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SHORT COMMUNICATION

Suspension Medium Influences Interaction of Mesenchymal Stromal Cells with Endothelium and Pulmonary Toxicity after Transplantation In Mice

ERIKA DEAK[1], BRIGITTE RÜSTER[1], LISA KELLER[1], KLAUS ECKERT[2], IDUNA FICHTNER[2], ERHARD SEIFRIED[1] & REINHARD HENSCHLER[1]

[1]*Stem Cell Biology Group, DRK Institute of Transfusion Medicine and Immune Hematology, Johann Wolfgang Goethe University, Frankfurt am Main, Germany, and* [2]*Experimental Pharmacology and Oncology, Max Delbrück Center for Molecular Medicine, Charite University Medicine Berlin, Germany.*

Abstract

Intravenous (i.v.) transplantation and subsequent homing of Mesenchymal Stromal Cells (MSC) may be adversely influenced by their relatively high adhesion capacity and their tendency to aggregate, leading to clogging of capillaries especially in the lungs. We evaluated the ability of murine MSC suspended in EDTA or heparin in buffered saline solution on their spontaneous adhesion to endothelial cells *in vitro,* under shear stress and their *in vivo* tolerability after i.v. injection. We show that suspension of MSC in heparin was highly beneficial, avoiding clinical symptoms in 95% of mice, whereas application of MSC suspended in PBS/EDTA or control buffer caused severe pulmonary reactions and partly, death. *In vitro* studies using parallel plate flow chambers revealed increased adhesion of MSC suspended in PBS/EDTA to endothelial cells compared with MSC in PBS/heparin. These data provide a means to predict and to interfere with toxicity of *i.v.* transplanted MSC.

Key Words: *lung toxicity; mesenchymal stromal cells, heparin, EDTA.*

Abbreviations: BSA, bovine serum albumin; DMEM, Dulbecco´s modified Eagle´s medium; EDTA, ethylenediaminetetraacetic acid; FITC, fluorescein isothiocyanate; HUVEC, human umbilical vein endothelial cells; MSC, mesenchymal stromal cells; NOD/SCID, non-obese diabetic/severe combined immunodeficiency; PBS, phosphate-buffered saline solution; VCAM-1, Vascular Cell Adhesion Molecule-1.

Background

Mesenchymal stromal cells (MSC) are a relatively rare population of plastic-adherent cells with multitissue regeneration capacity, which have been initially isolated from bone marrow and which have more recently been found to be present in various tissues (1). In cell culture, MSC display a fibroblastic morphology, can be expanded over several weeks by at least 4-6 logs, and can be differentiated into specific cell types including osteoblasts, chondrocytic cells, adipogenic cells or tendon and ligament type cells (2). In addition to concepts using MSC as a locally implanted cellular therapeutic, MSC have recently also been applied intravenously *(i.v.)* e.g. to confer cardiovascular regeneration, to improve hematopoietic regeneration after bone marrow transplantation, or to modulate immune responses such as graft-versus-host disease in patients after allogeneic stem cell transplantation (3,4). This way, the use of MSC in both, preclinical models and clinical studies has increased in order to further explore the mode of action of these cells and to better assess their potential toxicity and side effects.

A major goal of preclinical cell therapy models using MSC is to provide protocols to apply MSC safely and without hazard. However, specifically in transplantation models using *i.v.* applied rat or murine MSC, the transplanted cells may not be well tolerated, due to pulmonary embolism or unwanted incorporation into lung interstitium (5,6). On the

Correspondence to: Dr. **Med. Reinhard Henschler**, Institute of Transfusion Medicine and Immune Hematology, German Red Cross Blood Donor Center, Johann Wolfgang Goethe-University Frankfurt, Sandhofstrasse 1, D-60528 Frankfurt. Telephone: +49 69 6782 191; Fax: +49 69 6782 258; e-mail: r.henschler@blutspende.de

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other hand, a model for murine MSC lung damage is likely of relevance also in the human situation. Still, pulmonary effects of *i.v.* applied MSC have been difficult to assess in clinical trials since imaging technology with high resolution e.g. over lungs or liver tissue is currently not available, and since cases assessing acute tissue damage through histological diagnosis are rare. Therefore, exploration of the toxicity of MSC in the murine lungs is also of relevance for the development of preclinical and clinical models for therapies using *i.v.* applied MSC.

