Cytotherapy, 2018; 20: 232–244

International Society for Cellular Therapy
ISCT®

CrossMark

Intramuscular administration potentiates extended dwell time of mesenchymal stromal cells compared to other routes

LORENA R. BRAID[1], CATHERINE A. WOOD[1], DANIELLE M. WIESE[1] & BARRY N. FORD[2]

[1]Aurora BioSolutions Inc., Medicine Hat, Alberta, Canada, and [2]DRDC Suffield Research Centre, Casualty Management Section, Medicine Hat, Alberta, Canada

Abstract

Background. Mesenchymal stromal cells (MSCs) offer great potential for diverse clinical applications. However, conventional systemic infusion of MSCs limits their therapeutic benefit, since intravenously (IV) infused cells become entrapped in the lungs where their dwell time is short. *Methods.* To explore possible alternatives to IV infusion, we used *in vivo* optical imaging to track the bio-distribution and survival of 1 million bioluminescent MSCs administered IV, intraperitoneally (IP), subcutaneously (SC) and intramuscularly (IM) in healthy athymic mice. *Results.* IV-infused MSCs were undetectable within days of administration, whereas MSCs implanted IP or SC were only detected for 3 to 4 weeks. In contrast, MSCs sourced from human umbilical cord matrix or bone marrow survived more than 5 months *in situ* when administered IM. Long-term survival was optimally achieved using low passage cells delivered IM. However, MSCs could undergo approximately 30 doublings before their dwell time was compromised. Cryo-preserved MSCs administered IM promptly after thaw were predominantly cleared after 3 days, whereas equivalent cells cultured overnight prior to implantation survived more than 3 months. *Discussion.* The IM route supports prolonged cell survival of both neo-natal and adult-derived MSCs, although short-term MSC survival was comparable between all tested routes up to day 3. IM implantation presents a useful alternative to achieve clinical benefits from prolonged MSC dwell time at a homeostatic implant site and is a minimally invasive delivery route suitable for many applications. However, optimized thaw protocols that restore full biological potential of cryo-preserved MSC therapies prior to implantation must be developed.

Key Words: *bone marrow, cell survival, mesenchymal stromal cell, optical imaging, transplantation, umbilical cord*

Introduction

Mesenchymal stromal cells (MSCs) are a heterogeneous population of progenitor cells [1–5] exhibiting numerous therapeutically useful properties. Moreover, a growing body of evidence supports the notion that MSCs are highly suitable vectors for a range of therapeutic molecules, including growth factors, drugs and monoclonal antibodies [6–17]. Human umbilical cord perivascular cells (HUCPVCs) are a rich, well-characterized source of MSCs [18] highly amenable to engineering [6,7], stockpiling [19,20] and allogeneic transplantation [19,21,22]. HUCPVCs have exhibited robust clinical potential for a range of indications including inflammation [23–25], wound healing [24,26], myocardial infarction [25,27] and lung transplantation [28] and as gene therapy vectors for osteogenic repair [6] and bioweapons defense [7].

The consistent safety of administered MSCs [29–34] has been reported from numerous clinical trials. However, MSC therapies have had variable success in meeting required endpoints in phase 2 and phase 3 clinical trials [35], in part due to limited persistence of cell transplants. The current standard practice for delivering cell therapies is by intravenous (IV) infusion. However, numerous studies have consistently demonstrated that IV-infused cells largely become trapped in the capillaries of the lungs, where they fail to survive longer than a few days [34,36–40]. This phenomenon truncates the potential therapeutic benefit of applied MSCs, a limitation cited in both clinical trials and animal studies [32,41,42].

There have been recent experimental demonstrations of MSCs as an active secretion platform to modulate the pharmacokinetics of therapeutic factors, including increasing numbers of reports that natural and engineered MSCs can provide sustained, continuous delivery of innate biomolecules and exogenous drugs and antibodies [8–17,43–45]. The reported correlations between cell persistence and systemic circulation of MSC-derived factors [7,36] suggest that non-conventional protocols may be useful to achieve

Correspondence: **Lorena R. Braid**, PhD, 350 11th Street NE, Medicine Hat, Alberta T1A 5T2, Canada. E-mail: lorena@aurorabiosolutions.com

(Received 25 April 2017; accepted 27 September 2017)

https://doi.org/10.1016/j.jcyt.2017.09.013

optimal sustained benefit from MSC therapies. Few studies, however, have combined cell bio-distribution data with serum profiles of MSC-secreted factors. Cells administered IV exhibit an acutely truncated serum profile of secreted factors, lasting less than 3 days [36]. In contrast, we recently reported that engineered MSCs, administered intramuscularly (IM), were still detectable at the implant site more than 100 days after transplantation where they continued to secrete a functional antibody into circulation [7]. We selected the IM administration route over IV infusion because our concept of use is for administration outside the boundaries of the clinic, in the field and for mass casualty scenarios. Here we sought to identify the key aspects of the previous study that generated such an unexpected and useful result, postulating that the observed persistence may be a consequence of the administration route, the early passage of the cells used in that study or the increased survival potential of the neo-natal cord-derived MSCs compared with adult-sourced MSCs such as bone marrow (BM). We also sought to identify the organs preferentially populated by MSCs administered by different routes, to aid in developing organ- or target-specific therapies for various indications.

