# GLAUCOMA, APOPTOSE E SILDENAFIL: COMPREENSÃO DE UMA DOENÇA SEM SOLUÇÃO E UMA POSSÍVEL ABORDAGEM DE TRATAMENTO

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## RESUMO

Milhões de pacientes com glaucoma irão perder a visão, tornando-se uma necessidade desenvolver terapias neuroprotetoras que possam ser usadas, em conjunção com medicamentos hipotensores, para impedir a morte de células ganglionares da retina (CGR). Recentes avanços na compreensão da fisiopatologia do glaucoma são um fator chave na patogênese da neuropatia glaucomatosa. Com base nos achados em doenças semelhantes, a hipótese de que melhorando o fluxo sanguíneo do nervo óptico da retina possa resultar em uma proteção significativa nas CGR e prevenir a perda da visão no glaucoma. O sildenafil, uma droga vasodilatadora que inibe PDE5, aumentando assim os níveis de cGMP e prolongando seus efeitos, sendo que foi demonstrado melhorar a sobrevivência de várias lesões degenerativas. Esta breve revisão resume alguns dos avanços mais importantes que tiveram no entendimento sobre esta doença e um possível tratamento.

**Palavras-chave:** Apoptose, glaucoma, células ganglionares da retina.

# GLAUCOMA, APOPTOSIS AND SILDENAFIL: COMPREHENSION OF A DISEASE WITHOUT SOLUTION AND A POSSIBLE APPROACH OF TREATMENT

## ABSTRACT

As millions of glaucoma patients lose sight, it has become accepted that there is a need to develop neuroprotective therapies that can be used, in conjunction with hypotensive drugs, to prevent retinal ganglion cell (RGC) death. Recent advances in the understanding of the pathophysiology of glaucoma is a key factor in the pathogenesis of glaucomatous neuropathy. Based on findings in similar diseases, hypothesize that improving retinal and optic nerve blood flow can result in significant RGC protection and prevent vision loss in glaucoma. Sildenafil, a vasodilative drug that inhibits PDE5, thereby increasing cGMP levels and prolonging NO effects, has been shown to improve survival in several injury. This short review briefly summarizes some of the most important advances that have taken in the understand about this disease and a possible treatment.

**Keywords:** Apoptosis, glaucoma retinal ganglion cell.

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# GLAUCOMA, APOPTOSIS Y SILDENAFIL: COMPRENSIÓN DE UNA ENFERMEDAD SIN SOLUCIÓN Y UN POSIBLE ENFOQUE DE TRATAMIENTO


## RESUMEN

Millones de pacientes con glaucoma pierden la visión, por lo que es una necesidad para desarrollar terapias neuroprotectoras que pueden utilizarse en conjunción con medicamentos hipotensores, para prevenir la muerte de las células ganglionares de la retina (CGR). Los recientes avances en la comprensión de la fisiopatología de glaucoma es un factor clave en la patogénesis de la neuropatía glaucomatosa. Con base en los hallazgos en enfermedades similares, la hipótesis de que la mejora del flujo sanguíneo de la retina nervio óptico puede dar lugar a una protección significativa en CGR y prevenir la pérdida de visión en el glaucoma. Sildenafil, un fármaco vasodilatador que inhibe la PDE5, lo que aumenta los niveles de cGMP y la prolongación de sus efectos, y se ha demostrado que mejora la supervivencia de diversas lesiones degenerativas. Esta breve revisión resume los avances más importantes que tenían el conocimiento de esta enfermedad y su posible tratamiento.


**Palabras clave:** Apoptosis; Glaucoma; Células ganglionares de la retina.

## INTRODUCTION

Glaucoma is a leading cause of loss of vision and blindness in the world (1,2). It leads to visual impairment in 1-4% of the population over 40 years of age (3), though in some ethnic groups it may affect 20% or more of the population (4), with an estimated 80 million people worldwide by 2020 and, 8.4 million of them will be blind in both eyes (1). Despite these, glaucoma remains an "unsolved" disease, as evidenced by the growing number of patients, stressing the urgent need to develop new strategies to treat this blinding disease (1,4-6).

