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Intranasal administration of human umbilical cord mesenchymal stem cells-conditioned medium enhances vascular remodeling after stroke

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ABSTRACT

Stem cell-based treatments have been reported to be a potential strategy for stroke. However, tumorigenic potential and low survival rates of transplanted cells could attenuate the efficacy of the stem cell-based treatments. The application of stem cell-condition medium (CM) may be a practicable approach to conquer these limitations. In this study, we investigated whether intranasal administration of human umbilical cord mesenchymal stem cells (hUCMSCs)-CM has the therapeutic effects in rats after stroke. Adult male rats were subjected to middle cerebral artery occlusion (MCAo) and were treated by intranasal routine with or without hUCMSCs-CM (1 ml/kg/d), starting 24 h after MCAo and daily for 14 days. Neurological functional tests, blood brain barrier (BBB) leakage, were measured. Angiogenesis and angiogenic factor expression were measured by immunohistochemistry, and Western blot, respectively. hUCMSCs-CM treatment of stroke by intranasal routine starting 24 h after MCAo in rats significantly enhances BBB functional integrity and promotes functional outcome but does not decrease lesion volume compared to rats in DMEM/F12 medium control group and saline control group. Treatment of ischemic rats with hUCMSCs-CM by intranasal routine also significantly decreases the levels of Ang2 and increases the levels of both Ang1 and Tie2 in the ischemic brain. To take

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together, increased expression of Ang1 and Tie2 and decreased expression of Ang2, induced by hUCMSCs-CM treatment, contribute to vascular remodeling in the ischemic brain which plays an important role in functional outcome after stroke.

1. Introduction

Stroke is the third leading cause of morbidity worldwide and remains a major challenge to public health. Because of the limitation of effective therapeutics, it is also a leading cause of serious, long-term disability (Mozaffarian et al., 2015). Cell-based treatments of stroke include neural stem cells, bone marrow stromal cells, and umbilical cord mesenchymal stem cells (UCMSCs) (Zacharek et al., 2010). UCMSCs have potent therapeutic potential for stroke and umbilical cord blood, and implantation of the human UCMSCs (hUCMSCs) into the damaged hemisphere of ischemic stroke rats induce angiogenesis, and promote functional recovery after stroke (Chen et al., 2001; Koh et al., 2008; Lin et al., 2011). hUCMSCs treatment of stroke increases angiogenesis and vascular stabilization, partially mediated by Angiopoietin (Ang)/Tie system (Lin et al., 2011). Ang1 belongs to a family of endothelial growth factors and promotes migration, sprouting, and survival of endothelial cells and thereby mediates vascular remodeling through activation of signaling pathways triggered by Tie2 (Suri et al., 1996). Ang1 plays a role in the recruitment of vascular smooth muscle cells (VSMCs) and pericytes during vascular maturation and the remodeling processes (Sato et al., 1995; Suri et al., 1996). Angiopoietin-2 (Ang2), as an antagonist for Ang1, inhibits Ang1-promoted Tie2 signaling and decreases blood vessel maturation and stabilization (Cui et al., 2011; Ye et al., 2011a).

A major question involved in the use of stem cells for the treatment of stroke is stem cells within normal tissues might be of cancerous origin (Sell, 2010; Stagg, 2008). Therefore, to ensure the safety and efficiency of cell-based therapies, developing an alternative approach to direct transplantation of stem cells is necessary.

Previous studies have reported many growth factors and cytokines derived from the conditioned medium (CM) of various stem cells (Barcelos et al., 2009; Cai et al., 2009; Di Santo et al., 2009; Kinnaird et al., 2004; Perin and Silva, 2009; Yoon et al., 2010), which could be responsible for the paracrine protective effects of stem cells against several diseases. Consequently, the use of stem cells CM instead of direct implantation of stem cells may be a feasible approach to overcome the limitations of current cell-based therapy. In addition, administration of CM resolves the ethics issues involved with cell therapies, because CM is not a cell, but a conjugate of many growth factors such as TGF-β and VEGF (Cho et al., 2012; Inoue et al., 2013).

Despite the important neuroprotective roles of stem cells CM, delivery of it to brain is a significant challenge because of the presence of the blood–brain and the blood–cerebrospinal fluid barriers (Thorne and Frey, 2001). The methods for injecting CM directly into brain tissue in clinical practice are invasive, such as intracerebroventricular, intraparenchymal and intrathecal administration, which are limited to clinical application popularly (Thorne and Frey, 2001). Recently, a new noninvasive intranasal application (INA) drugs into CNS, bypassing the blood brain barrier was reported (Zhu et al., 2012). Previous studies have shown that CM can be treated by intranasal routine (Di Santo et al., 2009). However, whether CM administered by this route in ischemic stroke has neuroprotective effects remained unclear.

In the present study, we investigated whether INA of hUCMSCs-CM improves neurological functional outcome and vascular remodeling in a rat model of middle cerebral artery occlusion (MCAo). Molecular mechanisms underlying vascular remodeling induced by hUCMSCs-CM are described.

2. Results

2.1. hVEGF in hUCMSCs-CM

To tests whether VEGF was expressed in hUCMSCs-CM, Elisa tests have been performed. Fig. 1 shows that hUCMSCs-CM contained significantly higher amounts of hVEGF than DMEM/F12 medium.

2.2. Neurological outcome and lesion volume

To test whether intranasal administration of hUCMSCs-CM regulates functional outcome and lesion volume after stroke, a battery of functional tests was performed. Fig. 2 shows that hUCMSCs-CM group exhibited significantly improved functional recovery (A and B, $p<0.05$), but not reduced lesion volume (C, $p>0.05$) when compared with DMEM/F12 medium control group and saline control group. No significant differences of functional recovery and lesion volume were detected

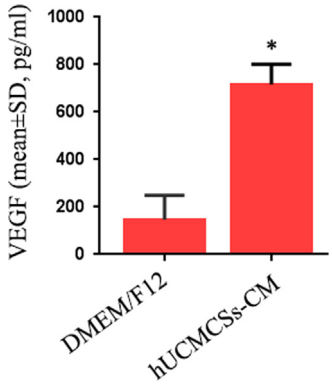

Fig. 1 – shows that hUCMSCs-CM contained significantly higher amounts of hVEGF than DMEM/F12 medium (*$p<0.05$, $n=3$/group).