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Research Article

Reduction of Neuro Degeneration in Glaucoma by Transplanted Stems Cells Precursors

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ABSTRACT

Introduction: Glaucoma is a common neurodegenerative disease for which current therapies are often insufficient. The purpose of our investigation was to determine whether Oligodendrocyte Precursor Cells (OPCs), a type of neural stem cell, can protect Retinal Ganglion Cells (RGCs) from glaucomatous damage in vivo.

Methods: Intraocular pressure was chronically increased by trabecular laser treatment delivered unilaterally to adult rat eyes. OPCs were isolated ***in vitro*** and then transplanted intra vitreally either before, or concurrent with, injury induction.

Results: Transplanted OPCs were found to survive within the eye for at least 12 weeks and to localize close to the RGCs. Moreover, OPCs significantly enhanced the survival of RGCs in the glaucomatous eye, but only when concomitantly activated by inflammation. Amelioration of RGC death was not attributable to inflammation but relied on an interaction between inflammatory cells and OPCs. Engrafted cells also displayed multipotentiality in vivo.

Keywords: Glaucoma, Neuroprotective, Oligodendrocyte Precursor Cells

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INTRODUCTION

Glaucoma is still one of the leading causes of blindness worldwide. In England and Wales glaucoma is a major or contributing factor in 12-14 % of all registrations for blindness and partial sight, only to macular degeneration (Bunce C, *et al.*, 2010). The worldwide burden is greater with glaucoma is the second leading cause of global blindness after cataract (Resnikoff S, *et al.*, 2004). It has been estimated that almost 60.5 million people worldwide affected by glaucoma in 2010, with the figure expected to rise to 80 million by 2020 (Quigley HA and Broman AT, 2006).

A neurodegenerative disease known as glaucoma, which is caused by the gradual occurring death of Retinal Ganglion Cells (rGCS). The pathophysiological changes can share with other neurodegenerative diseases, including axonal transport dysfunction (Martin KR, *et al.*, 2006), oxidative stress (Kumar DM and Agarwal N, 2007) increased the level of Intraocular Pressure (IOP) is the main risk factor (Vass C, *et al.*, 2007) and depression in the level of IOP is the only cure for this particular disease (Vass C, *et al.*, 2007). Whereas decreased levels of IOP can not suppress RGC degeneration in some patients with glaucoma, so there may be a new method of treatment to protect RGC. Stem cell technology is a newer tool in the treatment of neuroprotective treatment in the degeneration of CNS disease, then the increases neurodegeneration in the supply of neurotrophic factors. There is abundance Oligodendrocyte Precursor Cells (OPCs) in the Central Nervous System (CNS) of adult individuals, where they are the most widely used type of proliferative cell.

OPCs hold great responsibility for the generation of oligo dendrocytes in the developmental stage, whereas in adult individuals, they play a vital role in demyelinating pathologies and remyelinating of axons. OPCs appear to contain the majority of the stem cell characteristics and has been shown to be neuroprotective *in vitro*.

The aim of our study was to determine whether Oligodendrocyte Precursor Cells (OPC), a type of neural stem cell can protect Retinal Ganglion Cells (rGCS) from glaucomatous damage in vivo. Current treatments for glaucoma include lowering of intraocular pressure by eye drops, laser procedures or drainage surgery. However, as shown by the statistics above, many patients experience significant vision loss due to degeneration of Retinal Ganglion Cells (rGCS) despite advances in the treatments currently available. The need for new treatment options exist for these patients, especially those with end-stage glaucoma, where the maintenance of a small number of survivors rGCS perhaps even ensure a reasonable quality of life (Much JW, *et al.*, 2008). Stem cell treatment developed in the laboratory and translated into clinical practice provides an exciting and realistic hope for those affected by degenerative retinal diseases.

