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BRAIN RESEARCH

Research Report

Orally administered epigallocatechin gallate attenuates retinal neuronal death *in vivo* and light-induced apoptosis *in vitro*

Bo Zhang[a], *Dario Rusciano*[b], *Neville N. Osborne*[a,*]

[a]*Nuffield Laboratory of Ophthalmology, Walton Street, Oxford OX2 6AW, UK*
[b]*SIFI, Lavinaio-Aci S. Antonio, Catania, Italy*

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ABSTRACT

The aim of this study was to provide support for epigallocatechin gallate (EGCG), a component of green tea, to be considered in the context for neuroprotection in glaucoma, where administration by an oral route is required for adequate penetration into the retina. Ischemia was delivered to one eye of a number of rats by raising the intraocular pressure. EGCG was present in the drinking water of half of the animals 3 days before ischemia and also during the next 5 days of reperfusion. The electroretinograms (ERGs) of both eyes from all rats were recorded before ischemia and 5 days following ischemia. Seven days after ischemia retinas from both eyes of all rats were either analysed for the localisation of various antigens or extracts prepared for analysis for the level of specific proteins and mRNAs. Ischemia/reperfusion to the retina affected a number of parameters. These included the localisation of Thy-1 and choline acetyltransferase, the a- and b-wave amplitudes of the ERG, the content of certain retinal and optic nerve proteins and various mRNAs. Significantly, EGCG statistically blunted many of the effects induced by ischemia/reperfusion which included the activation of caspases. These studies demonstrate conclusively that orally administered EGCG attenuates injury to the retina caused by ischemia/reperfusion where caspases were activated. Studies were also conducted on a cell line (RGC-5 cells) where it was shown that white light (1000 lx, 48 h)-induced apoptosis is caspase-independent and can be blunted by EGCG. The present studies support the view for the use of EGCG in the treatment of glaucoma based on the premise that any potential neuroprotective agent must be administered orally, have a safe profile and poses a broad spectrum of properties that allows various risk factors (that include ischemia and light) to be attenuated.

1. Introduction

The unmyelinated retinal ganglion cell axons within the globe are richly provided with many mitochondria (Wang et al., 2003) to produce the high energy requirement for nerve conduction. These organelles are probably affected in the initiation of glaucoma due to an alteration in the blood flow dynamics in the optic nerve head region (Harris et al., 2005; Osborne et al., 2001). We have suggested that as a consequence of this initial insult ganglion cells exist in a compromised energetic state and then are susceptible to secondary insults that they would otherwise tolerate (Osborne et al., 2001). One secondary insult might be light impinging upon the many mitochondria in the ganglion cell axons (Osborne et al., 2006; Lascaratos et al., 2007). Other secondary insults might arise from chemicals in the extracellular environment caused by glial cell malfunction (Osborne et al.,

* *Corresponding author.*
E-mail address: neville.osborne@eye.ox.ac.uk (N.N. Osborne).

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2005; Tezel, 2006). Substances that need consideration in this respect include TNF-α (Tezel et al., 2001), glutamate (Osborne et al., 2001) and nitric oxide (Neufeld, 2004). These secondary insults ultimately trigger ganglion cell apoptosis (Osborne et al., 2001, 2006). Agents that are therefore targeted at nullifying the effects of secondary insults to energetically compromised ganglion cells might therefore slow-down their apoptosis in glaucoma.

Mitochondria provide the bulk of a neurone's energy since oxidation of reducing equivalents (e.g. NADH and $FADH_2$), via the electron transport chain, ultimately yields ATP. In addition to governing aerobic metabolism, mitochondria contribute to cytosolic calcium buffering (Steeghs et al., 1997), apoptosis (Green and Amarante-Mendes, 1998), excitotoxicity (Osborne et al., 2004) and generation of superoxide from iron–sulphur centres of complexes I and II and semiubiquinone and cytochrome *b* of complex III (McLennan and Degli Esposti, 2000). Also, mitochondrial flavin and cytochrome oxidases are affected by light impinging on the retina *in situ* (Godley et al., 2005; Chen et al., 1992; Lascaratos et al., 2007), and this potentially could stimulate a production of reactive oxygen species. Thus an alteration in any or a combination of the aforementioned processes might be the cause for retinal ganglion cells dying in glaucoma.

