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Transplantation of mesenchymal stem cells exerts anti-apoptotic effects in adult rats after spinal cord ischemia-reperfusion injury

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ABSTRACT

It is unknown whether transplantation of bone marrow mesenchymal stem cells (BM-MSCs) can repair spinal cord ischemia-reperfusion injury (SCII) in a rat model through an anti-apoptotic effect. Adult rats were divided into untreated or sham-operated controls, untreated models of SCII (uSCII) and BM-MSC-transplanted models of SCII (tSCII; labeled with CM-DiII transplanted at 1 h and 24 h after reperfusion). According to evaluation of hind-limb motor function, the motor functions of tSCII rats were significantly better than those of uSCII rats by the seventh day. H&E and TUNEL staining showed that the spinal cords of uSCII rats contained damaged neural cells with nuclear pyknosis and congestion of blood vessels, with a high percentage of apoptotic neural cells, while the spinal cords of tSCII rats were nearly normal with significantly fewer apoptotic neural cells. Immunohistochemistry and double immunofluorescence staining revealed that in tSCII rats CASP3 and neurofilament-H (NF-H) levels were 14.57% and 174% those of uSCII rats, respectively, and in tSCII rats the ratio of BAX to BCL2 was reduced by nearly 50%. The differentiation of transplanted CM-DiI-labeled BM-MSCs into neurons and astrocytes was observed in the spinal cords of the tSCII rats under laser scanning confocal microscopy. These results showed that transplantation of BM-MSCs improved functional recovery after SCII via anti-apoptosis.

Abbreviations: (BM-MSCs), bone marrow mesenchymal stem cells; (SCII), spinal cord ischemia-reperfusion injury; (tSCII), transplantation model of spinal cord ischemia-reperfusion injury; (uSCII), untreated SCII model

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1. Introduction

Spinal cord ischemia-reperfusion injury (SCII) is a devastating problem that leads to severe complications, including paralysis (Guerit and Dion, 2002). Many researchers have focused on the prevention and treatment of SCII with strategies such as induced hypothermia during surgical procedures (Cakir et al., 2003), ischemic preconditioning (Zvara et al., 1999), cerebrospinal fluid drainage (Cina et al., 2004) and pharmacological interventions with dapsone (Diaz-Ruiz et al., 2011), puerarin (Tian et al., 2011), or mesna (2-mercaptoethane sulfonate sodium) (Dolgun et al., 2010). Nevertheless, definitive prevention and therapy for SCII has yet to be determined.

Apoptosis includes both a receptor-dependent extrinsic pathway (Sakurai et al., 1998) and a mitochondria-associated intrinsic pathway (Springer et al., 1999), which are activated in spinal cord injuries. Mackey et al. (1997) and Hayashi et al. (1998) have shown that neuronal apoptosis occurs in a rabbit model of SCII. It has been shown that CASP3 and CASP9, effectors of apoptosis, are involved in mitochondria-associated apoptotic pathways (Li et al., 1997; Springer et al., 1999). Many other genes are involved in apoptosis, such as p53 (Kotipatruni et al., 2011), the anti-apoptosis gene Bcl2 (Kroemer, 1997; Woo et al., 2005) and apoptosis-inducer Bax (Kotipatruni et al., 2011).

It is known that the Bcl2 gene family includes genes for encoding the pro-apoptosis proteins BAX and BAK, and the genes for anti-apoptosis proteins BCL2 and BCLX (Kotipatruni et al., 2011; Kroemer, 1997; Sentman et al., 1991; Woo et al., 2005; Youle and Strasser, 2008). BCL2 is a 26-kDa protein located in mitochondrial membranes; overexpression of BCL2 inhibits apoptosis of neurons (Allsopp et al., 1993; Fan et al., 2010; Kane et al., 1993; Liu et al., 2011; Reed, 1997). Wang et al. (2011) demonstrated that overexpression of BCL2 inhibited neuronal apoptosis after spinal cord injury and improved recovery of neurological function in *Bcl2*-overexpressed transgenic mice.

