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Mesenchymal stromal cell secretomes are modulated by suspension time, delivery vehicle, passage through catheter, and exposure to adjuvants

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Abstract

Background aims. Extensive animal data indicate that mesenchymal stromal cells (MSCs) improve outcome in stroke models. Intra-arterial (IA) injection is a promising route of delivery for MSCs. Therapeutic effect of MSCs in stroke is likely based on the broad repertoire of secreted trophic and immunomodulatory cytokines produced by MSCs. We determined the differential effects of exposing MSCs to different types of clinically relevant vehicles, and/or different additives and passage through a catheter relevant to IA injections. *Methods.* MSCs derived from human bone marrow were tested in the following vehicles: 5% albumin (ALB), 6% Hextend (HEX) and 40% dextran (DEX). Each solution was tested (i) alone, (ii) with low-dose heparin, (iii) with 10% Omnipaque, or (iv) a combination of heparin and Omnipaque. Cells in vehicles were collected directly or passed through an IA catheter, and MSC viability and cytokine release profiles were assessed. *Results.* Cell viability remained above 90% under all tested conditions with albumin being the highest at 97%. Viability was slightly reduced after catheter passage or exposure to heparin or Omnipaque. Catheter passage had little effect on MSC cytokine secretion. ALB led to increased release of angiogenic factors such as vascular endothelial growth factor compared with other vehicles, while HEX and DEX led to suppression of pro-inflammatory cytokines such as interleukin-6. However, when these three vehicles were subjected to catheter passage and/or exposure to additives, the cytokine release profile varied depending on the combination of conditions to which MSCs were exposed. *Discussion.* Exposure of MSCs to certain types of vehicles or additives changes the profile of cytokine secretion. The activation phenotype of MSCs may therefore be affected by the vehicles used for these cells or the exposure to the adjuvants used in their administration.

Key Words: *cytokines, delivery, mesenchymal stromal cell (MSC), secretome*

Introduction

Bone marrow–derived mesenchymal stromal/stem cells (MSCs) are being actively explored for treatment of a range of medical disorders including acute neurological disorders, such as ischemic stroke [1–7]. MSCs have been shown to enhance recovery after stroke in several preclinical studies [8–10]. Their exact mechanism of therapeutic action remains unknown. However, in ischemic stroke, it may be attributed to the release of a wide array of cytokines, chemokines or growth factors, which regulate the tissue environment surrounding the injury [11–13]. These cytokines and growth factors enhance recovery by inducing endogenous angiogenesis, neurogenesis and white matter repair [14–19]. MSCs for ischemic stroke have been investigated in animal studies by administering the cells systemically, either via the intravenous (IV) [10] or

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the intra-arterial (IA) [20] route. Studies suggest that compared with IV delivery, IA administration leads to increased cell numbers delivered directly to the site of injury and lower loss of cells, which might translate to improved outcomes in cell therapy [12].

A number of clinical trials using MSCs are currently underway for stroke [21] using the IV route with different formulations [6,7]. However, an IA approach would differ in the following major aspects. First, the volume of the IA injection would be expected to be much smaller, requiring the cells to be suspended at a greater density. Second, IA administration will require the MSCs to be delivered via a catheter with lumen sizes far smaller than the IV infusion sets, with a greater possibility of mechanical shear/stress to the cells. Additionally, in the IA paradigm, there would be the need for anticoagulation (heparin) and also radio-opaque contrast dyes, such as Omnipaque/Visipaque, to visualize the precise location of cell delivery, thereby exposing the cells to compounds not involved in IV delivery. Previously, studies have been conducted to understand the effects of transcatheter passage of bone marrow–derived mononuclear cells and mesenchymal stromal cells on cellular viability and cytokine release, with results indicating an alteration in the secretome based on infusion rates and size of the catheter [22,23]. To our knowledge, there are no studies assessing each of the major variables involved in a planned endovascular trial delivering MSCs to a targeted organ, such as the brain. In anticipation of clinical trials evaluating the efficacy of IA delivery of MSCs in patients with ischemic stroke and other neurological disorders, it is essential to understand the combined effects of exposing MSCs to various delivery vehicles for different durations of time, the adjuvant chemicals needed for angiography and the actual catheter passage.

Therefore, our study was aimed at determining the combined effects of suspending MSCs in various delivery vehicles for increasing periods of time, their subsequent passage through an IA catheter and also exposure to heparin and Omnipaque. To avoid complexity in study design, we focused on MSCs from a single donor and thus aimed to explore the hypothesis that media and conditions needed to deliver cells intra-arterially would affect the secretome. After exposing MSCs to different conditions, we evaluated their viability and cytokine release profiles.

Methods

Bone marrow MSC isolation and culture

The MSCs were obtained from the bone marrow harvested from a single healthy volunteer. Twenty-five milliliters of bone marrow was cultured in a Quantum cell expansion system (Terumo), a closed system for cell culture, and expanded up to passage 2 (P2) to yield MSCs [24,25]. The cells were cultured in growth medium (Dulbecco's Modified Eagle's Medium) supplemented with 5% pooled human platelet lysate generated from screened blood donors, 2 IU/mL heparin (Fresenius), 2 mmol/L Glutamax (Life Sciences) and 10 mmol/L N-acetylcysteine (Sigma). P2 MSCs were frozen in Plasmalyte A (Baxter) containing 5% human serum albumin (Baxter) and 10% dimethyl sulfoxide (Cryoserv, Bioniche Pharma) and were used in all the subsequent experiments.

Experimental design

An initial phase 1 viability experiment was conducted to test five vehicles that could be used in a clinical trial (Table I). The vehicles tested were phosphate-buffered saline (PBS; Sigma), normal saline (NS; Baxter), 5% albumin in NS (CSL Behring), 6% Hextend (Hospira) and 40% dextran (Hospira). For the experiments, five million MSCs were suspended in 1 mL of respective vehicle for 5, 15, 30 or 60 min at room temperature to simulate the conditions in the endovascular suite for IA delivery. In this study, we

Table I. Conditions to which MSCs were exposed to in phase 1 and 2 experiments.

Phase	Vehicles	Conditions	Duration (min)
1	PBS	Vehicle only	5
	Normal saline	Vehicle only, followed by catheter passage	15
	5% Albumin	Vehicle containing heparin and Omnipaque followed by catheter passage	30
	6% Hextend		60
	40% dextran		
2	5% albumin	Vehicle only	5
	6% Hextend	Vehicle only, followed by catheter passage	30
	40% dextran	Vehicle containing heparin	60
		Vehicle containing heparin, followed by catheter passage	
		Vehicle containing Omnipaque	
		Vehicle containing Omnipaque, followed by catheter passage	

After subjecting MSCs to these conditions, viability and cytokine release assays were performed. Each vehicle was subjected to all sets of conditions (third column) for all exposure duration mentioned (fourth column).