A pathophysiological hallmark of glaucoma is the gradual loss of retinal ganglion cells (RGC) and their axons, the optic nerve (ON) fibers, like other neurons conditions such as Alzheimer's or Parkinson's disease (7-9). Generally is accepted that glaucomatous degeneration of the optic nerve and retina is linked to the development of elevated intraocular pressure (IOP) (10). Because this, for many years it has been accepted that increase in IOP is the cause of the disease (11).

There are evidences that in humans, in animals and, in models of experimental glaucoma, high IOP causes ganglion cell loss, and that lowering IOP in affected eyes can attenuate the degenerative process (10). However, today it is recognized that IOP elevation is the main risk factor in glaucoma, but evidence suggests that local ischemia, leading to reduced ON perfusion, is a key event in the pathogenesis of glaucomatous neuropathy (12,13). Reduced levels of nitric oxide (NO), a potent vasodilative mediator, have been demonstrated in glaucoma patients and animal models of the disease (as well as in other neurodegenerative diseases) and are implicated in the impairment of ocular blood flow, leading to neuronal death and loss of vision (14,15).

Several risk factors have been proposed to contribute to glaucoma progression including elevated intraocular pressure, age, genetic background, thinner corneal thickness and vascular degeneration (10). The existence of any of these factors might determine an individual risk to develop glaucoma, but they are not necessarily the cause of this condition (16). Research into the pathogenesis of glaucoma has been aided by the development of animal models that undergo apoptotic RGC death (15).

Sildenafil, an approved drug for the treatment of erectile dysfunction, is an inhibitor of phosphodiesterase (PDE), an enzyme that degrades cGMP, the secondary messenger of NO. Therefore, treatment with sildenafil has been shown to be neuroprotective in numerous models of neurodegenerative diseases, as elevated cGMP levels prolong the effect of NO and lead to vasodilation and improved circulation (17,18). Sildenafil treatment has resulted in increased neuronal survival, and even neurogenesis, in models of brain, spinal cord and cerebral injury (19,20).

This review aims to better understand the mechanism of cell death by apoptosis of the disease and its treatment adding.

## SCIENTIFIC BACKGROUND

### Glaucoma

Historically, glaucoma has been described as a disease caused by increased ocular pressure (IOP). Recently glaucoma has been considered a primary optic neuropathy, but this fact is not widely shared by the public and health-care professionals alike. An informal survey of mainstream dictionary definitions of glaucoma still reveals obsolete phrases such as 'increased intraocular pressure that results in a group of eye diseases characterized by pressure that is too high for the optic nerve to withstand' (21,22).

However, there is abundant evidence demonstrating that ocular hypertension alone is insufficient, but a necessary factor for the development or progression of glaucoma. The Cochrane Eyes and Vision Group define glaucoma as 'a disease characterized by defects in the visual field, damage to the nerve at the back of the eye, and usually raised pressure inside the eye.' This view of glaucoma as an optic neuropathy with elevated IOP as a modifiable risk factor rather than a causative agent for damage, although it still does not take into account the characteristic morphological and functional aspects of the disease (22).

Primary humans, glaucoma is classified into three principal types: primary open angle glaucoma (POAG), primary angle-closure glaucoma and congenital glaucoma. The POAG is the most common type in humans (23). However, for example, although high intraocular pressure is common among open-angle glaucoma patients, only a limited subset of individuals with ocular hypertension will develop this disease (23,24). Moreover, a significant number of patients presenting with glaucoma continue to lose vision despite responding well to therapies that lower eye pressure (16,24).

In dogs, the main protagonists of glaucoma in animals, can be classified based on the primary cause, in primary, secondary or congenital. Based on the appearance of gonioscopy drainage angle, open, closed or narrow and, according to the duration or with stage of evolution, chronic or acute. Chronic glaucoma is revealed with the progressive elevation of IOP. In contrast, the acute characterized by a sudden increase in IOP (25).

Glaucoma has been investigated for some 50 years in dogs. The highest incidence has been reported to Beagle, Welsh Springer Spaniel, and many other breeds. Uveitis and neoplasms are the most common eye diseases related to glaucoma. POAG studies in dogs showed that breeds Shiba-Inu and Shih-Tzu are the most affected, however, it is reported that the type of primary angle closure there is the most common in this breeds (25,26).

