

● COMMENTARIES

## Pathogenesis of glaucoma: how to prevent ganglion cell from axonal destruction?

Glaucoma is the main cause of irreversible blindness globally, but the pathogenesis of glaucoma is still pending consensus. In spite of great advances during the 20<sup>th</sup> and early 21<sup>st</sup> centuries in terms of detailed knowledge of pathological processes that take place in glaucoma, the exact pathogenesis is much less clear. Hypotheses abound, and the most researched areas are ischemia at the optic nerve head, blockade of ganglion cell axonal transport, peripapillary atrophy, and changes in the characteristics of the lamina cribrosa (Nucci et al., 2008).

Although it is a well-established clinical fact that very high intraocular pressure (IOP), higher than blood perfusion pressure, is able to induce ischemic damage, the presence of glaucomatous changes in patients with moderately increased and normal or even low pressure, without demonstrable pressure spikes, cannot directly be attributed to pressure, and additional explanations have been sought. Rather than one pathological process, the clinical recognition of low-pressure glaucoma has brought about complementary explanations, creating different categories of glaucoma with different pathogenesis.

Raised intraocular pressure has been dropped as “the” causative factor and now ranks alongside other risk factors. As the number of known risk factors multiplies, complex mathematical approaches are deemed necessary to tackle the tangled relationships between risk factors. Lowering IOP continues to be the only modifiable risk factor, and the mainstay of glaucoma treatment. But the progression of open angle glaucoma (OAG) in many cases, despite IOP treatment, strongly suggests that other factors must play a part in the development of the disease. Although dissatisfaction with a single approach based on ocular hypertension has helped promote direct anti-ischemic and neuroregenerative therapeutic approaches, these have been largely abandoned as sterile, when not outright dangerous (Caprioli et al. 2010).

With a wealth of data at hand, there is an apparent urgent need to seek new causal relationships between known deviations from the normal physiology both at the organ level and cell level, and propose, as a working hypothesis, a new pathogenetic model, simple, congruent with the clinical heritage, and with feasible therapeutic applications. But is it possible?

Focused on basic research on the pathogenesis of glaucoma, we have been working in our laboratory with cultured optic nerve tissue. Our approach to the pathology of glaucoma is condensed in a proposition based on histological evidence and presented as a chain of events. In short, we contemplate glaucoma as a result of the misdirection of the aqueous outflow.

The new hypothesis tries to solve some salient inconsistencies that form part of the narrative of the “hypertensive- mechanical-ischemic paradigm”. Only some of those inconsistencies, and their proposed solutions, will be considered here. It is frequent to find in the specialized literature that in the characterization of the pathology of glaucoma only the retinal ganglion cells (RGCs) and their axons are mentioned. Astrocytes are either plainly ignored, or even, in some cases, described as “reactive astrocytes” with newly arisen lethal abilities that would damage rather than protect the axons that depend on them. Other aspects that are difficult to interpret at the ultrastructural level

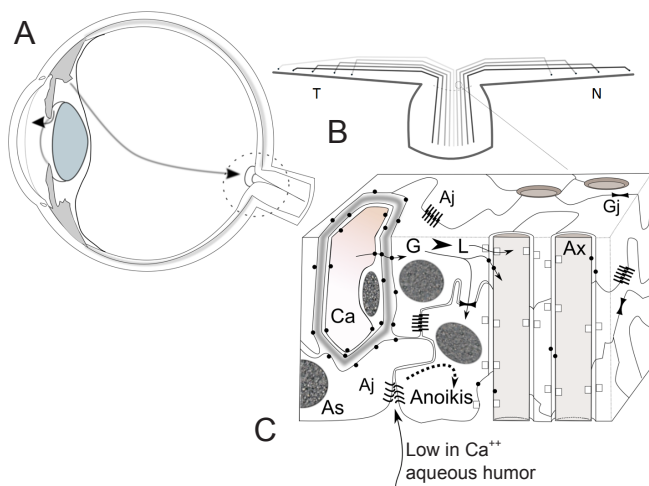
are how a process that causes progressive damage over decades, and projects a consistent diagnostic pattern in the visual fields is produced by pressure oscillations in a single, not lobulated, capillary bed in an area of 2.5 mm<sup>2</sup>, part of a vascular bed with autoregulatory capabilities. As a majority of glaucoma cases fall into the categories of moderate and low tension glaucoma, zeal to uncover pathological mechanisms in the hypertensive- mechanical-ischemic damage has led to difficulties to explain why normal individuals do not have glaucoma, or to dilute OAG in the murky waters of more complex syndromes, as the vascular dysregulation syndrome (Mozaffarieh and Flammer, 2009).

