (sGC) as a mediator for NO-induced increase in AH flow. The exposure of the tissue to the donor diethylenetriamine-NO (DETA-NO) increased the flow of AH up to 220%, being this effect mediated by the enzyme sGC that increased the production of cGMP, interfering in the response of these tissues to the presence of NO.[20] An additional study, this time on cell signaling in MT, demonstrated that DETA-NO is able to mediate the activation of calcium-activated high conductivity potassium channels, resulting in a reduction in tissue cell volume and facilitating the flow of AH.[21]

The endothelium within SC has a sensitivity that would regulate NO in AH and the maintenance of intraocular pressure (IOP). SC closure or narrowing would stimulate NO production by NOS3 (endothelial) in the endothelium. This NO promotes the muscular relaxation of the TM cells, besides promoting vascular influence and increasing permeability. This function contributes to the increase of the trabecular drainage.[21,22]

In the uveoscleral pathway, the presence of NO donor compounds promotes relaxation of the ciliary muscle, producing a contraction of TM and SC, decreasing AH flow through the trabecular route and facilitating uveoscleral flow. These mechanisms of action have been evidenced in studies conducted with NO donor compounds in ciliary muscles of bovine origin and also of Rhesus monkey.[23] Regarding the production of AH, there is controversy about results on the action of donors of NO.[24]

These findings are consistent with recent genetic studies showing that polymorphisms in NOS3 (endothelial) - the gene encoding NOS - are associated with increased risk of glaucoma.[25]

Nitric oxide and perfusion pressure

Studies involving humans and animals confirm that basal ocular blood flow is regulated by NO formed by NOS3 (endothelial) and NOS1 (neuronal). In one of these studies, the choroid, iris, ciliary body, optic nerve head, and ophthalmic arteries were influenced by NO.[26,27] Vascularization of the retina presents a vasodilatory response to the NO released by the neurons, being an important regulator of blood flow homeostasis, especially within the uveal tissues.[22,28] It has been described that eNOS plays a very important role mediating the induction of vascular patency and angiogenesis through vascular endothelial growth factor (VEGF).[29]

Endothelial dysfunction is understood as being associated with normal pressure glaucoma (NPG), perhaps through perfusion of the altered optic nerve. [30,31] This same group presented lower systolic and diastolic velocity of the ophthalmic artery when examined with Doppler.[31] In POAG, abnormal IOP and vascular dysregulation reducing ocular perfusion may together determine damage to the optic nerve.[10]

Patients with POAG show an abnormal blood flow response to systemic inhibition of NOS with L-NMMA at the optic and choroidal nerve head compared to healthy controls, despite a comparable increase in systemic blood pressure. This indicates local changes of the L-arginine / NO system in this disease.[32] Increased levels of NOS3 (endothelial) in vessels of the optic nerve head may be considered neuroprotection, causing vasodilation and thus increasing blood flow.[15]

Nitric Oxide Replacement

IOP is the only modifiable risk factor for glaucoma. It is determined by the balance in AH production by ciliary epithelium and elimination through TM and the unconventional uveoscleral tract.[33] Individuals with elevated IOP have an incompetent conventional flow system due to increased rigidity of TM by alteration in the extracellular matrix.[34] Because NO is a local mediator of contractility in the conventional outflow tract, its deficiency or dysfunctional signaling may be a cause of increased TM stiffness.[35, 36]

The impaired formation of NO may have a double negative effect on patients with glaucoma, acting on IOP and ocular perfusion pressure (OPP). [10] Low OPP is strongly associated with an increased prevalence of POAG.[37]

Estudos mostram que substancias doadoras de NO reduzem a PIO elevando o NO_2- na camara anterior, sugerindo envolvimento do NO na patogênese ou regulação da PIO no GPAA.[38] Another study demonstrated an increase in nerve head blood flow in healthy subjects with administration of an NO donor agent, giving rise to a possible role of NO in improving ocular perfusion in NPG.[39,40]

Some authors argue that patients with glaucoma receiving nitrate-based therapy under systemic conditions have less progression of glaucomatous optic neuropathy and visual field loss compared to patients who do not take these compounds.[41]

Topical NO therapy is a challenge due to the duration of efficacy, the short half-life, and the difficulty of ocular penetration, requiring a higher frequency of use. Given the growing evidence for the role of NO in aqueous flow modulation and the unmet need for a drainage modulator via TM / SC, NO has become an attractive molecule to be clinically developed for the treatment of glaucoma.[40]

In practice, the only compound with NO donor activity that has been used in clinical trials in eyedrops is the latanoprost bunode (LBN). The LBN eyedrops 0.024% was recently approved (November 2017) by the US Federal Drug Administration (FDA) because of its hypotensive effect in individuals with POAG or intraocular hypertension (IOP). After topical administration, LBN is hydrolyzed in latanoprostatic acid and 1,4-butanediol mononitrate, which in turn is converted to 1,4-butanediol and NO.[42] Thus, the IOP reduction is due to 2 independent mechanisms. On the one hand, latanoprost acid increases uveoscleral drainage by remodeling the extracellular matrix and relaxing the ciliary muscle. On the other hand, released NO decreases IOP by increasing AH flow through TM/SC by activating sGC-cGMP, as discussed above.[20]

Araie et al. published the results of a study with 24 healthy volunteers (26.8 ± 6.3 years) in Japan with LBN 0.024% and recording the evolution of IOP every 2-4 h, as well as the adverse or unexpected effects observed. Said study administered a single dose of eyedrops in both eyes at 8:00 p.m. for 14 consecutive days. The average IOP baseline was 13.6 ± 1.3 mmHg, and the average reduction over the 24-hour period was -3.6 ± 0.8 mmHg (-27%). The IOP maximum reduction was at 8:00 a.m. (-4.2 ± 1.8 mmHg, -30%), and the minimum expressed at 8:00 p.m. (-2.8 mmHg, -20%), that is, 12 and 24 hours after administration of the eyedrops. With regard to safety, the authors did not observe any serious adverse effects during the study. The most frequent adverse effects related to the administration of LBN 0.024% were conjunctival hyperemia (50%) and punctiform keratitis (54.2%).[43]

Another study compared LBN 0.024% used once daily and timolol maleate 0.5% used twice daily in 25 patients with POAG or IOP for 4 weeks, and showed that the administration of LBN 0.024% gave rise to a significant increase in daytime OPP with the individual sitting or lying down compared to the baseline (p <0.001 and p = 0.006, respectively). During the night period, these differences were detected between the treatment groups, that is, the OPP in the LBN group was higher than the OPP in the group that used timolol maleate (p = 0.010).[44] Also, the efficacy of IOP reduction was greater in users of LBN 0.024% when compared to users of latanoprost 0.005% after 28 days of treatment.[45]

Final Considerations

The clinical treatment for glaucoma aims to decrease IOP because it is currently the main modifiable risk factor involved. The medication available act mainly by decreasing the production of AH or increasing its drainage. In order to increase the arsenal of treatment, the interest in discovering a new hypotensive agent acting on different mechanisms or new application vehicles has increased significantly in recent years.

The use of NO donors is common in other areas such as cardiology. Despite this use in ophthalmology is still initial, safety and efficacy studies of these compounds indicate that they may be available in the near future. Studies to date have reported promising results, although they emphasize the need to improve certain aspects such as bioavailability, deeper understanding of the mechanism of action, long-term toxicity as well as the appropriate dose to achieve desired efficacy and safety levels to decrease the progression of glaucoma.

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