Neurodegeneration and Death of Retinal Ganglion Cells

Subjects: Ophthalmology

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Retinal ganglion cells (RGC) transmit light and visual information to the brain via the long axons that form the optic nerve. RGCs are the measurable endpoints in current research into experimental therapies and diagnosis in multiple ocular pathologies, like glaucoma. RGC subtype classifications are based on morphological, functional, genetical, and immunohistochemical aspects.

Keywords: retinal ganglion cells ; neurodegeneration

1. Retinal Images Techniques

Optical coherence tomography (OCT) can be temporal or spectral domain. This technique generates two-dimensional cross-sectional images from the optical backscattering of light and the time delay of the echo. If enough scans are available, it can generate three-dimensional images. In addition, its axial resolution is very high (1–15 μm) [1][2]. However, weak backscatter and low-contrast cell borders prevent direct visualization of individual RGC. The fundamental value of OCT is to provide high-resolution images of the thickness of the retinal nerve fiber layer (RNFL) [3][4][5][6]. Its performance has been improved by applying a Doppler development [7]; as well as with polarization-sensitive OCT, since ocular structures are capable of altering the polarization state of light, adding tissue-specific contrast to images, and allowing RGC axon densities to be measured [8]. Similarly, long-wavelength OCT, which reduces scatter [9], and swept-source OCT, which uses a tunable long wavelength can further increase resolution, represent significant improvements.

Confocal scanning laser ophthalmoscopy (cSLO) was established in 1980 [10], and is the most widely used retinal imaging technique. Its remarkable ability to adapt has been key to its survival as a diagnostic technique in glaucoma, providing high-resolution images with which subtle changes in the retina can be detected [11].

The cSLO methodology is based on the confocal microscopy technique, in which a laser scans the retina, and thanks to the pinhole, only light coming from certain depths is detected. In addition, as it is a narrow beam of light, no scattered light is produced; ensuring that only light from the desired focal plane is detected. In this way, a high lateral resolution is achieved, which allows the production of topographic images [10]. However, the axial resolution of conventional cSLO is poor. The key to improving the functionality of cSLO is the use of fluorescent markers. So, several research groups have used this combination to detect apoptotic RGCs in vivo using endogenous or exogenous markers and to be able to longitudinally monitor rodent RGCs in vivo [12][13][14][15][2][4]. In addition, the possibility of using various types of filters and laser wavelengths would allow double or multiple label detections. Limitations on its use only include pupil diameter and ocular opacities. However, the future of cSLO lies in adaptive optics (AO), in the ability to alter the scan amplitude speed, and in modifying the size of the pinhole.

One of these technical improvements in the ophthalmological field has been achieved through adaptive optics, since it allowed reducing optical aberrations [16]. Intrinsic optical aberrations were detected by analyzing the wavefront of light coming from the eye, and were corrected by electro-actuated deformable mirrors [17]. The main technical advantages of AO are improved lateral and axial resolution of retinal images, detection of smaller dots, and improved sensitivity to weak reflections. In combination with cSLO and OCT, it can improve fine detail resolution in vivo [16]. The association of AO with OCT increases the lateral resolution by five times compared to standard OCT, allowing direct visualization of individual cells without the need for an exogenous marker. This resolution allows individual nerve fiber bundles to be observed in humans in vivo [18][19]. Association with cSLO allows high-resolution in vivo visualization of fluorescently labeled rodent capillaries [20]; direct observation of cone-type photoreceptors through their intrinsic reflectance [21], and better resolution to analyze RNFL. The AO-SLO association was more accurate than the OCT [22]. All the above indicates that the future of cSLO lies in AO development. Although, in parallel, research is being carried out on small-aperture fast-scanning cSLO [23]. On the other hand, fluorescent labelers have an important role in further improving this technique.

