Role of Resveratrol in Neuroprotection Against Glutamate-Induced Cytotoxicity in Retinal Precursor Cells

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INTRODUCTION

Retinal ischemia is a common cause of visual impairment and blindness among individuals over 50 years of age in the United States. Ischemic insult to the human retina is frequently observed in open-angle glaucoma, diabetic retinopathies, and hypertensive retinopathies. Current therapies for retinal ischemic disease are not satisfactory and have little impact on preventing or slowing the molecular process that leads to retinal ganglion cell death at the time of ischemia.

The mechanism of cell death induced by retinal ischemia is not completely understood. It is known that ischemic retinal injury leads to energy dependent dysfunction, tissue edema, and eventual retinal ganglion cell death (1). Ischemia-induced neuronal injury is associated with enhanced production of endogenous substances such as glutamate, oxygen free radicals, nitric oxide (NO) and calcium (2, 3, 4). Interestingly, glutamate in particular acts as a normal neurotransmitter in the retina, but at high levels is neurotoxic *in vitro* and *in vivo,* resulting in apoptosis of retinal ganglion cells. The major causes of cell death from glutamate are the influx of calcium into cells and the generation of free radicals (5).

Resveratrol (trans-3, 5, 4'-trihydroxystilbene) is a polyphenol which is present at high levels in the skin and seeds of grapes, nuts, and pomegranates. It also constitutes one of the major components of red wine. Resveratrol has been reported to have anti-inflammatory and anti-aging, antioxidant, and anti-tumor activities as well as important protective effects in the nervous system. In a recent study, resveratrol was found to protect the spinal cord, kidneys, and heart from ischemia-reperfusion injury through upregulation of NO (6).

The role of resveratrol on retinal neurons during or after ischemia is unknown. In this study, we aimed to determine whether resveratrol has protective effects on retinal cells during simulated acute retinal ischemia *in vitro*.

METHODS

The R28 cell line is a rat-derived adherent retinal precursor cell line derived from postnatal day 6 Sprague-Dawley rat retinas immortalized with the 12S E1A gene of adenovirus. These cells, when grown with laminin and cAMP have a neuronlike phenotype. R28 cells have been used in numerous studies of retinal neuronal apoptosis. The methodology of this study was as follows:

1. The R28 cells with neuronal morphology were seeded in 96-well plates at 10,000 cells per well and allowed to culture.
2. Twenty four hours later, the cells were treated with fresh media containing either glutamate (3, 4, 5, or 6 mM) or glutamate (6 mM) plus resveratrol (1 or 2 µg/ml). A group of cells that received only fresh media served as the control group.
3. Another 24 hours later, cell viability was determined via spectrophotometric measurements using the neutral red dye uptake assay*. The cells were also photographed for morphologic changes.

*The Neutral Red Dye Uptake (NRU) Assay procedure is a cell survival/viability assay based on the ability of viable cells to incorporate and bind neutral red (NR), a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and predominantly accumulates intracellularly in lysosomes. Alterations of the cell surface or the lysosomal membrane caused by toxic substances lead to lysosomal fragility and other changes that gradually become irreversible. This leads to decreased uptake and binding of NR, making it possible to detect specifically viable cells via spectrophotometric measurements. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of NR after chemical exposure.

