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Research report

# Comparisons of the therapeutic effects of three different routes of bone marrow mesenchymal stem cell transplantation in cerebral ischemic rats

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ABSTRACT

Bone marrow mesenchymal stem cells (BMSCs) are mainly administered via three routes: intra-arterial, intravenous and intracerebral. It has been reported that BMSC administration via each route ameliorates the functional deficits after cerebral ischemia. However, there have been no comparisons of the therapeutic benefits of BMSC administration through different delivery routes. In this study, we injected BMSCs into a rat model of transient middle cerebral artery occlusion (MCAO) through the intra-arterial, intravenous, or intracerebral route at day 7 after MCAO. Control animals received only the vehicle. Neurological function was assessed at post-ischemic days (PIDs) 1, 7, 14, 21, 28 and 35 using behavioral tests (modified Neurological Severity Score (mNSS) and the adhesive removal test). At PID 35, the rat brain tissues were processed for histochemical and immunohistochemical staining. Our results showed that BMSC transplantation via the intra-arterial, intravenous, and intracerebral routes induced greater improvement in neurological functions than the control treatments; furthermore, the intra-arterial route showed the greatest degree and speed of neurological functional recovery. Moreover, BMSCs treatment through each route enhanced reconstruction of axonal myelination in the area of the corpus callosum on the infarct side of the cerebral hemisphere, increased the expression of SYN and Ki-67, and decreased the expression of Nogo-A in the brain. These effects were more apparent in the intra-arterial group than in the intravenous and intracerebral groups. These data suggest that BMSCs transplantation, especially through intra-arterial delivery, can effectively improve neurological function intra-arterial. The underlying mechanism may include the promotion of synaptogenesis, endogenous cell proliferation, and axonal regeneration.



## 1. Introduction

Stroke is a cerebrovascular disease with high mortality and morbidity. Patients who experience stroke often have long-term devastating and irreversible disability, but the treatment of nerve functional injuries is still very difficult, and there is no ideal therapeutic method (Howard and Goff, 2012). Therefore, it is very important to search for a new therapeutic method for stroke, especially in neurological rehabilitation.

BMSCs are pluripotent stem cells, that can be induced to differentiate into a variety of lineages in vitro, including smooth muscle cells, osteoblasts, adipocytes, hepatocytes and neural cells (Alimperti et al., 2014; Choi et al., 2014; Contador et al., 2015; Huat et al., 2014; Kim et al., 2015), and can secrete many kinds of growth cytokines (He et al., 2016; Li et al., 2014; Wang et al., 2015). Moreover, BMSCs have low immunogenicity in an allogeneic host (Dazzi and Krampera, 2011). Therefore, there is increasing evidence that BMSCs may be one of the most appropriate candidates to improve functional recovery after cerebral ischemia.

To date, BMSC administration has been employed in a small number of stroke patients (Miao et al., 2015; Zhou et al., 2017) and many different stroke models (Argibay et al., 2017; Chen et al., 2008; Du et al., 2014; Komatsu et al., 2010; Nam et al., 2015; Toyoshima et al., 2015; Uchida et al., 2017; Xiong et al., 2009; Yang et al., 2015; Zheng et al., 2010b), providing promising evidence that BMSC treatment can improve functional outcomes in stroke objects and is very safe, without inducing inflammation, immune rejection and tumorigenesis in the host. Currently, the intra-arterial, intravenous and intracerebral routes are the effec-

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tive modes of MSC transplantation after cerebral ischemia (Acosta et al., 2015a; Fukuda et al., 2014; Li et al., 2014; Sammali et al., 2017; Toyoshima et al., 2015; Uchida et al., 2017). However, each route has merits and drawbacks. Intravenous injection is the least traumatic among the three routes, but the biodistribution is broadest, and most transplanted cells home to other organs rather than to the lesioned brain (Acosta et al., 2015b; Sammali et al., 2017). However, the advantage of intracerebral transplantation is that there are no issues with the biodistribution and targeted migration of cells into the infarct brain tissue; however, it is more invasive and can lead to a physical mass of cells that could damage the healthy tissue. Therefore, the intracerebral transplanted cell suspension volume and dose should be as small as possible to alleviate the compression effect (Darsalia, 2011; Uchida et al., 2017). To achieve this goal, intra-arterial administration might be a promising therapeutic method because it may promote the migration of a significantly larger number of cells to the ischemic tissue than intravenous administration and is less invasive than intracerebral injection. However, intra-arterial delivery poses the risks of microembolization and bubble formation, which are reduced with the intravenous and intracerebral routes (Argibay et al., 2017; Janowski et al., 2015). To date, few studies have compared the effects of different transplantation routes, and the ideal route of stem cell transplantation remains unknown.