In humans, loss of vision in glaucoma patients is due to progressive death of RGCs and their axons, which constitute the ON fibers (7,8). Traditionally this neuronal and axonal death has been attributed to increase in intraocular pressure (IOP), but now recognized a significant portion of glaucoma cases present with normal or low IOP (27), and it is the main accepted that IOP elevation is one of several risk factors for the disease (13,27). Evidence suggests that

another, as local ischemia, leading to reduced ON perfusion cause glaucomatous neuropathy is reduced blood flow in the inner retina and ON (28).

Current evidence shows that patients suffer from low ocular perfusion pressure, as well as abnormal vascular auto regulation capacity, impairing the ability to adapt to increased IOP or decreased blood pressure (12,28). The result is RGC death and ON atrophy, as well as reperfusion injury and ON remodeling. Research into the pathogenesis of glaucoma has been aided by the development of animal models that undergo apoptotic RGC death (15,29,30).

The implication of these data is that current medical and surgical therapy, aimed at lowering IOP, has limited success in preserving vision in glaucoma patients. Therefore, it is evident that there is a pressing need to develop new therapeutic approaches that can be used, in conjunction with hypotensive drugs, to preserve vision (21).

## Apoptosis

Apoptosis is a modality of programmed cell death that can be identified by different morphological characteristics and the involvement of specific proteins that regulate it (29,30). Cell death also is part of normal development and maturation cycle, and is also component of many response patterns of living tissues to xenobiotic agents (i.e. micro organisms and chemicals) and to endogenous modulations, such as inflammation and disturbed blood supply (31).

Cell death is a fundamental cellular response that has a crucial role in shaping our bodies during development and in regulating tissue homeostasis by eliminating unwanted cells. The first form of regulated or programmed cell death to be characterized was apoptosis (32). The term 'apoptosis', defined as a controlled type of cell death that can be induced by a variety of physiologic and pharmacological agents, was first described by Kerr, Wyllie, and Currie in 1972 to describe a morphologically distinct form of cell death on the basis of the following main morphologic criteria: cellular shrinkage, condensation and margination of the nuclear chromatin, DNA fragmentation, cytoplasmic vacuolization, membrane blebbing, cell lysis, and the formation of apoptotic bodies (33).

However, the apoptosis pathway was described in Caenorhabitis elegans in the early 1990s. Subsequent genetic analysis of mammalian apoptosis presented a more complex, in which individual apoptosis genes from C. elegans have expanded into large multi-protein families. These findings suggest a redundancy, functional specialization and compensatory regulation of mammalian apoptotic signalling and execution might be important features of mammalian apoptosis (32).

In some cases there is the type of stimuli and/or the degree of stimuli that determines if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses. Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called "caspases" and a complex cascade of events that link the initiating stimuli to the final demise of the cell (34).

Using conventional histology, it is not always easy to distinguish apoptosis from necrosis, and they can occur simultaneously depending on factors such as the intensity and duration of the stimulus, the extent of ATP depletion and the availability of caspases. Necrosis is an uncontrolled and passive process that usually affects large fields of cells whereas apoptosis is controlled and energy-dependent and can affect individual or clusters of cells (34,35).

Necrosis proceses characteristic features, such as organelle swelling, mitochondrial dysfunction, massive oxidative stress and rapid plasma-membrane permeabilization that are

thought to be indicative of the catastrophic nature of cell death, rather than a result of cellular regulation. The general view of the relationship between apoptosis and necrosis is that milder insults to the cell cause apoptosis, whereas more intense insults induce uncontrollable necrosis. It is thought that apparently unregulated, the process accounts for the bulk of cell death events in acute pathologies (32,34).

The primary mechanism of RGC damage in glaucoma is not well understood, but there is evidence that neuronal loss in this disease occurs largely by apoptosis. This selfdestructive, genetically driven, death program is activated in all neurons. It is now widely accepted that neurotrophic factors promote neuronal survival by inhibiting default apoptotic pathways. During development of the nervous system, young neurons require trophic factors for their survival, differentiation and the establishment of synaptic connections (16,32).

Neurotrophic factors are produced in limited amounts; therefore only neurons exposed to optimal levels of these molecules survive, whereas less fortunate neurons are eliminated by apoptosis. In rodents, 65% of RGCs die during retinal development. Excess RGCs are eliminated in two successive phases of cell death in the retina: the first phase peaks at embryonic day 6, when RGCs differentiate; and the second phase coincides with the arrival of RGC axons to the brain, when these neurons become dependent on target-derived trophic support (16,36).