RESULTS

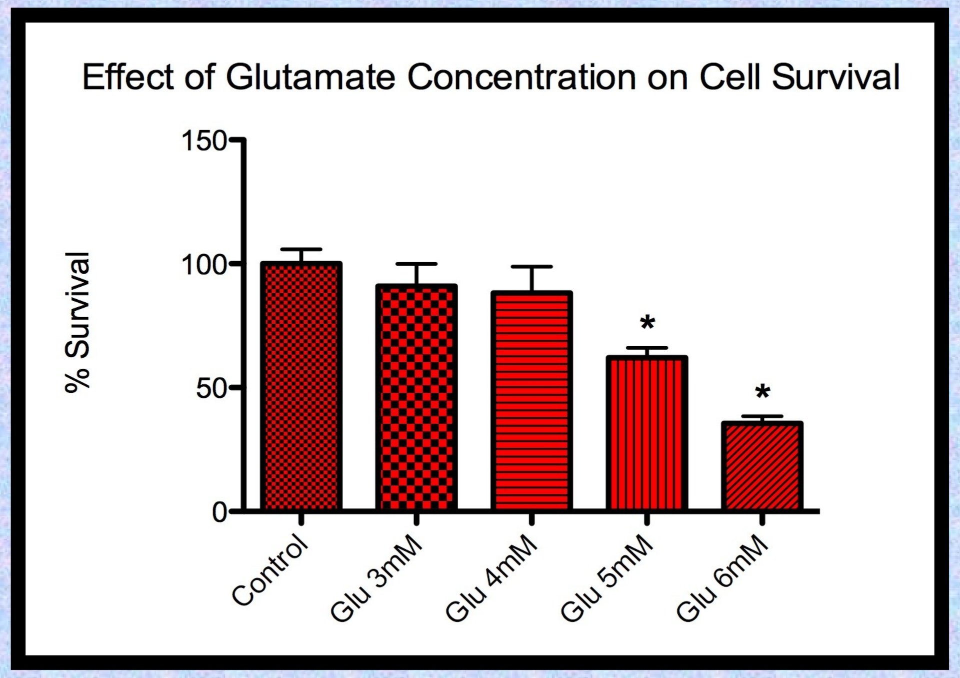


Figure 1. Effect of glutamate concentration on cell survival. Results of neutral red dye uptake assay on R28 cells (10,000 cells per well) treated with increasing doses of glutamate. Statistically significant changes in cell survival were observed at glutamate concentrations of 5mM and 6mM. * $p<0.001$, Student's *t-test* (n = 6 per group) comparing survival data between control and each glutamate treatment group.

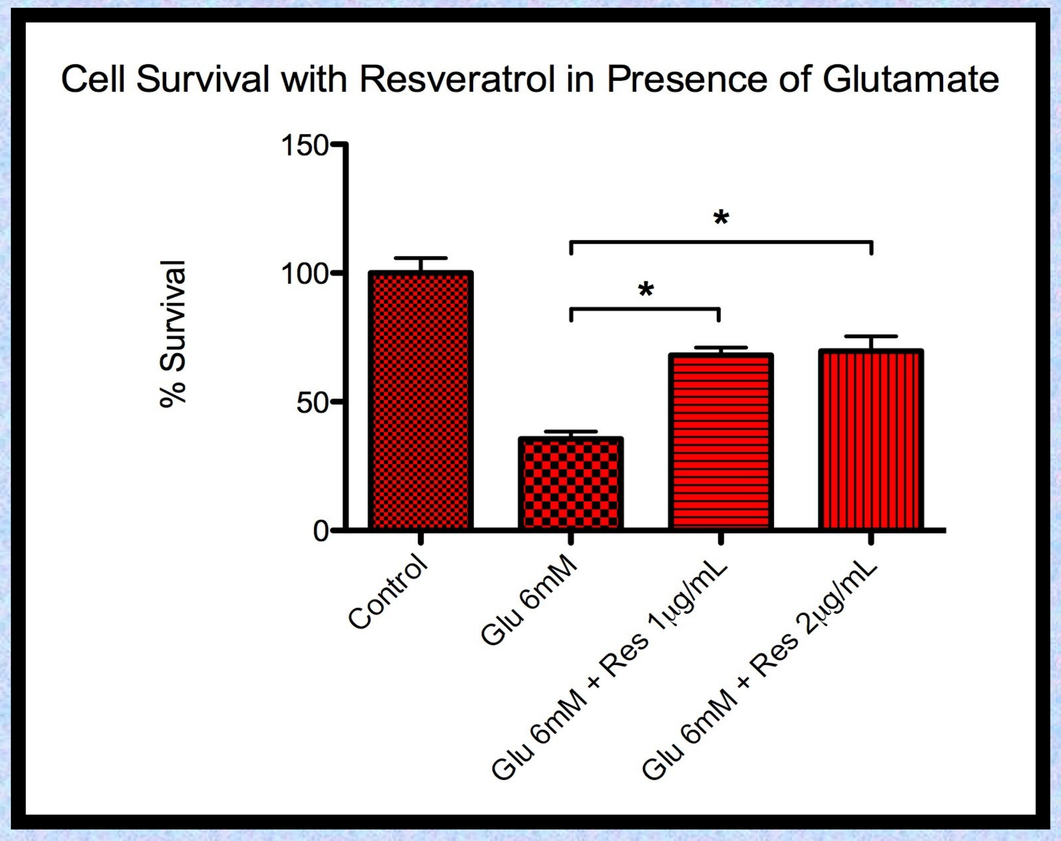
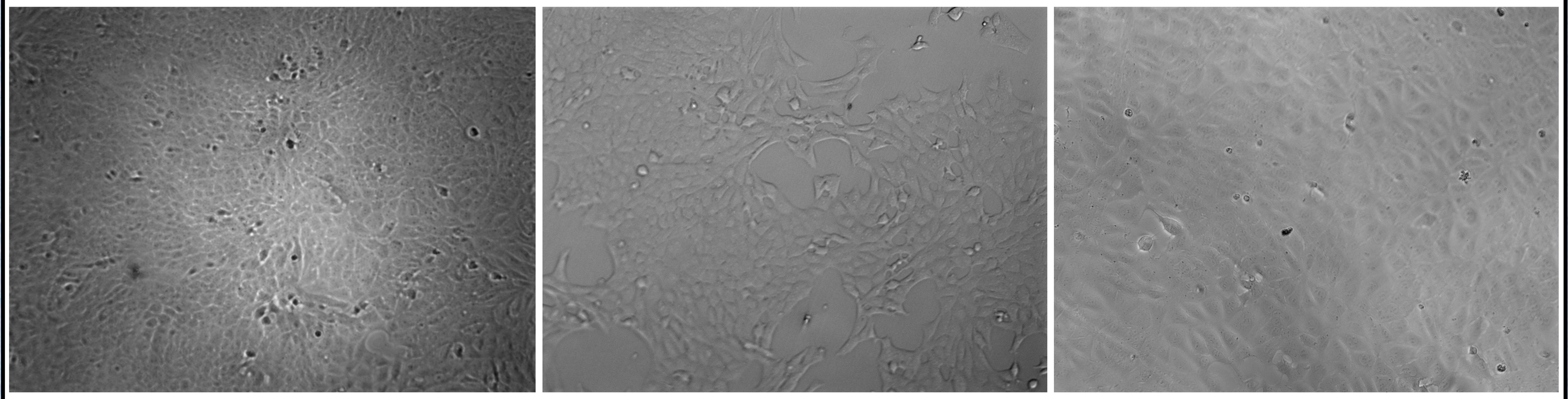