We demanded our hypothesis to face the following principles: in order to be considered, the hypothesis should be able to explain loss of axons under hypertensive and hypotensive situations. Axon loss should be able to occur in a very progressive manner, either slow or accelerated. It should be a process capable of leading to total blindness and also able to stop after some nerve loss, and either not progressing further or significantly slowing the rate of loss. At least the initial damage should take place in the optic nerve head (ONH) and should be based on the proven fact that the outflow of aqueous through the conventional and alternative pathways is altered during glaucoma development.

I will set out here a few structural details of the optic nerve head histological architecture that helped to shape our proposition. The surface of the vitreous surface of the optic nerve is formed by astrocytic projections. Around the main vessels there is a net of wide interconnected intercellular spaces; those passages are absent in the areas only occupied by axons and axon-wrapping astrocytes. No tight junctions are present connecting the astrocytic projections, only occasional adherens and gap junctions are present (Carreras et al., 2009). Excess extracellular fluid can be interchanged with the vitreous by the network of interconnected peri-vascular and para-axonal spaces (Carreras et al., 2010a). That the vitreous body itself is permeable in mammals to fluids and solutes has been known since the late 19<sup>th</sup> century. It means there is no obstacle to aqueous humor diverted to the vitreous cavity being evacuated through the optic nerve head (Carreras et al., 2010b). As **Figure 1A** shows, in case of increased resistance to outflow through the anterior pathways, the natural exit for aqueous is the optic nerve head. The colloid osmotic pressure of the vitreous body, the only obstacle to posterior outflow, is variable and changes with age.

Modeling of the retina and optic nerve has shown that following a model of development of the optic stalk, in which order of axonal growth and fasciculation is strictly followed, reproduces the path of the axons in the retina with up to 90% fidelity (Carreras et al., 2011). These models show the existence of a singularity for the fibers coming temporarily to the macula that enter the optic disc centrally rather than peripherally. If a concentric erosion of the optic cup is implemented, then the early visual defect of nasal step is reproduced (Carreras et al., 2010b). As a diagrammatic depiction of the ordering of the axons in the neuroretinal rim shows (**Figure 1B**), antero-posterior elimination of axons is coincident with an enlarging central cup. Both could be the result of a noxa (the aqueous humor) acting from front to rear. The mentioned singularity would explain the early appearance of the nasal step in glaucomatous fields (Carreras et al., 2011a).

The nutrition of RGCs, axons depends on the transport across the astrocytes of lactate and, in lesser measure, glucose. Metabolic coupled molecules, monocarboxylate transporters (MCTs) and glucose transporters (GLUTs), need the presence of gap junctions along with CAMs and adherens junctions to keep the energy shuttle in place (Carreras et al. 2011b). **Figure**