The induction of apoptosis can occur by external or internal stimulus. Two major general pathways of induction of apoptosis: receptor or extrinsic pathway and the mitochondrial or intrinsic pathway (29). The extrinsic pathway promotes the activation of caspase 8, which activates caspase 3 or cleaves at pro-apoptotic gene bcl-2 family, by linking to the Fas receptor and TNFR (37). The intrinsic pathway is mediated by internal stimuli of intracellular stress, as well as DNA lesion or disruption of the cell cycle or in metabolic pathways (30).

Once activated, the majority of the caspases have the ability to catalyze the activation of multiple other members of this family, resulting in amplification of the proteolytic cascade (37). The caspases is classified in two groups: the initiator caspases (caspase-2, 8, 9 and 10) and executors caspases (caspase-3, 6 and 7). The functional forms of the initiator caspases promote directly or indirectly the activation of executors caspases (29,30,37).

Both extrinsic and intrinsic pathways and at the point of the execution phase, considered the final pathway of apoptosis. It is the activation of the execution caspases that begins this phase of apoptosis. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins (34).

In the extrinsic pathway, an active death receptor recruits the intracellular adaptor protein Fas-associated death domain which in turn recruits procaspase-8 to form a signaling complex. Caspase-8 is cleaved and activated through autoproteolysis leading to subsequent activation of caspase-3 and caspase-6. The expression of both initiator and effector caspases has been investigated in RGCs following acute or chronic optic nerve injury. Active, cleaved caspases- 3, -8 and -9 have been detected after optic nerve transaction or crush, ocular hypertension as well as ischemic injury (16,30,38). Although caspase-3 was implicated in the primary and secondary waves of RGC apoptosis, it was active for a long period of time and with greater intensity during the primary wave of RGC loss (39).

Extrinsic apoptotic signals include an array of death-receptor ligands: Tnf-α, Fas-l, and TNF-related apoptosis-inducing ligands (TRAIL) that bind to their respective receptors to induce cell death. Death receptor activation results in the recruitment of the intracellular adaptor Fas-associated death domain (FADD), which typically recruits the initiator procaspase-8 leading to caspase-8 activation followed by executioner caspase-3 activation and cell death (16).

In the intrinsic pathway, cytochrome C is released from the mitochondria and together with Apaf-1 and procaspase-9 forms the apoptosome, which facilitates caspase-9 activation and downstream cleavage of caspase-3. Cytochrome C, which is released from damaged mitochondria, promotes the formation of a heptameric 'apoptosome' megacomplex of APAF1 and caspase-9 (a member of the CED 3-like Cys protease family). This leads to the conformational change and activation of caspase-9. Activated caspase-9 in turn cleaves and activates downstream caspases, including caspase-3, caspase-6 and caspase-7 that carry out the execution phase of apoptosis (16,32).

The BAX protein is normally present in the cytoplasm of a cell, but upon activation of the cell death signal, it will translocate and insert into the mitochondrial outer membrane. Several studies suggest that BAX monomers can form a multisubunit pore structure large enough to allow the escape of molecules like cytochrome C. Knock-out mice lacking a functional Bax gene exhibit several supernumerary populations of neurons, including retinal ganglion cells, indicating the importance of Bax in regulating neuronal programmed cell death during development (40,41).

The central hypothesis of excitotoxic injury is that excess glutamate binds to cell surface ionotropic glutamate receptors, primarily N-Methyl-D-Aspartate (NMDA) receptors (NMDAR), triggering massive Ca influx and activation of pro-apoptotic signaling cascades in neurons. Elevation of endogenous glutamate and activation of glutamate receptors have been shown to contribute to a variety of acute and chronic neurological disorders, including stroke, trauma, seizures, and various forms of dementia and neurodegeneration (42,43)

In the retina, excess glutamate has been proposed to underlie common neurodegenerative disorders such as retinal artery occlusion and glaucoma. A vast number of studies have now demonstrated that adult RGCs are exquisitely sensitive to exogenously applied NMDA, which triggers rapid death of these neurons, and that inhibitors of NMDAR and/or downstream pathways are neuroprotective in experimental models of retinal ischemia and glaucoma (16,43,44).