2. Apoptosis Detection Techniques

The Detection of Retinal Apoptotic Cells (DARC) is a recent methodology that, through real-time non-invasive imaging technique applied to the detection of apoptotic RGC cells in vivo, has the potential to identify diseases in their early stages [12]. It consists of the injection of intravenous annexin-5 marked with fluorescence (ANX776). Annexin 5 has a high calcium-dependent affinity for negatively charged phosphatidylserine [24]. It has been observed that, during the early phase of apoptosis, phosphatildylserine is externalized in the outer membrane of neurons. [25]. Binding of phosphatidylserine to annexin 5 in the plasma membranes of apoptotic cells is detected by cSLO retinal examination. This examination allows counting the number of fluorescent spots representing each simple apoptotic RGC bound to annexin-5 to be calculated. During the cellular stress of early apoptosis, phosphatidyserine, normally intracellular, translocate to the outer plasma membrane, and is exposed to the outside of the cell, signaling it to be removed by phagocytic cells [26]. As this is one of the initial steps of the apoptotic cascade, it constitutes a much earlier marker than others, such as DNA fragmentation detected by the terminal labeling of deoxynucleotidyl transferase dUTP Nick (TUNEL) [12][27][17][19].

Detection of annexin-5 labeling by confocal scanning ophthalmoscopy (cSLO) focused on the RGC layer [28] allows for obtaining high-contrast fluorescent images that span between 35° and 55° of the retinal field. On the other hand, the ability of annexin 5 to cross the blood-brain barrier [29] allows the assessment of diseases in other areas of the nervous system, in addition to the retina.

This technique has proven useful in the investigation of neuroprotective therapeutic agents in glaucoma models, as well as in the relationship between glaucoma and Alzheimer's disease [12][30][31] or the correlation between the number of apoptotic cells and axonal loss in RGC [32]. It has also served to establish the relationship between increased intraocular pressure and RGC apoptosis [30], and to verify in an intraocular hypertension model the reduction of apoptosis in vivo by the use of coenzyme Q10 [33]. Likewise, in the retina of diabetic mice, an increase in DARC counts was observed before vascular changes were perceived in the eye [34]; in a model of blue light exposure in rats this technique served to determine photoreceptors loss [35]; it made possible to verify the neuroprotective effect of brimonidine in glaucoma and the prevention of the formation of amyloid plaques [36], especially useful for early visualization of Alzheimer's disease; it was used to determine the protective effect of modulating glutamatergic excitotoxicity [37]; or to demonstrate the therapeutic capacity of 2-Cl-IB-MECA to reduce apoptosis in vivo after partial transection of the optic nerve [38]; it was essential to detect the regeneration of injured axons after placing Schwann cells on the damaged optic nerve sheath [39]; in the same model, topical recombinant human nerve growth factor (rh-NGF) was found to be able to decrease apoptosis [40] by using this technique.

Especially interesting is the fact that this technique allows, through the retina, to observe the evolution of other diseases whose main symptoms affect other systems. Thus, the analysis of amyloid plaques in the retina and their relationship with apoptosis have shown a dose- and time-dependent relationship, so that, by preventing their formation or increasing their elimination, survival was increased [41]. In general, a close relationship has been observed between the retina and diseases in other parts of the nervous system, such as Alzheimer's [42][43] or Parkinson's diseases [44]. Therefore, the study of the retina seems to be a good way to control the evolution of these other diseases of the nervous system.

3. Caspase Activation Detection

Caspases are essential endoproteases in apoptotic and inflammatory processes. Caspase activation occurs through extrinsic or intrinsic signals. The extrinsic pathway is triggered by ligands that bind to extracellular death receptors, while the intrinsic pathway responds to intracellular stress signals such as hypoxia, DNA damage, reactive oxygen species, accumulation of misfolded proteins, and mitochondrial damage. Regardless of the trigger, the cascade begins with the activation of "starter" caspases capable of cleaving and activating "executer" caspases, which cleave DNA leading to cell death. Detection of these caspases is a reliable indicator of apoptosis. On the other hand, some data suggest that different types of neurons use different death messages. Thus, in the retina, caspase-1 plays an important role in photoreceptor death, while caspase-3 is important in the inner nuclear layer, and caspase-2 is the main caspase involved in neuron death in the retinal ganglion cell layer [45].

