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Research Article

Analysis of Neuro degeneration in Glaucoma patients by Transplanted different Cell Precursors in Pakistan

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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 intravitreally 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

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[1]. The worldwide burden is greater with glaucoma is the second leading cause of global blindness after cataract[2]. 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[3].

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, oxidative stress[4] increased the level of intraocular pressure (IOP) is the main risk factor and depression in the level of IOP is the only cure for this particular disease. Whereas decreased levels of IOP cannot 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 (Corti et al. 2007) , (Madhavan et al. 2008) , (Yasuhara et al. 2006) . 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[5].

OPCs hold great responsibility for the generation of oligodendrocytes in the developmental stage, whereas in adult individuals, they play a vital role in demyelinating pathologies and remyelinatingof axon[6]. OPCs appear to contain the majority of the stem cell characteristics and has been shown to be neuroprotective in vitro[7].

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. 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[8].

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 and Kaufman., 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 and retinal pigmented epithelium (RPE)[9].

2.0 MATERIAL AND METHODS

2.1 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.

RESULTS

According to Figure 1 oligodendrocyte 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-cytochemicallabeling 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.

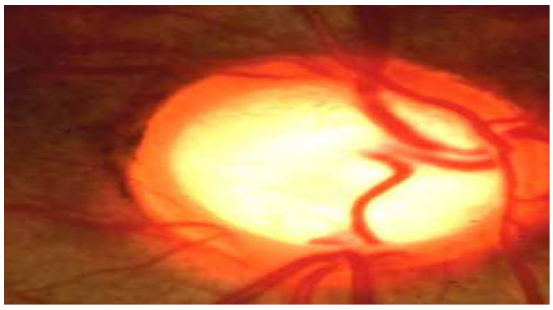

Figure1. Characterization of OPCs cultured for transplantation.

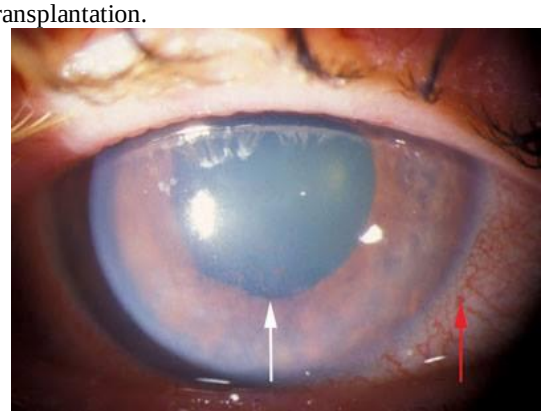

Figure 2: Intravitreal transplantation of OPCs did not affect intraocular pressure changes following ocular hypertension induction.

Table 2: Experimental Glaucoma Group

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

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.

4.0 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 responsible for reducing RGC loss, rather than neuroprotection occurs as a side effect of inflammatory processes[10].

This is a key point that cytokine activated astrocyte can support injured neurons[11]. However, the inflammatory stimulus was needed to induce OPC mediated neuroprotection as we also found that OPCs injected into the eye does not remedy glaucomatous neuronal degeneration. These results indicate that neuroprotection 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[12].

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[13].

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 neuroprotection[14]. 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 intravitreal population[15].

5.0 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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