Embryonic Stem Cells (ESC) occur from the inner cell mass of the blastocyst, which is formed in about five days post-fertilization in humans. Such cells are often derived from excess tissue obtained from embryo donation and fertility treatments, and has been associated with ethical objections because controversies regarding the use of such tissue for research. However, they have an unlimited capacity for self - renewal with an ability to differentiate into any of the cell types in the human body (Evans MJ and Kaufman MH, 1981). ESCs have been proposed as ideal candidates for cell based therapies to treat human retinal diseases, due to their ability to migrate and differentiate into various cell types. ESC have been differentiated *in vitro* into neurons (Bibel M, *et al.*, 2004) and Retinal Pigmented Epithelium (RPE) (Hirano M, *et al.*, 2003), but control their differentiation has been challenging. In the absence of appropriate intracellular signals, like ESCs to differentiate toward a neuronal fate as standard (Hemmati-Brivanlou A and Melton D, 1997), even though the differentiation in the retina-specific precursors often involves complex laboratory protocols (Osakada F, *et al.*, 2009). One drawback of pluripotent cell type is the risk of teratoma formation of uncontrolled

growth of transplanted ESCs (Hentze H, *et al.*, 2007), there is still a significant concern. Moreover, strains safety concerns from the observed chromosomal instability of cultured ESCs (Moon SH, *et al.*, 2011) require further investigation.

MATERIAL AND METHODS

Preparation of culture medium

First of all, primary mixed glial cultures generated from the cortex dissected from postnatal day 0 Lewis rat pups and grown on poly-D-lysine-coated tissue culture flasks in DMEM and 1% penicillin/streptomycin. OPC was isolated from the mixed glial cultures by a series of mechanical shaking steps and then kept in OPC expansion medium and analyzed directly after isolation.

Design of animal models

All animal experiments were conducted in accordance with the ethics committee university. Young adult (8 weeks old) male Lewis rats were used (n=83). Animals had free access to food and water and were maintained on a 12-hour light/dark cycle.

Experimental design

Transplantation of naive OPCs were performed either at the time of glaucoma induction (acute group), or 8 weeks prior to (chronic group). A group experienced transplantation of activated OPCs 8 weeks before glaucoma induction and the OPC acute transplant experiment, the animals received one uniocular 3UL intra-vitreous injection of PBS alone.

Treatment of tissue sample

Animals were perfused lower terminal anesthesia with 0.1 M PBS, followed by 4% paraformaldehyde/0.1 M Phosphate Buffer Saline (PBS). For immunohistochemal analysis tissues were fixed in 4% PFA for 2 hours. Posterior eyecups were cryopreserved with 30% sucrose and embedded in optimal cutting temperature compound for frozen sectioning at 40 um.

RESULTS

According to Figure 1 Oligo Dendrocyte Progenitor Cell (OPC) culture were analyzed for expression of A2B5 explaining the purity of the culture prior to transplantation. A2B5 is a cell surface antigen expressed by immature OPCs in culture (23). Immuno-cytochemical analysis showed that 93.9% ± 2.9% (mean ± SD, n=7) of the transplanted cells was OPCs. In addition, the OPC cell cultures show appropriate morphology in culture. Immuno- cytochemical labeling of either ED1 or GFAP in OPC cultures showed a 2.37 % ± 1.9 % (mean ± SD) contamination of microglia and 2.34% ± 0.9% (mean ± SD) contamination of astrocytes, respectively. Parallel staining in microglial cultures showed 1.15% ± 1.2% (mean ± SD) contamination of OPCs and 0.84% ± 0.6% (mean ± SD) contamination of astrocytes *(Figure 1 and Table 1)*.

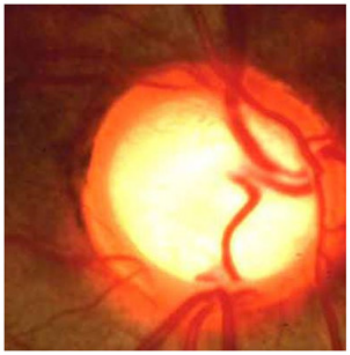

Figure 1: Characterization of OPCs cultured for transplantation

Table 1: Characteristics of animals (Acute transplant)

	Duration	Perfusion
Acute transplant	8 weeks	4 weeks
Chronic transplant	8 weeks	4 weeks

According to *Figure 2*, IOP was elevated in the entire laser-treated eyes *(Figure 2)*. There was no difference in peak IOP or integral IOP, a measure of IOP exposure over time previously shown. Peak IOP measurements were similar in the chronic OPC and OPC enabled transplantation experiments in which laser treatment was initiated at 16 weeks but significantly lower in the acute OPC transplantation experiment in which glaucoma was induced at 8 weeks of age *(Figure 3 and Table 2)*.