Figure 2. Cell survival with resveratrol in the presence of glutamate. Results of neutral red dye uptake assay on R28 cells (10,000 cells per well) treated with 6mM glutamate and either 1µg/mL or 2µg/mL resveratrol. * $p < 0.001$ Student's *t-test* (n = 6 per group) comparing cells + glutamate only data to cells + glutamate + resveratrol data.

Resveratrol at concentrations of 1µg/mL or 2µg/mL has a statistically significant protective effect on survival of R28 cells (at 10,000 cells per well) treated with 6mM glutamate.



Control Cells | **Control + Glutamate** | **Control + Glutamate + Resveratrol**

Figure 3. Effect of glutamate and resveratrol on the morphology of R28 cells. Shown are representative photographs of R28 cells (20,000 cells per well) treated with glutamate 2.5mM or glutamate 2.5mM + resveratrol 5µg/mL.

DISCUSSION

The major finding of this preliminary study is that resveratrol has a statistically significant protective effect against glutamate-induced cytotoxicity.

Recent studies have shown that resveratrol protects tissues of the heart, kidney, and brain from ischemia / reperfusion injury (7,8,9). Resveratrol significantly protected rat brain tissue against focal cerebral ischemia (10). A recent study also showed that resveratrol has a preventative effect in antibody-induced apoptotic death of retinal cells (11).

There is much left to discover in exploring the use of resveratrol in ischemic retinal disease. The exact mechanism of action of resveratrol such as observed in this study remains unknown. Studies have suggested that a mechanism along the nitric oxide pathway may play a role (6). Furthermore, *in vivo* role of resveratrol on retinal neurons after ischemia is unknown. These studies are forthcoming.

These preliminary findings raise awareness for the potential of resveratrol to protect against ischemic retinal disease-related retinal ganglion cell death.

CONCLUSIONS

Resveratrol has a statistically significant protective effect against glutamate-induced cytotoxicity. These preliminary findings suggest that resveratrol may have neuroprotective effects in this experimental model of ischemic retinal disease.

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