Present evidence therefore suggests that retinal ganglion cell death in glaucoma is related to mitochondrial failure. Pharmacological strategies that would directly enhance the metabolic state of ganglion cell mitochondria are likely therefore to sustain the survival of retinal ganglion cells in glaucoma. This would necessitate the use of an orally administered prophylactic agent that can reach the retina to positively influence the metabolic state of retinal ganglion cells without having a negative affect on healthy cells. One substance proposed to fulfill such characteristics is epigallocatechin gallate (EGCG), a flavonoid present in a variety of plants with green tea containing a particularly rich concentration. Various studies have shown that when EGCG is orally consumed it reaches all tissues, that include the central nervous system, and has a half-life of around 8.6 h (Swezey et al., 2003). Moreover, numerous beneficial effects of EGCG have been demonstrated in laboratory studies with no evidence for it being toxic even when consumed in appreciable amounts (Isbrucker et al., 2006a,b). The beneficial effects of EGCG are both experimentally and clinical evident on multiple targets (Zaweri, 2006) and especially important in cancer chemoprevention (Khan et al., 2006) and neuroprotection (Mandel et al., 2005).

EGCG appears in many respects to be an ideal candidate drug for use in glaucoma. The vasodilatory and anti-thrombotic properties should reduce the effects of the initial insult in glaucoma which is thought to originate from a compromised ocular blood flow in the optic nerve head (Osborne et al., 2006; Flammer et al., 2002; Pache and Flammer, 2006). Also its powerful antioxidant influence will enhance mitochondrial function and counteract the secondary insults that trigger the onset of ganglion cell apoptosis. The purpose of this study was therefore to provide substance to this supposition by orally administering EGCG to rats and determine whether this will result in attenuating the effect of retinal ischemia. In our previous study we showed that when EGCG is administered by a systemic route it was effective at counteracting retinal ischemia (Zhang et al., 2007) but felt it necessary to see whether orally administered EGCG when placed in the drinking water of rats at a realistic concentration reached sufficient quantities in the retina to be effective at counteracting ischemia. Moreover, we investigated whether the flavonoid attenuates light-induced apoptosis to ganglion cells in culture as we believe that in glaucoma light is one of the secondary insults that initiate the process of apoptosis (Osborne et al., 2006).

2. Results

2.1. *In vitro studies*

Exposure of RGC-5 cell cultures to 1000 lx light for 48 h caused a 23% decrease in cell viability (by the MTT assay, Fig.1A) and a 20% reduction in mitochondrial dehydrogenase activity (by the

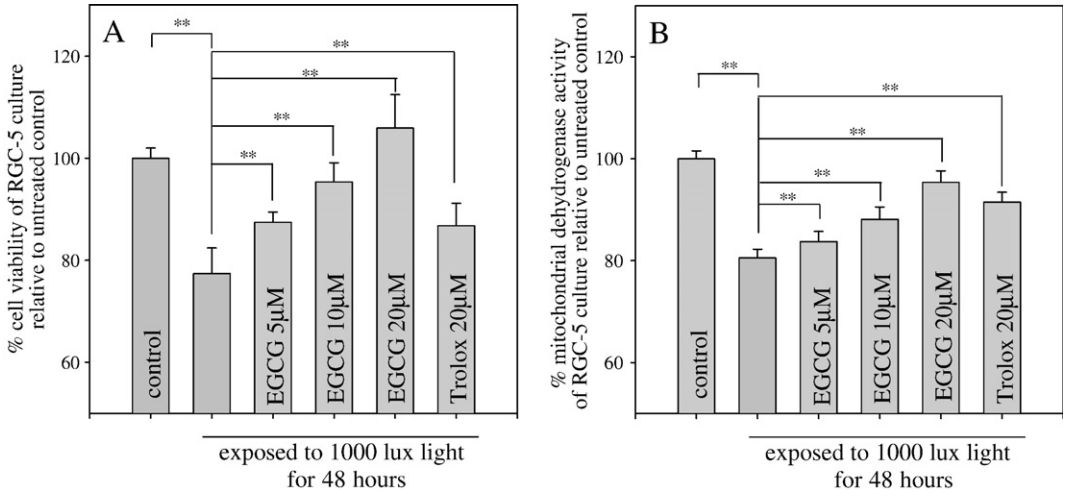

Fig. 1 – This figure shows that the negative effect of light exposure (1000 lx over a period of 48 h) on RGC-5 cells in culture can be attenuated by the presence of EGCG, dose-dependently (5–20 μM) and also trolox (20 μM), determined by the MTT viability assay (A) or for mitochondrial dehydrogenase activity (B). Light on its own decreases cell viability and mitochondrial dehydrogenase activity by approximately 23% and 20% respectively, relative to cells maintained in the dark (100%). Results are mean values ± S.E.M. where $n = 6$. $^{**}p < 0.01$ by one-way ANOVA followed by Tukey multi comparison test.