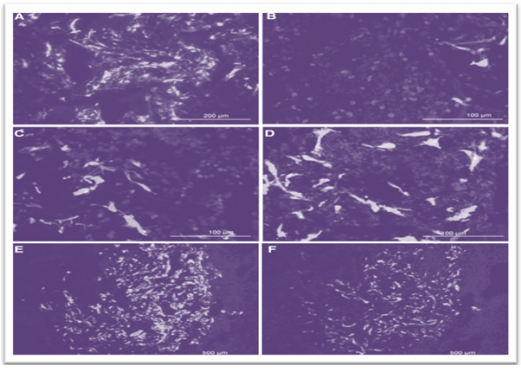

Figure 2: Acute intravitreal transplantation of OPCs was not neuro protective in glaucoma, however, engrafted cells survived and initiated differentiation

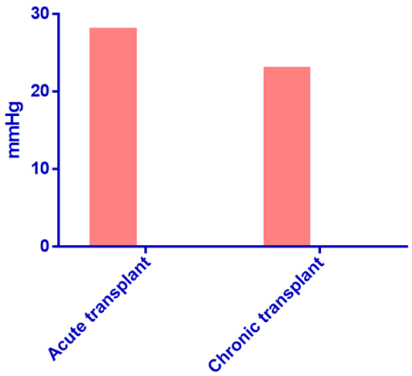

Figure 3: Graphical representation of groups

Table 2: Experimental glaucoma group

Group	Peak (mmHg)
Acute transplant	23 ± 1.8
Chronic transplant	28 ± 2.3

According to Quantification of optic nerve axonal survival after 4 weeks of exposure to ocular hypertension showed that intravitreal transplantation of OPCs in glaucoma induction does not offer protection to rGCS from glaucomatous death. Immunohistochemical analysis showed that a large number of OPCs, positive for the nuclear marker Olig2, survived in the vitreous, proximal to the inner surface of the retina, R glaucomatous eye for up to 4 weeks.

DISCUSSION

In the present study, we found that intra-vitreous transplantation of OPCs protected rGCS from glaucoma-induced death in vivo, but not until the engrafted cells had been activated by co-stimulation of inflammatory cells. Thus, we can deduce that the grafted OPCs were responsi-

ble for reducing RGC loss, rather than neuroprotection occurs as a side effect of inflammatory processes.

This is a key point that cytokine activated astrocyte can support injured neurons. However, the inflammatory stimulus was needed to induce OPC mediated neuro protection as we also found that OPCs injected into the eye does not remedy glaucomatous neuronal degeneration. These results indicate that neuro protection was mediated by the engrafted OPCs and it was triggered by a signal or signals transmitted by reactive immune cells. It seems unlikely that OPCs responded directly to zymosan, where they seem to lack TLR2, the innate receptor responsible for detecting zymosan, and failed to respond to zymosan exposure *in vitro* (Quigley HA and Green WR, 1979).

As previously reported, the throat also been shown to increase expression of MBP by OPCs in vivo and optionally OPC-mediated myelination of RGC axons normally unmyelinated retinal. We observed less OPC differentiation into MBP-expressing cells in the retina than previously reported. It is not clear why this difference in myelin production was detected, but it may be due to the use of different breeds of rats which strain differences in the inflammatory response and protective autoimmunity has been documented.

OPCs were injected into the vitreous of both injured and glaucomatous eye were found to survive well in all experiments. In addition, the grafted OPCs observed to spread across the inner retinal surface, which puts them in the ideal location for mediating the observed neuro protection. The number of grafted cells was lower in chronic graft which had been OPCs in vivo for 12 weeks, compared with acute graft in vivo for only 4 weeks. Long-term survival of healthy engrafted OPCs may have been relieved of their observed low levels in vivo proliferation, which may have preserved their intra vitreal population.

Interesting was engrafted cells were found to initiate neuronal and glial differentiation in the glaucomatous eye, both cutely and chronic. As expected, OPCs possessed the ability to differentiate into MBP expressing oligodendrocytes in the retina. On this basis, the logic of transplanting cells with the potential to produce reactive astrocytes in the eye is questioned. But the harmful effects mediated by native astrocytes in the optic nerve in glaucoma, including effects on local homeostasis and integrity of neural and connective tissue, appear to be highly localized.

CONCLUSION

We have demonstrated that transplantation of OPCs may relieve RGC death in vivo and that this neuroprotective capacity depends on inflammatory cell activation of OPCs. Such long-term relief of RGC death in glaucoma at an experimental intervention is rarely seen, suggesting a new approach to the development of neuroprotective strategy.

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