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UMBILICAL CORD CELLS

Neuroprotective effects of human umbilical cord–derived mesenchymal stromal cells combined with nimodipine against radiation-induced brain injury through inhibition of apoptosis

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) possess the ability to repair brain injuries. Additionally, nimodipine is a neuroprotective agent that increases cerebral blood flow and may help with the homing of MSCs to the injury site. Here we investigate the effectiveness of a combined human umbilical cord–derived MSCs and nimodipine therapy in radiation-induced brain injury (RIBI). *Methods.* Female mice received whole brain irradiation (WBI) and were treated with saline, nimodipine, hUC-MSCs, or hUC-MSCs combined with nimodipine. Body weight was measured weekly. An open field test for locomotor activity and a step-down avoidance test for learning and memory function were conducted at week 4 and week 12 post-WBI. The histological damage was evaluated by hematoxylin and eosin staining and glial fibrillary acidic protein immunohistochemistry. Quantitative polymerase chain reaction and Western blotting were used to detect apoptosis-related mediators (p53, Bax and Bcl-2). *Results.* In mice receiving the hUC-MSCs or the combined treatment, their body weight recovered, their locomotor and cognitive ability improved, and the percentage of necrotic neurons and astrocytes was reduced. The combined therapy was significantly ($P < 0.05$) more effective than hUC-MSCs alone; these mice showed decreased expression of pro-apoptotic indicators (p53, Bax) and increased expression of an anti-apoptotic indicator (Bcl-2), which may protect brain cells. *Conclusions.* We demonstrated that hUC-MSCs therapy helps recover body weight loss and behavior dysfunction in a mice model of RIBI. Moreover, the effectiveness of the combined hUC-MSCs and nimodipine therapy is due to apoptosis inhibition and enhancing homing of MSCs to the injured brain.

Key Words: *apoptosis, mesenchymal stromal cells, nimodipine, radiation-induced brain injury*

Introduction

Radiation encephalopathy is a serious complication in primary and metastatic brain tumors of the central nervous system after radiotherapy. Radiation-induced brain injury (RIBI) leads to neuronal apoptosis, declining neurogenesis, and cognitive impairment [1–3]. The primary approach to manage RIBI in the clinic is symptomatic treatments, including hormone and neurotrophic drugs, hyperbaric oxygenation therapy and surgery [4,5]. However, more effective methods for the treatment of RIBI are required.

One potential option for managing RIBI is to use mesenchymal stromal cells (MSCs), which are multipotent stem cells that aid recovery of many injured tissues, including brain and spinal cord tissues [6,7]. MSCs have previously been shown to inhibit apoptosis during neurological disorders [8] or irradiation-induced lung injury [9,10]. For example, the treatment of rat spinal cord injury with MSCs inhibited apoptosis of spinal neurons by down-regulating the apoptosis-inducing factors p53 and Bax and up-regulating the anti-apoptotic factor Bcl-2 [11]. MSCs can also promote learning and memory cognitive dysfunction recovery

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in Alzheimer disease [12] and Parkinson disease [13]. Additionally, in a mouse model of ischemic stroke, MSCs can promote functional recovery by increasing neuronal and astrocyte survival and reducing neurological impairment [14,15]. In hypoxic-ischemic brain damage, MSCs show long-lasting effects on the motor, cognitive and histological outcomes up to 9 weeks after hypoxic-ischemic brain damage [16]. Similarly, human embryonic stem cells ameliorate cognitive impairment 8 months after irradiated brain injury [17,18]. Therefore, MSCs may be useful for repairing cerebral nerve injury or preventing apoptosis in RIBI.

Despite these benefits of MSC-based therapy, the blood–brain barrier may limit the homing of MSCs to the injury sites, thereby reducing its therapeutic efficacy. Homing to the injured site is extremely important, as was indicated in previous experiments using adipose-derived MSCs for the treatment of experimental autoimmune encephalomyelitis [19]. In our study, we hypothesized that the addition of nimodipine may reduce the limitation of blood–brain barrier on MSC-based therapy for central nervous system injuries. Nimodipine, a neuroprotective lipophilic calcium channel antagonist, can dilate cerebral blood vessels and increase cerebral blood flow [20] and may enhance the homing of transplanted MSCs to the cerebral injury sites by selectively dilating cerebral arterioles. Therefore, combining MSC-based therapy with nimodipine might be a beneficial for RIBI.

To test our hypothesis, we built a whole brain irradiation (WBI)-induced brain injury mice model and compared the therapeutic effects of human umbilical cord (UC)-derived MSCs (hUC-MSCs) alone or combined with nimodipine on body weight, behavior function and histopathology. We also examined changes in expression of apoptosis-related factors (i.e., p53, Bax and Bcl-2) using the different therapies. This study provides beneficial evidences and promising insights for the cytotherapy of RIBI.

Methods

Experimental design

The overall experimental design is shown in Figure 1. Briefly, mice were fed with their respective diets for 7 days before WBI and maintained on these diets until they were euthanized at 12 weeks post-WBI. Fifty mice were randomly divided into five groups: a sham irradiation control group (CON), a WBI and saline group (WBI), a WBI and nimodipine group (NIMO), a WBI and hUC-MSCs group (MSC) and a WBI and combined hUC-MSCs and nimodipine group (MSC + NIMO).

The irradiation procedure has been described previously [21]. Briefly, WBI was performed using a ^{60}Coγ-rays irradiator using lead shielding devices so that the whole brain, including the brainstem, was irradiated, while the rest of the body was shielded. Irradiated mice received a single dose of 15 Gy at a dose rate of 1.17 Gy/min. Sham-irradiated control mice were anesthetized but not irradiated. Body weight was measured weekly during the experimental course (12 weeks).

Nimodipine injection (Baier) was purchased from the General Hospital of Chinese People's Liberation Army (Beijing, China). Nimodipine was injected intraperitoneally (1 mg/kg) 30 min before the MSCs were administrated (i.e., at 2 hours, 1 week, and 2 weeks post-WBI). For the hUC-MSCs transplantation, approximately 1×10^6 hUC-MSCs in 200 µL saline were injected into each animal through the lateral tail vein immediately after irradiation. The mice in the control group were injected with 200 µL of saline. Behavioral tests were performed at weeks 4 and 12 after WBI.

Ethical approval

The female C57BL/6 mice aged 6–8 weeks (weight 16–18 ± 0.3 g) were obtained from the Animal Center of the Academy of Military Medical Sciences (Beijing, China). All experiments involving animal subjects were performed in accordance with guidelines. The Institutional Animal Care and Use Committee of the Academy of Military Medical Sciences approved these experiments.

Isolation and culture of hUC-MSCs

All UC samples were obtained from local hospitals with the donors' informed consent, and hUC-MSCs were isolated as described previously [22]. Briefly, under sterile conditions, UCs were rinsed three times with phosphate-buffered saline (PBS) to remove contami-

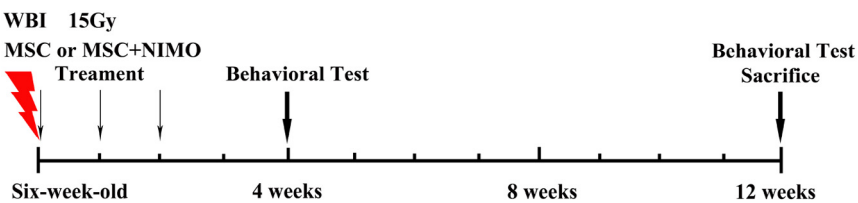

Figure 1. Schematic overview of the experiment showing radiation exposure and treatment programs in the mice model.