Elevated hydrostatic pressure increases the sensitivity of optic nerve head astrocytes to an oxidative challenge

Vidhya R. Rao, 1, 2, 3 Alexandra D. Hegel, 1, 2, 3 Jonathan Lautz, 1, 4 Jamie C. Floss, 1 Vicki Husak, 1 Evan B. Stubbs Jr. 1, 3 Simon Kaja 1, 2, 3

1 Department of Ophthalmology, 2 Department of Molecular Pharmacology & Therapeutics, 3 Graduate Program in Neuroscience, Loyola University Chicago, Maywood, IL, U.S.A. 4 Research Service, Edward Hines Jr. Veterans Administration Hospital, Hines, IL, U.S.A.

Corresponding author: Simon Kaja, Ph.D. (skaja@luc.edu)

Abstract

Primary open angle glaucoma (POAG) is often associated with elevated intraocular pressure (IOP), manifesting in a pathological triad of optic nerve head remodelling, damage to the optic nerve, and retinal ganglion cell loss.

Optic nerve head astrocytes (ONHAs), the primary cell type in the optic nerve head, undergo significant pathological changes in POAG.

Increased levels of oxidative stress, secondary to elevated IOP, are strongly implicated in the pathophysiology of POAG.

Here, the cellular and molecular consequences of elevated hydrostatic pressure on cultured ONHAs responses to an exogenous oxidative challenge were investigated.

Materials and Methods

Cell Culture: Primary adult rat optic nerve head astrocyte (ONHA) culture was prepared and maintained as described by us previously (Kaja et al., Exp. Neurol. 2015; 256: 59-68 and Kaja et al., Exp. Eye Res. 2015; 138: 159-166).

Exposure to elevated hydrostatic pressure: ONHA culture was exposed to control ambient pressure (AP) or elevated hydrostatic pressure (25-30 mm Hg above ambient pressure) using a custom-built cell culture pressure chamber.

Exposure to oxidative stress: ONHAs were subsequently challenged with chemically-induced oxidative stress using tert-butyldihydroperoxide (tBHP; 0-500 µM for 5h). For proof-of-concept experiments, some ONHAs cultures were pre-treated with the prototypic antioxidant Trolox (100 µM) dissolved in ethanol.

Cell viability assays and detection of Reactive Oxygen Species (ROS): Cell viability was measured using MTT and LDH assays; levels of oxidative stress were quantified using the fluorescent indicator dye, CellROX®, or by using the cell-permeable 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA), which is converted to the fluorescent 2',7'-dichlorofluorescin (DCF) by endogenous esterases and oxidation (Kaja et al., Exp. Eye Res. 2015; 138: 59-66).

Quantitative PCR and immunoblotting: qPCR was performed using standard Taqman® chemistry in an AriaMX Realtime PCR System machine (Agilent Genomics). Immunoblotting for NOS2 was performed using a validated antibody to NOS2 (sc-650; 1,500 dilution) using standard protocols and Luminata Forte HRP substrate (Millipore).

Data analysis: Data was graphed and analyzed in Prism 6.0 (GraphPad Software, Inc.) and is presented as mean ± s.e.m or S.D., as indicated.

Results

1. Exposure to elevated hydrostatic pressure sensitizes primary ONHAs to subsequent oxidative insults

In a subsequent series of experiments, we exposed cells to the prototypic antioxidant, Trolox (100 µM in 100% ethanol), or vehicle (100% ethanol), for 1 h prior to and during the exposure to oxidative stress. Trolox shifted the EC50 equally in ambient and elevated hydrostatic pressure-treated groups (see Table 1).

2. The prototypic antioxidant, Trolox, can prevent hydrostatic pressure-induced sensitization to subsequent oxidative stress insult

Table 1: Parameters from non-linear fit of MTT assay. Data is presented as mean ± s.e.m.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 (µM)</th>
<th>Vehicle</th>
<th>EHP</th>
<th>Trolox</th>
<th>EHP + Trolox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (plates)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>n (%)</td>
<td>0.946</td>
<td>0.980</td>
<td>0.954</td>
<td>0.975</td>
<td></td>
</tr>
</tbody>
</table>

3. Exposure to hydrostatic pressure generates elevated levels of oxidative stress in ONHAs

Exposure to elevated hydrostatic pressure (30 mm Hg for 16 h) did not alter cell viability. Absolute LDH release was unaltered (n = 6; P = 0.651). However, subsequent exposure to chemically-induced oxidative stress revealed significantly increased LDH release and reduced conversion of MTT, indicative of reduced cell viability.

4. Hydrostatic pressure increases nitric oxide synthase 2 expression in ONHAs

Table 1: Parameters from non-linear fit of MTT assay. Data is presented as mean ± s.e.m.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 (µM)</th>
<th>Vehicle</th>
<th>EHP</th>
<th>Trolox</th>
<th>EHP + Trolox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (plates)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>n (%)</td>
<td>0.946</td>
<td>0.980</td>
<td>0.954</td>
<td>0.975</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Parameters from non-linear fit of MTT assay. Data is presented as mean ± s.e.m.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 (µM)</th>
<th>Vehicle</th>
<th>EHP</th>
<th>Trolox</th>
<th>EHP + Trolox</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (plates)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>n (%)</td>
<td>0.946</td>
<td>0.980</td>
<td>0.954</td>
<td>0.975</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

• Our data suggest that even modest exposure to elevated IOP in POAG may significantly alter the oxidative response of ONHAs.

• Our experimental system provides a standardized in vitro model to study the intracellular pathways leading to the generation of hydrostatic pressure-induced increases in ROS and NO.

• In our model is amenable to high-throughput screening approaches for the testing of novel glioprotectants for POAG.

Acknowledgements

Research reported on this poster was supported, in part, by grants from the Illinois Society for the Prevention of Blindness (SK, EBS), the Glaucoma Research Foundation Shaffer Grant (EBS), the Richard A. Pettit Charitable Foundation (SK), EBRP Grant from Loyola University Chicago (SK and the Dr. John L. and Theresa E. Mulloy Endowed Professorship in Ophthalmology (SK). Additional support from the Department of Veterans Affairs Grant (I21RX001593 to EBS) is gratefully acknowledged. VRR is the recipient of a travel grant from the Chicago Association for Research and Education in Science (CARES). This material is the result of work supported with resources and the use of facilities at the Edward Hines Jr. VA Hospital, Hines, IL. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

References


Disclosures
