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A combination of taxol infusion and human umbilical cord mesenchymal stem cells transplantation for the treatment of rat spinal cord injury

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ABSTRACT

Background and purpose: Studies have shown that the administration of Taxol, an anti-cancer drug, inhibited scar formation, promoted axonal elongation and improved locomotor recovery in rats after spinal cord injury (SCI). We hypothesized that combining Taxol with another promising therapy, transplantation of human umbilical mesenchymal stem cells (hUCMSCs), might further improve the degree of locomotor recovery. The present study examined whether Taxol combined with transplantation of hUCMSCs would produce synergistic effects on recovery and which mechanisms were involved in the effect.

Methods: A total of 32 rats subjected to SCI procedures were assigned to one of the following four treatment groups: phosphate-buffered saline (PBS, control), hUCMSCs, Taxol, or Taxol+hUCMSCs. Immediately after injury, hUCMSCs were transplanted into the injury site and Taxol was administered intrathecally for 4 weeks. Locomotor recovery was evaluated using the Basso, Beattie and Bresnahan locomotor (BBB) rating scale. Survival of the transplanted human cells and the host glial reaction in the injured spinal cord were studied by immunohistochemistry.

Results: Treatment with Taxol, hUCMSCs or Taxol+hUCMSCs reduced the extent of astrocytic activation, increased axonal preservation and decreased the number of caspase-3^+ and ED-1^+ cells, but these effects were more pronounced in the Taxol+hUCMSCs group. Behavioral analyses showed that rats in the Taxol+hUCMSCs group showed better motor performance than rats treated with hUCMSCs or Taxol only.

Conclusions: The combination of Taxol and hUCMSCs produced beneficial effects in rats with regard to functional recovery following SCI through the enhancement of anti-inflammatory, anti-astrogliosis, anti-apoptotic and axonal preservation effects.

1. Introduction

Traumatic spinal cord injury (SCI) induces neural cell death, axonal degeneration, and destruction of the microvasculature. These events trigger a subsequent cascade of pathological changes (so-called secondary events), including free-radical release, hyperplasia of astrocytes, calcium-mediated damage, hemorrhagic necrosis, mitochondrial dysfunction,

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and inflammatory responses, that lead to delayed cellular dysfunction and death (Guest et al., 2005; Waxman et al., 1991). Moreover, the poor trophic support and growth-inhibitory nature of the environment of the adult central nervous system (CNS) is hostile to endogenous spinal cord regeneration. Intensive efforts have been made to develop therapeutic strategies to minimize the extent of neurological disabilities and to promote the recovery of function after SCI. However, no single neuroprotective agent has been approved for clinical use by the US Food and Drug Administration, underlining the need to focus on strategies that simultaneously affect multiple injury mechanisms. In this context, we hypothesized that a combination of therapeutic strategies might be more effective than a single strategy for promoting functional recovery after SCI.

Taxol is a clinically approved anti-cancer drug. Taxol stabilizes microtubules and, as a result, interferes with the normal breakdown of microtubules during cell division (Vyas and Kadow, 1995). Taxol can prevent the formation of axon retraction bulbs and decrease axonal degeneration after SCI and can overcome myelin inhibition of neurite outgrowth in vitro (Erturk et al., 2007). Taxol also exhibits immunomodulatory action on immune cells, such as microglia and macrophages. For example, Taxol can reduce the infiltration/activation of ED-1$^+$ cells (macrophages/microglia) after optic nerve injury (Sengottuvel et al., 2011). A recent study also indicated that moderate microtubule stabilization by Taxol decreased scar formation and prevented accumulation of chondroitin sulfate proteoglycans (CSPGs) in an experimental model of rat SCI (Hellal et al., 2011).