In contrast, BAX, a cytosolic protein in normal living cells, can induce apoptosis and quickly translocates to mitochondria at an early stage of the apoptotic process (Gross et al., 1998; Hsu and Youle, 1997). The anti-apoptotic effect of BCL2 hinders the activity of the BAX pro-apoptotic protein. The ratio of BAX and BCL2 in the outer membrane of the mitochondrion is a determining factor for the release of cytochrome C (Adams and Cory, 1998), and its binding to cytosolic Apaf-1 (apoptotic protease activating factor 1) and pro-CASP 9 to form complexes activates CASP9, which then activates CASP3 and ultimately initiates apoptosis (Li et al., 1997).

Kotipatruni et al. (2011) showed upregulation of phospho-p53 and BAX, downregulation of BCL2, and the release of cytochrome C in rat spinal cords after spinal cord injury. These results point to a mitochondrial-mediated apoptotic pathway after spinal cord injury. Further studies have confirmed that the expressions of CASP9 and CASP3 observed after spinal cord injury are intrinsic key mediators of apoptosis (Barut et al., 2005; Kakinohana et al., 2011; Kanellopoulos et al., 1997; Kotipatruni et al., 2011; Yakovlev et al., 1997).

Raisova et al. (2001) demonstrated the importance of the ratio of BAX to BCL2 in apoptosis. The ratios in 11 human melanoma cells were evaluated by Western blot and the results revealed that susceptibility to CD95/Fas-mediated apoptosis in melanoma cells was directly associated with the BAX/BCL2 ratio. Moreover, the results showed that cells resistant to apoptosis had a characteristically low BAX/BCL2 ratio while cells sensitive to apoptosis had a high ratio.

A previous study showed the anti-apoptotic effects of transplantation of bone marrow mesenchymal stem cells (BM-MSCs) in acutely-induced Alzheimer's disease mice brains (Lee et al., 2010). Moreover, BM-MSCs can migrate and integrate into damaged organs or tissues (Jin et al., 2013; Sato et al., 2011; Soler et al., 2012; Stamm et al., 2003) where they differentiate into cell lineages such as myocardiocytes (Guan et al., 2011) and neurons (Wei et al., 2012).

It remains unclear whether transplantation of BM-MSCs has an anti-apoptotic effect in the injured spinal cord of an animal model of spinal cord ischemia. Therefore, in the present study, we investigated the effects of BM-MSCs on apoptosis of nerve cells after transplantation of BM-MSCs into adult rat with SCII.

2. Results

2.1. *Establishment of rat BM-MSCs culture*

Cells attached to flasks 3 h after seeding. The number of attached cells increased after change of the culture medium on day 3. The majority of cells appeared spindle-shaped, only a few cells were triangular. After two passages, morphologically homogenous populations of fibroblast-like cells were observed.

Few of the cultured cells expressed CD45 antigen (1.01%). However, these cells were strongly positive for CD44 (99.35%) and CD90 antigen (99.16%). Results showing BMSCs positive for CD44 and CD90 but negative for CD45 indicated that they were not hematopoietic (Fig. 1).

2.2. *Efficacy of CM-Dil labeled BM-MSCs*

Red fluorescence was observed in the cytoplasm of BM-MSCs after the cells were labeled, the percentage of the BM-MSCs labeled by CM-Dil was 90.4% assessed by flow cytometry assay.

2.3. *Transplantation of BM-MSCs significantly improved neurological deficit in the animals with SCII*

To examine effects of BM-MSCs on functional recovery, we used the BBB locomotor rating scale to compare hind-limb motor functions among groups during 7 days immediately after ischemia-reperfusion (Fig. 2). We observed that the hind-limb motor functions were normal in the controls (non-operated and uSCII model; the normal score was 21) and sham-operated groups. During the first 3 days, the animals in both the uSCII model and MSC-transplanted (tSCII) groups scored <14.5 on the BBB scale, indicating that the animals in both these two groups had developed unambiguous neurological deficit. However, on the fourth day, the BBB scores of the animals in the uSCII model group were