**Figure 1** Misdirection of aqueous outflow in glaucoma and ionic stress at the optic nerve head level.

(A) Schematic section of the human eye. Short arrow shows the normal flow of aqueous towards the anterior chamber and anterior outflow pathways. Long arrow shows the misdirection of aqueous outflow towards the posterior pole and optic nerve head when anterior pathways increase the resistance to outflow. Note that no epithelial sheet impedes the posterior flow of aqueous. (B) Ordering of the axons in the retinal nerve fiber layer and optic nerve head. Retinal ganglion cells near the disc enter the nerve centrally, ganglion cells from the periphery of the retina enter the nerve peripherally. The singularity of the axons coming from the temporal periphery entering the disc centrally is also depicted. Shadows of grey in the fibers highlight that antero-posterior disappearance of axons and centrifugal enlargement of the optic cup are equivalent. (C) A cubic portion of the prelaminar optic nerve shows a capillary surrounded by astrocytes and 4 axons from the axonal bundle wrapped by astrocytic expansions. Glucose transporters help carry glucose (G) from the capillary lumen into the astrocyte. Part is consumed, part is transported to the axons (through GLUT1 and GLUT3, black dots) and the main part is transformed into lactate (arrowhead). Lactate (L) is transported (long bifid arrow) into the axons through MCT 1&2 (white squares). Gap junctions (macula comunicans) allow astrocytes to share glucose and lactate. Critical to the maintenance of correct cellular relationships is the presence of adherens junctions (zonula adherens) rich in N-Cadherine, a calcium dependent adhesion molecule. If aqueous humor, poor in Ca<sup>++</sup> relative to plasma displaces extracellular fluids, the adherens junctions separate and trigger a form of apoptosis known as anoikis. The consequence is initially axonal compartmentalized destruction and secondarily retinal ganglion cell apoptosis. As: Astrocyte; Ax: axon; Ca: capillary; GLUTs: glucose transporters; MCTs: monocarboxylate transporters; Black dots: GLUTs; white squares: MCTs; G: glucose; L: lactate; Aj: adherens junction; Gj: gap junction; For convenience figure C is inverted in relation to B. Not to scale.

IC shows the elements that participate in the energy shuttle from the capillaries to the axons. Key elements are represented: gap junctions, adherens junctions, MCTs and GLUTs that cooperate in the metabolic energy transfer.

The previous structural data gain relevance when the ionic composition of the aqueous humor is recalled. Ionic composition of aqueous humor is critically different from that of the plasma and interstitial tissue. Both in vitreous and aqueous, the concentration of ion calcium is lower than in plasma, varying from 49% to 57% of plasma levels, while the concentration of potassium ion is similar to that of plasma (Carreras et al., 2009). This combination is crucial to adherens junctions, which require calcium to establish homophilic cadherin interactions, and potassium, a smaller cation, interferes with the union. The levels of calcium and potassium present in the aqueous have been shown to trigger a salvo of apoptosis in prelaminar tissue culture (Car-

reras et al., unpublished results). Due to their signalling role, separation of adherens junctions by a drop in calcium concentration is able to trigger a form of apoptosis known as anoikis. The axons fall secondarily to astrocyte support deprivation. Axonal compartmentalized destruction is followed secondarily by somatic RGC apoptosis. Anteroposterior thinning of the fiber layer and erosion of the central cup would ensue, giving rise to the characteristic glaucoma progression in the visual fields. This process is summarized in Figure 1C. This process of “silent” tissue destruction would explain, among others signs, splinter disc hemorrhages as a consequence of capillary breakdowns due to lack of tissue support.

In brief, this hypothesis blames not the raised intraocular pressure, but the misdirection of the flow of aqueous towards the posterior pole of the eye as the main culprit in glaucoma. If proven, this might have huge consequences both in the diagnosis of the disease and follow-up and treatment. From this perspective, the hypothesis would suggest interfering with the preservation of the integrity and colloid osmotic properties of the vitreous body might have in the prevention of this blinding and widespread disease.

The crucible of this proposition will be the clinical tests to relate quantitatively posterior flow of aqueous and glaucoma progression, and for that purpose the adaptation of new optic technology would be required. If successful, new clinical tests could advance the diagnosis of glaucoma to precede severe fiber loss.

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