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Mesenchymal stromal cell–mediated neuroprotection and functional preservation of retinal ganglion cells in a rodent model of glaucoma

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Abstract

Background aims. Glaucoma is a leading cause of irreversible blindness involving loss of retinal ganglion cells (RGC). Mesenchymal stromal cells (MSC) have shown promise as a paracrine-mediated therapy for compromised neurons. It is, however, unknown whether dental pulp stem cells (DPSC) are effective as a cellular therapy in glaucoma and how their hypothesized influence compares with other more widely researched MSC sources. The present study aimed to compare the efficacy of adipose-derived stem cells, bone marrow–derived MSC (BMSC) and DPSC in preventing the loss of RGC and visual function when transplanted into the vitreous of glaucomatous rodent eyes. *Methods.* Thirty-five days after raised intraocular pressure (IOP) and intravitreal stem cell transplantation, Brn3a$^+$ RGC numbers, retinal nerve fibre layer thickness (RNFL) and RGC function were evaluated by immunohistochemistry, optical coherence tomography and electroretinography, respectively. *Results.* Control glaucomatous eyes that were sham-treated with heat-killed DPSC had a significant loss of RGC numbers, RNFL thickness and function compared with intact eyes. BMSC and, to a greater extent, DPSC provided significant protection from RGC loss and RNFL thinning and preserved RGC function. *Discussion.* The study supports the use of DPSC as a neuroprotective cellular therapy in retinal degenerative disease such as glaucoma.

Key Words: *dental pulp stem cells, glaucoma, stem cell transplantation, mesenchymal stromal cells, neuroprotection, retinal ganglion cells*

Introduction

Glaucoma is a common cause of irreversible blindness and is characterised by a degenerative loss of retinal ganglion cells (RGC) and their axons, leading to optic disc cupping and reduced visual acuity [1]. Current treatments are designed to reduce intraocular pressure (IOP) to slow disease progression, whereas neuroprotective treatments that directly target the injured RGC are still in their infancy. Neurotrophic factors (NTF), particularly neurotrophins, are neuronal survival factors that are retrogradely transported along a functionally connected axon to the soma maintaining the survival of connected neurons, but unconnected neurons die by apoptosis [2]. Elevation of IOP significantly inhibits retrograde transport of NTF [3] and is one of the mechanisms involved in RGC death [4]. NTF, especially when delivered in combinations, promote the survival of injured RGC *in vitro* [5,6]. However, their neuroprotective efficacy *in vitro* is not easily translatable to *in vivo* models as a constant delivery of multiple NTF is required for maintaining therapeutic effect [7,8].

Mesenchymal stromal cells (MSC) are multipotent self-replicating stromal cells that are being evaluated as a cellular therapy for treating glaucoma because of their secretion of a wide array of NTF [9–12]. MSC-derived NTF protect injured RGC, protecting them from death and ultimately preserving vision [13], and several clinical trials evaluating their neuroprotective efficacy are ongoing [14]. MSC can be isolated from a variety of adult tissues, such as bone marrow (BMSC) and adipose tissue (ADSC), but here we focus on the use of MSC-like cells from the dental pulp (DPSC). DPSC are multipotent cranial neural crest–derived stem cells [15,16] that secrete significantly more NTF, including nerve growth factor, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) than ADSC and BMSC [9,10]. We and others have demonstrated significant RGC neuroprotection by

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DPSC [9] and BMSC [17,18] after traumatic optic neuropathy and shown *in vitro* that this neuroprotective effect is most likely due to NTF, including neurotrophins [9,10] and platelet-derived growth factor (PDGF) [12]. These findings indicated that MSC, and in particular DPSC, may be more effective as a treatment for neurodegenerative conditions such as glaucoma.