We show here that suspension of murine MSC in buffered media before transplantation results in a major difference in tolerability and acute toxicity. Murine MSC suspended in heparin-substituted PBS are comparable in their toxicity with human MSC suspended in PBS/EDTA, resulting in no detectable toxicity. In contrast, application of murine MSC suspended in PBS/EDTA or buffer alone leads to pulmonary embolism, suffering and death in a proportion of the transplanted mice. *In vitro* shear stress studies confirm increased endothelial adhesion of MSC suspended in PBS/EDTA or PBS alone, and indicate a role of increased binding of MSC to the endothelial integrin ligand, VCAM-1.

Materials and Methods

Chemicals and reagents. Antibodies against murine CD45 (clone 30-F11), CD73 (clone TY/23), CD31 (clone MEC 13.3), or against human CD45 (clone 2D1), CD73 (AD2), and CD105 (266) were obtained from BD Pharmingen, San Jose, CA. Anti-murine CD 105 (clone 209710) was from R&D Systems (Wiesbaden, Germany) and the function blocking antibody against vascular cell adhesion molecule-1 (VCAM-1, CD106, clone 1G11) from Immunotech (Marseille, France). Recombinant murine VCAM-1--Ig fusion protein was obtained from R&D Systems and basic Fibroblast Growth Factor (bFGF) from TeBU-bio (Offenbach, Germany). The PKH-26 fluorescente cell linker kit and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (Steinheim, Germany); 0.5% Trypsin /EDTA (10x) solution was from Invitrogen (Karlsruhe, Germany) and heparin-Natrium 5000 I.E./0.2 ml was obtained from Ratiopharm (Ulm, Germany). C57/BL6 were purchased from Charles River Laboratories (Sulzfeld, Germany) and NOD/SCID mice from Jackson Laboratory, Bar Harbor, USA.

Cells. Murine MSC were isolated by the adherence method. Therefore, bone marrow cells were harvested from femurs and tibiae of C57Bl\6 donor mice after cervical dislocation. Bone marrow cells were plated at a density of 5×10^5 nucleated cells/cm^2 in DMEM supplemented with 10% FCS, 100 IU/ml penicillin, 0.1 mg/ml streptomycin and 2 ng/ml bFGF, and incubated in a humidified atmosphere at 37°C and 5%CO_2. The medium was changed completely every 3 - 4 days. After 3 - 4 weeks, a layer of adherent spindle-shaped cells was obtained. These cells were stained negative for CD45 and positive for mesenchymal markers CD105 and CD73 by flow-cytometric analysis. Their multipotency was confirmed by successful induction of osteoblastic and adipogenic differentiation upon addition of the respective media, NH AdipoDiff Medium and NH OsteoDiff Medium (Miltenyi Biotec, Bergisch Gladbach, Germany) and subsequent positive staining for fat cells using oil red O, or osteoblasts using von Kossa stain (7). Human MSC were isolated and cultured as described previously (7,8) from bone marrow samples harvested from the iliac crest of patients undergoing hip replacement surgery after informed consent under a procedure approved by the local ethics committee. Human MSC were also negative for CD45, positive for CD105 and CD73 upon flow-cytometric analysis, and displayed trilineage differentiation potential (adipogenic, chondrogenic and osteogenic) using NH AdipoDiff Medium, NH ChondroDiff Medium and NH OsteoDiff Medium (Miltenyi Biotec) (7). Human umbilical vein endothelial cells (HUVEC), purchased from Cambrex (Brussels, Belgium) were thawed and passaged according to the manufacturers´ protocols. HUVEC were seeded into µ slide parallel plate flow chambers (Ibidi, München, Germany) and used for experiments until passage 6 as described previously (7).

In vitro aggregation studies. Murine MSC were trypsinized and after centrifugation were immediately suspended in PBS alone, PBS supplemented with 10 mM EDTA or PBS supplemented with 100 U/ml heparin, seeded at 5×10^5 nucleated cells/cm^2 into 6 well plates and incubated in a humidified atmosphere at 37°C and 5%CO_2 for 0, 10 or 20 min. Cell aggregate formation on the bottom of the plates was analyzed under a light microscope under 40 fold magnification and photodocumented using a charge coupled device (CCD). camera. Evaluation was done using a size determination grid (Eppendorf, Hamburg, Germany) and measurement of the diameters of the resulting aggregates. Statistical analysis was performed using an unpaired two-sided student´s t-test.

Immunohistochemistry. Lung tissue sections were prepared from C57Bl/6 mice which were killed by cervical dislocation 1h after i.v. injection of 1×10^6 PKH26 labelled murine MSC. The organs were embedded and frozen in Tissue Teck (Sakura Finetek Europe, Zoeterwoude, The Netherlands) at –80°C. For immunohistochemistry, 6µm sections were prepared on glass slides. After thawing, slides