Cell transplantation is another promising strategy for the treatment of SCI. The grafted cells could provide trophic support for neurons and manipulate the environment within the damaged spinal cord to facilitate axon regeneration or to promote plasticity in the lesioned spinal cord. Various types of multipotent stem cells, including embryonic stem cells (ES) (Deshpande et al., 2006), neural stem cells (NSC) (Yasuda et al., 2011) and mesenchymal stem cells (MSC) (Ide et al., 2010) are currently under investigation as potential alternative cell sources for cell transplantation. Theoretically, ES cells are the best candidates for treating SCI; however, their potential is limited by ethical issues. The number of NSCs decreases in neurogenesis, and NSCs undergo replicative senescence over time. In this context, mesenchymal stem cells (MSC) are promising candidates as cumulative evidence shows both the multipotency of MSCs and their capability to exert a neuroprotective effect after CNS injury through the paracrine production of mitogenic, anti-apoptotic, and trophic factors (Nakajima et al., 2012; Sakai et al., 2011; Schira et al., 2011). Among the MSCs, human umbilical mesenchymal stem cells (hUCMSCs) appear to have several advantages. They offer a noncontroversial, readily available source of cells and can be obtained through a low-cost, noninvasive collection method (Yang et al., 2012). Furthermore, hUCMSCs are isolated from fetal structures during the perinatal period and are better tolerated following transplantation, resulting in a lower incidence of graft versus host disease compared with other types of postnatal MSCs (Cho et al., 2008).

The treatment of SCI requires a multifaceted strategy because of the multiple potential mechanisms that hinder spinal cord recovery (Kim et al., 2007). The aim of this study is to evaluate whether a therapeutic combination of hUCMSCs and Taxol enhances the beneficial effects of treatment.

2. Results

2.1. *Characterization of hUCMSCs*

The isolated hUCMSCs demonstrated a fibroblast-like morphology in confluent layers in culture. In agreement with previous observations (Novikova et al., 2011), all of the hUCMSCs were immunopositive for vimentin, laminin and fibronectin. The percentage of hUCMSCs expressing Nestin and Ki67 was $9.3\pm1.4\%$ and $56.65\pm5.35\%$, respectively. The hUCMSCs did not express CD34, indicating that they were of non-hematopoietic origin (Fig. 1).

2.2. *Cell survival and migration in vivo*

SCI was induced in female Sprague-Dawley rats by dropping a 10 g metal rod from a height of 7.5 cm using an NYU impactor. Immediately after injury, 2×10^5 hUCMSCs were transplanted at a distance of 2 mm rostral and 2 mm caudal to the site of injury, respectively. The survival and migration of hUCMSCs in the hUCMSCs group and in the hUCMSCs+Taxol group was assessed 1 week after transplantation in harvested sagittal tissue sections. Grafted human stem cells were clearly detected by immunohistochemical staining with a specific anti-human nuclei antibody (hNu, MAB1281). One week after transplantation, we found extensive survival of the human cells (Fig. 2A). The percentage of surviving cells versus total transplanted cells was $37.8\pm10.5\%$ and $32.5\pm9.2\%$ for the hUCMSCs group and the hUCMSCs+Taxol group, respectively ($p>0.05$, $N=4$ for each group) (Fig. 2B). The majority of the surviving cells were observed at the epicenter of the lesion, suggesting that the grafted cells migrated toward the injury sites. We investigated whether the human stem cells exhibited active cell division/proliferation after transplantation by double-labeling for hNu and Ki67. Most of the grafted cells did not express Ki67 ($3.1\pm1.6\%$ and $2.5\pm1.2\%$ for the hUCMSCs group and the hUCMSCs+Taxol group, respectively, $p>0.05$, $N=4$ for each group) (Fig. 2C), indicating that they ceased proliferation after transplantation into the injured spinal cord. Ki67-positive cells were randomly dispersed across the graft area without evidence of clustering in specific sites.

2.3. *Fate of transplanted cells*

Histological examination revealed that the hUCMSCs survived for 4 weeks after transplantation. To evaluate the neural differentiation potential of the hUCMSCs in the spinal cord environment, the expression of neuronal and glial marker proteins was analyzed using immunohistochemistry. The results showed that, whether they were with within or outside of the lesion zone, the hUCMSCs failed to express the