We have demonstrated a significantly greater neuroprotective effect of DPSC compared with BMSC/ADSC in an *in vitro* model of RGC injury, with multiple secreted NTF being the mechanism behind the effect [10]. We now hypothesize that DPSC may also be a candidate cellular therapy for protecting RGC from loss in glaucoma and determined to test this using an *in vivo* model. Accordingly, three widely researched stem cells for ocular repair [14]—BMSC, ADSC and DPSC—were transplanted into the vitreous body of rats in which ocular hypertension was induced using exogenously administered transforming growth factor-β1 (TGF-β). Administration of TGF-β1 [19] or TGF-β2 [20,21] are both accepted models that induce sustained elevations in IOP leading to significant RGC loss. In this study, RGC survival was assessed using immunohistochemical quantification of $Brn3^+$ RGC and retinal imaging using optical coherence tomography (OCT) of the retinal nerve fibre layer (RNFL) thickness. Changes in retinal function were measured using electroretinography (ERG).

Methods

All reagents were purchased from Sigma unless otherwise specified.

Human DPSC/BMSC/ADSC cultures

Human DPSC were obtained from AllCell, and both human BMSC and human ADSC from Lonza. Each MSC batch represented pooled samples from three donors. The MSC were characterized by $CD29^+$/$CD44^+$/$CD73^+$/$CD90^+$/$CD45^-$ profile (confirmed by the supplier) and demonstrated multi-differentiation (osteogenic, adipogenic and chondrogenic) capability. The stem cells were cultured into T25/T75 flasks (Corning) in both a total volume of 5 mL/15 mL Dulbecco's Modified Eagle's Medium containing 1% penicillin/streptomycin and 10% fetal bovine serum (Hyclone Laboratories) and at a density of 1×10^6 cells/2×10^6 cells, respectively. Cultures were maintained at 37°C in 5% CO_2, the supplemented medium was changed every 3 days, and the cells were passaged when 80% confluent using 0.05% trypsin/ethylenediaminetetraacetic acid. One week before transplantation, cells were transfected with a *gfp* plasmid using lipofectamine 3000 (Life Technologies, Invitrogen) according to the manufacturer's protocol. This study used MSC at passage 2–4, when 80% confluent and with no observable physical differences between the three cell types.

Experimental design

Twelve rats (24 eyes) received bi-weekly (twice a week) bilateral intra-cameral (IC) injections of TGF-β_{0-35d} (for 5 weeks) and were separated into two groups of six. On day 0, Group 1 received an intravitreal transplantation of DPSC into one eye and dead DPSC (sham control) into the other eye (left and right eyes, respectively, in three rats, and vice versa in the remaining three rats). On day 0, Group 2 received an intravitreal transplantation of BMSC into one eye and ADSC into the other eye (left and right eyes, respectively, in three rats, and vice versa in the remaining three rats). All rats in Group 1 and 2 received ERG and OCT recordings on day 35, before culling and tissue processing for immunohistochemistry. Animal numbers in each group were determined using a previously published power calculation [22,23]. A separate group of six rats received bi-weekly unilateral IC injections of phosphate-buffered saline (PBS) on days 0–35, and these rats are referred to as the Intact Group.

Animals

All animal procedures were performed in strict accordance to the UK Home Office Animals Scientific Procedures Act of 1986 and approved by the University of Birmingham Ethical Review Sub-Committee. Eighteen adult female Sprague Dawley rats weighing 150–200 g (Charles River) were housed in conditions of 21°C and 55% humidity under a 12 h light/dark cycle with a daytime luminance of 80 lux, given food/water *ad libitum* and were monitored by welfare staff. Gaseous anaesthesia was induced with 5% isoflurane/1.5 L/min O_2 (National Veterinary Supplies) and maintained at 3.5% during surgery and 2% during ERG recording.

Surgery for IC injections to induce ocular hypertension and intravitreal transplantation of MSC

Following anaesthetic induction, IOP were recorded for all rats using an icare tonometer (Tonolab). Rats were then secured in a head-holding frame for IC injections of TGF-β1 (Peprotech) through a single corneal incision, 2 mm anterior to the limbus using a 15° blade (BD Ophthalmic System). Using the same incision site a glass micropipette, produced in-house from a glass capillary rod (Harvard Apparatus) using a Flaming-Brown micropipette puller (Sutter Instruments) was used to inject 3.5 µL of 5 µg/mL activated TGF-β1 IC into all 12 rats. Contemporaneously, while the animals were still anaesthetized, a glass