Loss of cholinergic amacrine cells in an ischemia-reperfusion animal model

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Purpose: Ocular ischemic injuries, like ocular vein occlusion, are huge risk factors for damage of retinal neurons and often lead to loss of vision. A lot of different animal models are used to study the effects of ischemia-reperfusion (I/R), often only investigating one or two different cell types. Currently, little is known about the global effect of ischemia on different retinal neurons. Therefore, we investigated functional and morphological changes of different cell types of the retina after I/R.

Methods: I/R was induced by raising the intraocular pressure in one eye of rats to 140 mmHg for 1h (N=5). 21 days after ischemia, scotopic flash electroretinogram (ERG) measurements were made using intensities from 0.1-25 cd*s/m^2. H&E staining was performed to measure the retinal layer thickness. Changes of RGC, amacrine-, rod bipolar-, glia as well as apoptotic cells were analyzed using immunohistochemistry.

Results:

- The known initial apoptosis peaking at 48 hrs after ischemic damage is presumably followed by a degenerative process like a second wave of apoptosis later on, causing progressive damage in the retina.
- A significant loss of ChAT^+ cells was noted in I/R retinas (p<0.001). GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer. Scale bar: 20 µm.

Conclusions:

The study allowed a comparison of effects on different retinal cell types at an advanced disease state. RGC and amacrine cells were primarily affected by ischemia, while rod bipolar cells and photoreceptors were more resistant.

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Ischemia-reperfusion leads to a loss of retinal functionality

A: Experimental setup: 1 ground-, 2 reference-, 3 recording electrode. B: Representative ERG recording at 3 cd*s/m^2 of a control eye (grey) and a contralateral I/R eye (black). The arrow represents the start of the light stimulus. C: Changes in the amplitude of the a-wave of ischemic eyes at increasing light intensities. D: Alterations of the b-wave amplitude of control and ischemic eyes at different light intensities. E: A-wave latencies of control and I/R. F: Response latency in the b-wave in control and I/R.

Changes in the morphology of the inner retina

A: H&E staining 21 days after I/R. B: The thickness of the GCL and IPL of the ischemic eyes was significantly reduced (GCL: p=0.0022; IPL: p<0.001). No differences were observed in the INL, OPL, ONL, and the overall thickness of the retina. GCL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer. Scale bar: 20 µm.

Loss of retinal ganglion cells after ischemia-reperfusion

A: Bm-3a stained retinal ganglion cells (RGC, green) and cell nuclei (DAPI, blue) at 21 days. B: RGC numbers were significantly reduced in the ischemic eyes (p=0.0016). GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer. Scale bar: 20 µm.

Increased apoptosis in the inner retina

A: Double-staining with TRADD (green) and TNF-R1 (red). B: Significantly less co-localization between TRADD and TNF-R1 was visible (p=0.0012). C: Fasl^+ cells were predominantly detected in the INL (arrows). D: Fasl was significantly increased in ischemic retinas (p=0.001). GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer. Scale bars: 20 µm.

Rod bipolar cells unaffected

A: Staining of rod bipolar cells with PKCα.
B: No difference was observed between the two groups (p=0.9).

Increase of Müller cell and astrocyte activity

A: Macroglia (GFAP) staining in both groups. B: Müller glia stain with vimentin. C: The vimentin stained Müller cells and GFAP labeled showed more co-localisation in I/R retinas (asterix).

Loss of cholinergic amacrine cells

A: CHAT staining (red) in control and ischemic eyes. B: A significant loss of CHAT^+ cells was noted in I/R retinas (p<0.001). GCL: ganglion cell layer, INL: inner nuclear layer, ONL: outer nuclear layer. Scale bar: 20 µm.