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Safety and biodistribution study of bone marrow–derived mesenchymal stromal cells and mononuclear cells and the impact of the administration route in an intact porcine model

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Abstract

Background aims. Bone marrow mononuclear cells (BM-MNCs) and bone marrow–derived mesenchymal stem stromal cells (BM-MSCs) could have therapeutic potential for numerous conditions, including ischemia-related injury. Cells transplanted intravascularly may become entrapped in the lungs, which potentially decreases their therapeutic effect and increases the risk for embolism. *Methods.* Twelve pigs were divided into groups of 3 and received ^{99m}Tc- hydroxymethyl-propylene-amine-oxime–labeled autologous BM-MNCs or allogeneic BM-MSCs by either intravenous (IV) or intra-arterial (IA) transplantation. A whole body scan and single photon emission computed tomography/computed tomography (SPECT/CT) were performed 8 h later, and tissue biopsies were collected for gamma counting. A helical CT scan was also performed on 4 pigs to detect possible pulmonary embolism, 2 after IV BM-MSC injection and 2 after saline injection. *Results.* The transplantation route had a greater impact on the biodistribution of the BM-MSCs than the BM-MNCs. The BM-MNCs accumulated in the spleen and bones, irrespective of the administration route. The BM-MSCs had relatively higher uptake in the kidneys. The IA transplantation decreased the deposition of BM-MSCs in the lungs and increased uptake in other organs, especially in the liver. Lung atelectases were frequent due to mechanical ventilation and attracted transplanted cells. CT did not reveal any pulmonary embolism. *Conclusions.* Both administration routes were found to be safe, but iatrogenic atelectasis might be an issue when cells accumulate in the lungs. The IA administration is effective in avoiding pulmonary entrapment of BM-MSCs. The cell type and administration method both have a major impact on the acute homing.

Key Words: *biodistribution, imaging, stem cells*

Introduction

During the past decade, bone marrow–derived mesenchymal stromal cells (BM-MSCs) have been used in various forms of restorative and preventive medicine and have shown positive effects in ischemic stroke and cardiovascular diseases, which are major causes of morbidity and mortality [1]. Results from preclinical studies and initial clinical trials have been intriguing as BM-MSCs appear to act as immunomodulatory and regenerative agents and could offer major advantages in the clinical treatment of a variety of diseases [2–6]. Immunomodulation is considered a major factor in the effectiveness of BM-MSCs [1], and promising results have been reported when they are used in steroid-resistant graft-versus-host disease or renal transplant rejection [8]. Despite a growing number of studies using BM-MSCs, there is a lack of fundamental information about how administration of stem cells should be executed and optimized [9].

The systemic administration of *in vitro* expanded cells to the vascular system is the main focus of the therapeutic approach because it would provide a simple way to use stem cells in clinical practice; in addition, systemic administration is the only feasible option in such therapeutic implications as graft-versus-host disease [7]. We have no knowledge how

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nature uses mesenchymal stem cells. Hence, more detailed information about factors contributing to the success of stem cell therapy is needed [10]. It is thought that the administration route and chosen cell lineage can have a major impact on the biodistribution and organ targeting after administration [11]. The clinical potency of bone marrow mononuclear cells (BM-MNCs) has also been observed [12]. The therapeutic results obtained with BM-MSCs have differed from those obtained with BM-MNCs [13]. Yet BM-MNCs are more easily obtainable from an autologous source, and a better understanding of their biodynamic characteristics is needed.

It is known that intravenously (IV) transplanted bone marrow cells accumulate in the lungs in large numbers [14,15]. This is considered an unwanted result that limits the therapeutic effectiveness of the transplanted cells at the targeted site [14,16,17]. It has been suggested that transplanted bone marrow stromal cells can be trapped in the lung capillaries purely due to their size [18]. This could be speculated to have adverse effects on the lungs and even cause embolus formation [19,20]. Cell entrapment at the precapillary level and microembolus formation have also been associated with intra-arterial (IA) administration of BM-MSCs [19,21]; in addition, decreased blood flow and myocardial infarction have been observed when injecting BM-MSCs intracoronarily [16,22]. The deposition and clearance of transplanted cells in lungs may be far more complex than pure mechanical obstruction with various cellular level interactions participating in tissue distribution [15].

The primary aim of our study was to use the porcine model to assess the biodistribution of freshly isolated BM-MNCs and cultivated BM-MSCs using 3 radionuclide methods. The second aim was to study possible adverse effects of administration, such as pulmonary emboli. To our knowledge, this is the first study to assess the effect of an administration route on the biodistribution of bone marrow derived cells in a large animal model without any experimental trauma. We believe these data will provide fundamental information for future studies and clinical trials.

Methods

Animals and study layout

Twelve female domestic pigs with a median weight of 22.9 kg (interquartile range: 20.73–24.1 kg) underwent surgery. Before surgery, the animals were kept in specific porcine housing facilities of University of Oulu Laboratory animal center and euthanized after the experiment. The animals were divided into 4 study groups. Six animals received BM-MNCs and the other six BM-MSCs. These groups were further divided so that half of the animals received ^{99m}technetium hydroxymethyl-propylene-amine-oxime (^{99m}Tc-HMPAO)-labeled cells by intravenous (IV) administration and the other half by intra-arterial (IA) administration. There were 3 animals in each group. Animals were followed for 8 h before radionuclide imaging, and biopsies were taken for gamma counting. In addition, 2 pigs underwent helical computed tomography (CT) after IV BM-MSC injection and 2 after saline injection to detect possible pulmonary embolism. A single animal with saline injection underwent identical protocol. Including a larger number of animals in each group was considered unethical because of the descriptive nature of the study.

All the studied animals received humane care in accordance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council (published by the National Academy Press, revised in 1996). The study was approved by the Research Animal Care and Use Committee of the University of Oulu, Oulu, Finland.

Anesthesia and surgical procedures

The animals were sedated with ketamine hydrochloride (15 mg/kg) and midazolam (2 mg/kg). Anesthesia was induced with thiopental and fentanyl followed by endotracheal intubation. The anesthesia was maintained by a continuous infusion of fentanyl (25 μg/kg/h), midazolam (0.25 mg/kg/h), pancuronium (0.2 mg/kg/h) and inhaled isoflurane (1.0%). The animals received intravenous Ringer's acetate (15 mL/kg/h) throughout the experiment. A continuous electrocardiogram was recorded. The left femoral artery and vein were approached through a surgical opening and carefully exposed and ligated. A Swan-Ganz type triluminal catheter was openly inserted in the femoral vein, and the catheter's tip was led to the pulmonary artery by assessing blood pressure curves. The described method is commonly used in clinical practice. The arterial needle was inserted in the femoral artery and the urinary catheter was installed. Blood samples were collected frequently from a central venous catheter for arterial blood gas and serum electrolyte analyses. Mean arterial pressure was maintained between 60 and 70 mm Hg using crystalloid fluids and L-norepinephrine- dopamine infusion, if necessary.