Another option to detect caspase activity is the use of apoptotic probes (CapQ) activated by caspases. These penetrate cells and mark those that are in apoptosis [27]. This technology consists of a cell-penetrating peptide conjugated to an effector caspase recognition sequence, joint to a pair of fluorophores. This set is activated by effector caspases in apoptotic cells, and its fluorescence is detected by cSLO. After intravitreal injection of the TcapQ488 probe, RGCs showing apoptosis in vivo were detected using cSLO in mouse retinal degeneration models [46]. However, this probe had minimal toxicity capable of activating the probe, even in the eyes of wild-type rodents, and therefore causing apoptosis.

The use of caspase inhibitors with fluorescence (FLIVO, fluorescence in vivo) allows the visualization of apoptosis in vivo and in vitro. These tracers injected into the circulation selectively accumulate in apoptotic cells. Being able to cross the blood-brain barrier, they can be used in the study of brain and eye neurodegenerative diseases, selectively targeting cells that undergo caspase-dependent apoptosis [47]. These methodologies have been used in animal models (in vitro and in vivo) and in the clinic to monitor the activity of Diabetic Retinopathy [48], glaucoma [49], retinitis pigmentosa [50], blue light-induced retinal damage, and AMD [51].

Luciferins are bioluminescent molecules that when activated by luciferases release energy by emitting light. Z-DEVD-aminoluciferin is a luciferin modified to be activated by specific caspases, which allows it to detect the activity of these caspases in vitro [52]. In this way, it serves as a marker of apoptosis. Apoptosis has also been detected in vivo in mouse models of tumor xenografts [53].

4. Detection of Changes in the Apoptotic Membrane

Certain imaging techniques to detect apoptotic cells also use low molecular weight (300 to 700 Da) amphipathic molecules that selectively cross the apoptotic plasma membrane and accumulate in its cytoplasm as the Aposense family of compounds [54][55]. The anchoring is made to the hydrophobic (lipid) region of the cell membrane, passing into the cell interior, unlike what happens in non-apoptotic cells, in which the hydrophilic region blocks their entry into the cytoplasm. Examples of these compounds are those containing the dansyl group, like N,N′-didansyl-L-cystine, NST-732, and NST-729; or those containing an alkyl-malonate molecule, such as ML-9 and ML-10 [54].In apoptosis, the accumulation of these molecules in the cytoplasm, exposure to phosphatidylserine, activation of caspases, and loss of mitochondrial membrane potentials have been observed. These compounds may be intrinsically fluorescent or may be labeled with a radioactive moiety. These molecules have demonstrated their usefulness in the field of experimentation in models of Alzheimer's disease, amyotrophic lateral sclerosis [56], melanomas [57], chemotherapy-induced enteropathy [58], and models of reperfusion-induced damage [55]. In clinical practice, a radiolabeled version of ML-10 has been used to monitor the response of brain metastases to radiotherapy [59]. Although these molecules can cross the blood-brain barrier, and therefore could be used in neurodegenerative conditions such as Alzheimer's, Parkinson's, and glaucoma, their toxicity could be a problem.

Glaucoma ocular biomarkers are endogenous biochemical, physiological, and anatomical indicators associated with specific pathological states [60][61]. They provide an objective measure to detect the disease early and monitor therapeutic efficacy. The optimal biomarker must be specific, sensitive, and reproducible, as well as inexpensive and non-invasive. A glaucoma biomarker should indicate the rate of RGC loss and the number of remaining or apoptotic RGCs with high sensitivity. Recent advances in fluorescent technology have improved the ability to identify individual RGCs undergoing apoptosis. These specific markings will be valuable both in experimental models and in the clinic.

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