**Neuroprotection and sildenafil**

Though therapy for glaucoma is focused on reducing IOP, much has been dedicated to the development of drugs that provide neural protection. Substances have been suggested as candidates for neuroprotective therapy based on inhibition mechanisms of degeneration and apoptosis of RGCs, in order to promote their survival (45). Nitric oxide (NO) is a gaseous molecule labile released from endothelial cells. It induces vasodilation, increased blood flow and decreased vascular resistance. Its inhibition leads to reduced perfusion. It is not therefore surprising that vascular endothelial dysfunction, resulting in decreased levels of NO, is found in glaucoma patients developing increased retinal vascular resistance. Deficient production of NO is therefore a participant in the pathogenesis of glaucoma, while the increased synthesis and release may prevent the progression of harmful manifestations (14,17,18,46).

Vasoactive agents were tested, including tadalafil and sildenafil employed in the treatment of erectile dysfunction. Sildenafil is a new vasoactive drug that has been developed for the treatment of erectile dysfunction. It increases intracellular cGMP through inhibition of PDE enzyme as well as by enhancement of NO/cGMP mediated signaling. There is experimental evidence that increasing intracellular cGMP can prevent oxidative stress induction and lipid peroxidation (47,48).

Therefore, treatment with sildenafil has been shown to be neuroprotective in numerous models of neurodegenerative diseases, as elevated cGMP levels prolong the effect of NO and lead to vasodilation and improved circulation (17,18). Sildenafil treatment has resulted in

increased neuronal survival, and even neurogenesis, in models of brain, spinal cord and cerebral injury (19,20).

It enhances the vasodilator effect of nitric oxide (NO) on the sinusoidal and vessel smooth muscles of the corpus cavernous and increases the arterial blood flow into the sinusoids. Therefore, treatment with sildenafil has been shown to be neuroprotective in numerous models of neurodegenerative diseases, as elevated cGMP levels prolong the effect of NO and lead to vasodilation and improved circulation (17,18). Sildenafil treatment has resulted in increased neuronal survival, and even neurogenesis, in models of brain, spinal cord and cerebral injury (19,20).

The sildenafil has powerful cardioprotective effects and could reduce apoptosis and necrosis in cardiac tissues after ischemia–reperfusion injury (49). Ebrahimi et al. (50) observed that sildenafil reduced diabetes-induced cardiac cell apoptosis at the end of the first and second weeks, when it reached its peak value. Previous studies have also shown the cardioprotective effects of sildenafil in different experimental models. Fisher et al. (51) demonstrated that sildenafil attenuated cardiomyocyte apoptosis in a chronic model of doxorubicin cardiotoxicity. Salloum et al. (49) also showed that acute and prolonged treatment with sildenafil during myocardial infarction (MI) was associated with myocardial salvage from necrosis, reduction of apoptosis, prevention of adverse cardiac remodeling and heart failure, and improved survival. In the eye the sildenafil can affect ocular blood flow and choroidal volume due to its effects on vascular smooth muscles. It may also have vasodilator effects on choroidal sinusoids and retinal vessels similar to the vasodilation of corpora cavernosa sinusoids (52).

This drug is frequently used in older patients with erectile dysfunction and vascular diseases. This population is also at risk of many ophthalmological diseases, such as senile macular degeneration, glaucoma, diabetic retinopathy or ischaemic ocular problems (53). In contrast, studies showed that NO causes increased DNA damage, apoptosis, neurotoxicity and inflammation. When the concentration of NO is more than 1 mM, the predominant effects mediated by him include deamidation, oxidation or nitration of DNA via interaction of NO with superoxide or oxygen radicals. When the concentration is less than 1μM, their actions are direct, without interacting with superoxide radicals or oxygen, may regulate physiological activities by different signaling pathways (54).

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## FINAL CONSIDERATIONS

Glaucoma is a common eye disease that is usually associated with na elevated IOP. As our understanding of the process of ganglion cell death in glaucoma becomes more comprehensive to develop new therapeutic strategies that target this process. The focus of this new strategies include prevent damage to the axons in the optic nerve, prevention of ganglion cell soma death, and the reactivation of surviving ganglion cell somas to initiate the regrowth or repair of damaged axons. As scientific investigation, a more precise definition of these targets and development of the therapies needed to treat them will be included in the advancements of glaucoma research.

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