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ARVO Annual Meeting Abstract | July 2019

Glucose protects cultured retinal cells from oxidative injury via pentose phosphate pathway activation and maintenance of reduced glutathione

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Investigative Ophthalmology & Visual Science July 2019, Vol.60, 1676. doi:

Abstract

Purpose : Oxidative injury has been implicated to play a role in a range of retinal neurodegenerative conditions. Thus, protecting retinal cells in vivo from such an insult is extremely beneficial. We therefore sought to investigate whether glucose, acting via the pentose phosphate pathway (PPP) was able to counteract oxidative stress to retinal cells in culture.

Methods : Mixed retinal neuron-glial cultures were prepared from 2 day old rat pups and used at 7 days in vitro. Neuron-only and primary Muller cell cultures were prepared from mixed cultures, by treatment with cytosine arabinoside to kill dividing cells, or, by regular medium changes for 30 days, respectively. At appropriate stages, cultures were treated with t-butyl hydroperoxide (tbH; 100nM-10mM) in energy substrate-free DMEM to induce oxidative stress. Some cultures were co-treated with glucose (100μM-25mM) or other metabolic substrates (5mM; pyruvate, lactate, glutamine, fructose-1,6-bisphosphate). Glycolysis was inhibited with iodoacetate (IOA; 10μM) or 2-deoxy-D-glucose (2-DG; 1mM). PPP was inhibited with 6-aminonicotinamide (6NA; 500μM) and glutathione biosynthesis with buthionine sulphoxamine (BSO; 100μM). Cell viability, immunocytochemistry and Western blot were employed to assess cellular outcomes after treatments. An antioxidant assay identified which, if any, of the metabolic substrates tested had intrinsic antioxidant properties.

Results : Oxidative stress resulted in loss of viability to mixed retinal cells and primary Muller cells: the EC_{50} for tbH was approximately 35μM in each case. Glucose dose-dependently reduced the toxicity of tbH with a maximal effect at 5mM (EC_{50} of tbH elevated to approximately 250μM). Pyruvate was also partially protective, but had intrinsic antioxidant properties. Glycolytic blockade had no effect on the protective effect of glucose but both 6NA and BSO inhibited the protective response. When cultured alone, neurons were equally susceptible to tbH-toxicity but could not be protected by glucose.

Conclusions : Glucose prevented oxidative stress to retinal cells via the PPP and the consequent generation of reduced glutathione. Neurons were not subjected to glucose-induced protection except when glial cells were present, implying the passage of a transmissible protective factor between the two cell types.

This abstract was presented at the 2019 ARVO Annual Meeting, held in Vancouver, Canada, April 28 - May 2, 2019.