Direct comparative evidence for MSC survival and bio-distribution following implantation by various routes is currently lacking. The parameters of individual studies, including cell source, isolation and expansion conditions, implantation routes and delivery vehicles, immune-competency, disease or injury state and type of animal model, combined with various cell labeling and detection methods preclude reliable comparisons between existing data sets.

The aim of this study was to execute a controlled, side-by-side comparison of the effects of delivery route and passage number (i.e., time in culture prior to implantation) on survival and distribution of a clinically relevant population of cells—HUCPVCs. In the present work, we performed a longitudinal comparison of the dwell time and bio-distribution of HUCPVCs administered by four clinically relevant routes: IV, intraperitoneal (IP), subcutaneous (SC) and IM. Next, we examined the effects of *in vitro* expansion and cryopreservation on the dwell time of IM implanted HUCPVCs. Finally, we tested whether IM implantation also potentiates extended dwell time of human BM-derived MSCs. Taken together, our data identify IM implantation as an optimal route to achieve prolonged post-transplantation survival of MSCs, a critical factor in achieving controlled, sustained therapeutic benefit of applied MSCs. Finally, these data reveal that HUCPVCs can be subject to considerable expansion *in vitro* and still retain their persistence after IM implantation, and confirm a recent report that MSCs administered directly from cryogenic storage may be functionally compromised [46].

Materials and methods

Cell culture

HUCPVCs [22] cryo-preserved at passage 2 were provided by Tissue Regeneration Therapeutics (TRT), Inc. HUCPVCs were thawed according to TRT's proprietary standard operating procedures and expanded in Mesenchymal Stem Cell Growth Media—Chemically Defined (MSCGM-CD; Lonza). Passage 1 human BM-MSCs were also provided by TRT. BM-MSCs were recovered in isolation media–Alpha-Minimum Essential Medium (MEM; Life Technologies) supplemented with 15% MSC-Fetal Bovine Serum (FBS; Life Technologies)–then weaned to MSCGM-CD to facilitate direct comparison with HUCPVCs. At 70–80% confluence, MSCs were enzymatically detached from the culture vessel by brief incubation with TrypLE Select (Life Technologies), and re-seeded at a density of 4000 cells/cm^2. Culture conditions were maintained at 37°C, 5% CO_2, 80% relative humidity, with media replacement every 3–4 days.

For cryo-preservation, cells were enzymatically detached using TrypLE Select, pelleted by centrifugation at 149*g*, then resuspended in 50% MSCGM-CD and 50% EZ-CPZ (InCell) cryo-preservation media. Cryogenic vials were rapidly transferred to a CoolCell (Biocision) controlled-rate freezer and stored at -80°C overnight, then transferred to liquid nitrogen for cryogenic storage.

Bioluminescent MSCs

For transient engineering of MSCs with the *firefly luciferase (ffluc)* gene, HUCPVCs and BM-MSCs at approximately 70% confluence in T150 flasks were incubated with a recombinant adenovirus serotype 5 encoding *ffluc* (Vector BioLabs), at 200 multiplicity of infection (MOI), suspended in 7.5 mL of MSCGM-CD (Lonza) without antibiotics for 3 h. Transduction media was removed and replaced with MSCGM-CD supplemented with antibiotic-antimycotic (Life Technologies) without washing. Twenty-four hours after transduction, cells were enzymatically detached using TrypLE Select, counted using a Millipore Scepter 2.0 (EMD Millipore), washed once in excess Hank's Balanced Salt Solution (HBSS; Life Technologies), then resuspended in an appropriate volume of HBSS to generate doses of 1 million cells per 75 μL. Cell viability in the dose cell suspension was verified by Trypan blue exclusion using a TC20 cell counter (BioRad) prior to implantation and again after administration of the final dose. Athymic mice received a single injection of 1 million cells in HBSS by IV infusion or IP, SC or IM injection.

To engineer MSCs with an integrated bioluminescent reporter gene, 2 million P2 HUCPVCs at