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

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Abstract

Primary open angle glaucoma (POAG) is often associated with elevated intracellular pressure (IOP), manifesting in a pathological triad of optic nerve head remodeling, 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 pathophysiological 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, 265: 59-680 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 (EHP; 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-butylhydroperoxide (tBHP; 500-5000 nM 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'-dichlorofluorescein (DCF) by endogenous esterases and oxidation (Kaja et al., Exp. Eye Res. 2015; 138: 59-660).

Quantitative PCR and immunoblotting: qPCR was performed using standard TaqMan® chemistry in an Applied Real Time System machine (Agilent Genomics). Immunoblotting for NOS2 was performed using a validated rabbit polyclonal anti-NOS2 antibody (sc-650; 1:500 dilution) and is presented as mean ± s.d. or S.D., as indicated.

Table 1: Parameters from non-linear fit of MTT assays. Data is presented as mean ± s.e.m. and was generated from three separate experiments (n=3), with eight technical replicates each.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC50 (µM)</th>
<th>S.D.</th>
<th>N</th>
<th>p</th>
<th>q</th>
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<tbody>
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<td>EHP</td>
<td>50.0 ± 1.9</td>
<td>99.1 ± 2.2</td>
<td>73.0 ± 2.4</td>
<td>135.2 ± 2.6</td>
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In a separate experiment, we exposed ONHAs to elevated hydrostatic pressure for 2 hours in cells pre-treated with sublethal conditions of tBHP (100 µM) and/or Trolox (100 µM) and quantified ROS usingDCFDA fluorescence. Elevated hydrostatic pressure challenge significantly increased ROS to levels comparable to those of sublethal tBHP exposure. Trolox completely prevented generation of hydrostatic pressure and tBHP-induced ROS. Data is mean ± S.D.

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 hr 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

Figure 1: Effect of elevated hydrostatic pressure on cell viability.

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

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

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Summary

• Short-term exposure of cultured ONHA to elevated hydrostatic pressure does not cause cell viability, but sensitizes to subsequent oxidative challenge.

• Elevated hydrostatic pressure leads to a statistically significant increase in Reactive Oxygen Species, as determined by quantification of CellROX® and DCFDA fluorescence.

• Elevated hydrostatic pressure increases NO2 expression, as described previously.

• Trolox protects ONHAs against elevated hydrostatic pressure and tBHP-induced ROS.

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.

• Our in vitro model is amenable to high-throughput screening approaches for the testing of novel glioprotectants for POAG.

References


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Disclosures

Disclosures VRR- none, ADH- none, JL- none, JCF- none, VH- none, EBS- none.