Although the mechanism responsible for the neuroprotection of BMSC administration is uncertain, it may include the secretion of growth factors, neural replacement, promotion of endogenous regeneration, reduction of inflammatory responses and demyelination, and so on; nevertheless, the promotion of endogenous regeneration may be the most important one (Acosta et al., 2015a; Chen et al., 2013; Sammali et al., 2017; Yang et al., 2015). Some studies have found that BMSC transplantation may advance synaptogenesis by affecting the expression of synaptophysin (SYN) and neurite outgrowth inhibitor-A (Nogo-A), which is a protein that can inhibit neurite outgrowth (Alvarez-Castelao and Schuman, 2015; Feng et al., 2016). Recent studies confirmed that BMSC transplantation after ischemic stroke encouraged angiogenesis by increasing the vascular density and vascular endothelial growth factor (VEGF) expression in infarct brain tissue (Arutyunyan et al., 2016; Sammali et al., 2017). Moreover, BMSC delivery may promote the proliferation of endogenous nerve stem cells and astrocytes (Uchida et al., 2017) and reduce the thickness of the glial scar in the infarct zone (Karamyan et al., 2013; Shen et al., 2008).

In the present study, we aimed to determine the ideal route of BMSC delivery in MCAO rats. The therapeutic effects on neurological function were examined using mNSS and adhesive removal tests (Toyoshima et al., 2015), and the neuroprotective mechanisms of BMSC transplantation were determined by measuring neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), Ki-67, SYN, Nogo-A and VEGF expression.

## 2. Results

### 2.1. Morphology, BrdU labeling rate and phenotypic characterization of BMSCs

Under a phasecontrast microscope, BMSCs on the first day in primary culture showed growth of the cell suspension (Fig. 1A). On the 3rd day they changed, showing adherent growth and a thin spindle shape (Fig. 1B). After the BMSCs from the second generation were cultured with 10 μm/L BrdU for 48 h, the percentage of $BrdU^{+}$BMSCs was 95.0 ± 8.40% (Fig. 1C). The surface antigen positivity rates in the transplanted BMSCs for CD90, CD29, CD106, CD34, CD45 and CD11b were 91.7 ± 4.23%, 88.4 ± 5.12%, 52.2 ± 3.12%, 2.7 ± 0.41%, 5.7 ± 0.68% and 7.8 ± 0.85%, respectively (Fig. 1D). The BMSCs from the second generation showed high surface antigen expression levels of CD90, CD29 and CD106 and low expression levels of CD34, CD45 and CD11b.

### 2.2. Variability and mortality of MCAO

MCAO rats with an mNSS below7 (n = 9) or above 12 (n = 4) at PID 1 were excluded from the experiment. Some rats died within 24 h (n = 6: 4cases died of subarachnoid hemorrhage, 2 cases died of cerebral edema) and within 2 to 7 days before transplantation (n = 3: 1 cases died of subarachnoid hemorrhage, 1 case died of infection, 1 case died of cerebral parenchymal hemorrhage). Unfortunately, some animals died after transplantation through three different routes, including one rat that died of infection in the intracerebral transplantation group, one rat that died of cerebral parenchymal hemorrhage in the intracerebral control group and one rat that died of malnutrition in the intra-arterial transplantation control group. Thus, 42 rats completed the whole experiment.

### 2.3. BMSC transplantation via different routes leads to behavioral recovery

Before the middle cerebral artery occlusion (MCAO) rats underwent BMSC transplantation, there were no significant differences in the mNSS, the adhesive removal test, and body weight among the control, intracerebral, intravenous, and intra-arterial groups (Fig. 2A, B, C). However, over time, the nerve function of the rats in each group gradually recovered. The mNSS was significantly different between the intra-arterial group (n = 8) and the control group (n = 18) at PIDs 14, 21, 28, and 35 ($p < 0.05$), between the intravenous group (n = 8) and the control group (n = 18) at PIDs 21, 28, and 35 ($p < 0.05$), and between the intracerebral group (n = 8) and the control group (n = 18) at PIDs 28 and 35 ($p < 0.05$) (Fig. 2A). The mNSS was significantly higher in the intra-arterial group than in the intravenous and intracerebral groups at PID 35 ($p < 0.05$) (Fig. 2A). For the adhesive removal test, significantly less time was spent in the intra-arterial group (n = 8) than in the control group (n = 18) at PIDs 21, 28, and 35 ($p < 0.05$); it was also lower in the intravenous (n = 8) and intracerebral (n = 8) groups than in the control group (n = 18) at PIDs 28 and 35 ($p < 0.05$) (Fig. 2B). The time to remove the adhesive paper was shorter in the intra-arterial group than in the intravenous group and the intracerebral group at PID 35 ($p < 0.05$) (Fig. 2B). Over time, the rat weight in each group gradually increased, with a greater increase in the intra-arterial group (n = 8) compared with the control group (n = 18) at PIDs 14, 21, and 28 ($p < 0.05$) and in the intravenous group (n = 8) compared with the control group (n = 18) at PID 14 ($p < 0.05$) (Fig. 2C).

### 2.4. Effects of BMSC transplantation on infarct volume

The volume of cerebral infarction at 35 days after the experimental procedure was measured by H&E staining. Although there were no significant differences in the volume of cerebral infarction in rats transplanted with BMSCs through the intra-arterial (n = 8, 31.5 ± 0.83), intravenous (n = 8, 30.0 ± 0.98) and intracerebral (n = 8, 31.9 ± 0.96) routes compared with the control rats (n = 18, 32.7 ± 0.69), the infarction volumes remained smaller in the rats transplanted with BMSCs than in the control rats ($F = 1.985$; $p = 0.134$, Fig. 3). Moreover, the infarction volume in intravenous group was smaller than that in intra-arterial and intracerebral groups, but there were no statistical differences among them.