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Research Report

Sustained effect of bone marrow mononuclear cell therapy in axonal regeneration in a model of optic nerve crush

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ABSTRACT

In adult mammals, the regeneration of the optic nerve is very limited and at the moment there are several groups trying different approaches to increase retinal ganglion cell (RGC) survival and axonal outgrowth. One promising approach is cell therapy. In previous work, we performed intravitreal transplantation of bone-marrow mononuclear cells (BMMCs) after optic nerve crush in adult rats and we demonstrated an increase in RGC survival and axon outgrowth 14 days after injury. In the present work, we investigated if these results could be sustained for a longer period of time. Optic nerve crush was performed in Lister-hooded adult rats and BMMC or saline injections were performed shortly after injury. Neuronal survival and regeneration were evaluated in rats' retina and optic nerve after 28 days. We demonstrated an increase of 5.2 fold in the axon outgrowth 28 days after lesion, but the BMMCs had no effect on RGC survival. In an attempt to prolong RGC survival, we established a new protocol with two BMMC injections, the second one 7 days after the injury. Untreated animals received two injections of saline. We

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observed that although the axonal outgrowth was still increased after the second BMMC injection, the RGC survival was not significantly different from untreated animals. These results demonstrate that BMMCs transplantation promotes neuroregeneration at least until 28 days after injury. However, the effects on RGC survival previously observed by us at 14 days were not sustained at 28 days and could not be prolonged with a second dose of BMMC.

1. Introduction

It is well described that in adult mammals most of the retinal ganglion cells (RGCs) fail to regenerate their axons after optic nerve crush or transection and undergo apoptosis. This regeneration failure has been attributable both to the inhibitory environment of the central nervous system and the intrinsic inability of RGCs to regenerate (Schwab et al., 1993; Goldberg, 2004). Because of that, many groups have been testing different approaches in order to promote RGC survival and axonal outgrowth following optic nerve injury.

Among these approaches, there is induction of inflammation either by lens puncture or Zymosan injection (Fischer et al., 2000; Leon et al., 2000; Yin et al., 2003) and the stimulation of RGCs intrinsic program to regenerate through, for example, the deletion of the phosphatase and tensin (PTEN) (Park et al., 2008) and/or the deletion of the homolog suppressor of cytokine signaling 3 (SOCS3) (Smith et al., 2009; Sun et al., 2011). The combination of PTEN deletion with Zymosan injection and elevation of intracellular cAMP leads to long distance regeneration and functional recovery (Kurimoto et al., 2010; de Lima et al., 2012).

However, these different approaches are not easily translated to clinic. Regarding this, a developing strategy involves cell therapy with the transplantation of bone marrow derived cells or other cell types. Cell therapy has been used in different animal models of neurological diseases and lesions with interesting results (Giraldi-Guimaraes et al., 2009; Ribeiro-Resende et al., 2009; Johnson et al., 2010; Moraes et al., 2012; Suzuki et al., 2012; de Vasconcelos dos Santos et al., 2010; Vasconcelos-dos-Santos et al., 2012) and it is beginning to be used in clinical trials (Barbosa da Fonseca et al., 2009, 2010; Battistella et al., 2011; Friedrich et al., 2012; Rost et al., 2012). Interestingly, data from clinical studies suggests that an intravitreal injection of autologous bone-marrow-derived cells into the vitreous cavity is technically feasible and safe (Jonas et al., 2008, Siqueira et al., 2011).

In the visual system, bone marrow mesenchymal cells have been transplanted intravitreally in two different models of glaucoma and showed neuroprotective results (Yu et al., 2006; Johnson et al., 2010). It has also been shown that brain-derived neurotrophic factor (BDNF)-secreting mesenchymal cells increases RGC survival in chronically hypertensive rat eyes (Harper et al., 2011) and that modified bone marrow mesenchymal cells increased neuroprotection after optic nerve transection (Levkovitch-Verbin et al., 2010). Increased neuronal survival was also obtained after bone marrow mesenchymal cells transplantation in a model of lesion by ischemia/reperfusion (Li et al., 2009).

In previous work, our group demonstrated increase in RGC survival and axonal outgrowth 14 days after optic nerve crush and BMMC transplantation (Zaverucha-do-Valle et al., 2011). We have also identified two proteins—Tax1-binding protein 1 and Synaptotagmin IV—that were up-regulated after optic nerve crush and cell therapy (Mesentier-Louro et al., 2012). However, in most of these studies the effects were analyzed 14 days after treatment and it was important to investigate whether BMMC transplantation effects could still be present after a longer period of time. Therefore, in the present work, we analyzed neuroprotection and axonal regeneration 28 days after nerve injury and BMMC transplantation.

2. Results

2.1. *BMMC transplantation increases axonal outgrowth 28 days after injury*

To evaluate if the effect of BMMC transplantation on regeneration (Zaverucha-do-Valle et al., 2011) was sustained, we analyzed the axonal outgrowth 28 days after injury and cell therapy. For that, we used cholera toxin subunit B conjugated to Alexa-488 (CTB) as anterograde marker to visualize axons. In untreated animals (Fig. 1(A)), we observed a very small number of axons crossing the injury site (*). After BMMC transplantation, we noticed an increase in the number of axons distal to the lesion (Fig. 1(B)) when compared with the untreated group.

We quantified the number of CTB-labeled axons extending to 0.5, 1.0, 1.5 and 2 mm from the crush site using the formula described by Leon and co-workers (Leon et al., 2000). The untreated animals had a median of 163.9 axons per nerve at 0.5 mm; 52.8 axons at 1 mm; 2.3 axons at 1.5 mm and 0 axons at 2 mm from the crush site ($n=8$). The animals that received BMMC transplantation had a median of 819.2 axons per nerve at 0.5 mm ($n=5$); 454.9 axons at 1 mm ($n=5$); 304.5 axons at 1.5 mm ($n=4$) and 15.8 axons at 2 mm ($n=3$) from the crush site. BMMC transplantation generated a 5-fold significant increase ($p<0.01$) in the number of CTB-labeled axons at 0.5 mm from the crush site, a 8.6-fold significant increase ($p<0.01$) in the number of CTB-labeled axons at 1 mm from the crush site and a 129.8-fold significant increase ($p<0.05$) in the number of CTB-labeled axons at 1.5 mm from the crush site. At the distance of 2 mm from the injury site, there was no statistically significant difference between the two conditions ($p>0.05$). Fig. 1(C) shows the quantified data.

These results were confirmed by using GAP-43 staining. We quantified the number of GAP-43^{+} axons located at 0.5, 1.0, 1.5