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# Early neuroprotective effect with lack of long-term cell replacement effect on experimental stroke after intra-arterial transplantation of adipose-derived mesenchymal stromal cells

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**Abstract**

*Background aims.* Adipose-derived mesenchymal stromal cells (AD-MSCs) have high proliferative capacity and ability to secrete trophic factors. Although intra-arterial (IA) transplantation of stem cells induces efficient engraftment to the host brain, it is unclear whether engrafted cells exert their long-term therapeutic effects through a bystander mechanism or a cell replacement mechanism. *Methods.* After induction of ischemia in rats by middle cerebral artery occlusion, we transplanted human AD-MSCs into their carotid arteries with the use of a micro-needle, and we then investigated the therapeutic effects during the early and late phases of ischemia by means of *in vivo* magnetic resonance imaging, functional and histological analyses. *Results.* During the early phase of cerebral ischemia, IA transplantation of AD-MSCs attenuated inflammation and enhanced endogenous neurogenesis. Transplanted animals showed a marked improvement in functional tests during the early phase of cerebral ischemia that was less prominent but still significant during the late phase of cerebral ischemia. Although the transplanted cells effectively migrated to the infarct area, only a small number of engrafted cells survived at 8 weeks after transplantation and differentiated into neuronal, glial and endothelial cells. *Conclusions.* IA transplantation of human AD-MSCs provides an effective therapeutic modality in a rodent model of stroke, of which the main effects are mediated by a bystander mechanism at the early phase of ischemia.

**Key Words:** *adipose tissue, intra-arterial, mesenchymal stromal cells, neuroprotection, stroke, transplantation*

## Introduction

Stroke is one of the major causes of death and disability worldwide. Except for recanalization therapy within 4.5 h of stroke onset [1], there is no established treatment for acute stroke [2]. Stem cell therapy is a promising approach to improve functional deficits in stroke patients, as has been shown with the use of the rodent stroke model [3]. The majority of previous experimental studies have demonstrated that systemically administered adult stem cells exert a neuroprotective effect on the host brain by reducing inflammation or enhancement of angiogenesis through a bystander effect rather than cell replacement [4,5]. Recently, the first clinical trial that used intravenous (IV) administration of bone marrow–derived mesenchymal stromal cells (BM-MSCs) was reported to show a trend toward functional improvement during a 5-year-follow-up study [6,7].

Adipose tissue–derived mesenchymal stromal cells (AD-MSCs) have several advantages over BM-MSCs. AD-MSCs are easy to obtain with minimal invasiveness and are readily cultured to a sufficient number of cells for autologous transplantation without ethical issues [8,9]. Under specific conditions, AD-MSCs have the capacity for differentiation

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into neuronal and glial lineages [10–12]. Moreover, they secrete multiple trophic factors, including granulocyte monocyte colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and brain-derived neurotrophic factor (BDNF) [13–15], all of which potentially benefit brain function after a stroke. Moreover, comparative analysis between AD-MSCs and BM-MSCs showed that AD-MSCs have a higher proliferative activity and a greater production of trophic factors than BM-MSCs [8]. Several studies showed that intravenous or intracerebral transplantation of AD-MSCs or adipose tissue–derived neuronal progenitors ameliorates functional deficits in the rodent stroke model [8,11].

The delivery mode of stem cells is one of the major issues for the clinical application of cell therapy to treat stroke patients [3]. The formation of functional networks between the grafted cells and the host brain is a prerequisite for achieving a long-term effect of stem cells in stroke patients [3]. Although IV administration of stem cells shows promising results in stroke animal models, nearly 90% of the injected cells become trapped in the lung, liver or spleen, which limits their effectiveness in treating brain injury [16–18]. Intracerebral implantation could circumvent this problem, but it is methodologically invasive, especially in the acute phase of stroke when patients are hemodynamically unstable. In contrast, intra-arterial (IA) transplantation proximal to the damaged tissue is an alternative delivery mode for stem cell therapy, because IA-transplanted cells can bypass the peripheral filtering organs, thereby resulting in a higher engraftment rate to the target organ [19–21]. Several experimental studies have demonstrated that IA transplantation efficiently targets transplanted cells around the infarct lesion, even with low doses of cells [16–18]. However, blood flow blockade and intravascular embolism lead to a mortality rate of approximately 40% when the micro-catheter method is used [18]. A recent report of IA transplantation of stem cells with the use of a micro-needle injection method into the carotid artery showed decreased mortality compared with the micro-catheter method, without cerebral blood flow blockade or micro-embolism induction [22].

Although the IA transplantation method can provide a more efficient delivery route to the target area in the ischemic brain, the mechanism by which IA-transplanted cells improve brain function in cerebral ischemia is currently uncertain, because most previous studies have investigated the effects at only a single time point [16,18,22]. If cells engrafted through IA transplantation survive and integrate in the host brain long-term, functional improvement may be sustained, or even further increased, at later stages after transplantation, which would suggest that the therapeutic effects are largely due to a cell replacement mechanism. However, if the engrafted cells do not survive or integrate over the long term, functional improvement would be limited to the early stage, which would suggest that the therapeutic effects are largely due to a bystander mechanism. In this study, we investigated whether IA transplantation of human AD-MSCs with the use of a micro-needle at 1 day after middle cerebral artery occlusion (MCAo) can lead to a functional improvement. To investigate the transplantation effects, we performed histological analyses at different time points after transplantation.

## Methods

### *Ethics statement*

This study was conducted in accordance with the institutional review board of CHA Bundang Medical Center on the use of human AD-MSCs (BD2011-152D), as well as the CHA University Institutional Animal Care and Use Committee on animal experiment (IACUC090012). Human AD-MSCs were obtained from elective liposuction of 23-year-old healthy women, with informed consent.

### *Preparation and characterization of human AD-MSCs*

Isolation and expansion of AD-MSCs were performed as previously described [23]. Briefly, the liposuction waste tissues were digested through the use of 250 U/mL of type I collagenase for 90 min at 37°C and centrifuged at 300*g* for 10 min to obtain the stromal vascular fraction. The cell suspension was layered onto Histopaque-1077 (Sigma-Aldrich) and centrifuged at 840*g* for 10 min. The supernatant was discarded, and the cell band buoyant over Histopaque was collected. The retrieved cell fraction was cultured overnight at 37°C with 5% $CO_2$ in expansion medium [Dulbecco's modified Eagle's medium (DMEM)-HG supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 mg/mL of streptomycin (BioWhittaker)]. The resulting cell population was maintained for 3 to 5 days to reach 80% to 90% confluence. AD-MSCs were sub-cultured after reaching 85% confluence and were used for experiments at passage 5.

For fluorescence-activated cell sorting (FACS) analysis, AD-MSCs cultured in expansion medium for 48 h were washed with phosphate buffered saline (PBS) and incubated with fluorescein isothiocyanate–conjugated antibodies for human CD44, CD73, CD34, CD45, HLA-DR (BD Bioscience) CD90 and CD105 (R&D Systems) for 30 min