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Safety evaluation of exosomes derived from human umbilical cord mesenchymal stromal cell

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Abstract

Background aims. Mounting evidence shows that non–cell-based transplantation of exosomes derived from mesenchymal stromal cells (MSCs) has more potential protective and reparative effects than MSCs have. However, whether it is safe to transplant MSC exosomes into tissues is still not clear. In this study, we evaluated the safety of transplantation of exosomes derived from human umbilical cord MSCs (hucMSC exosomes). *Methods.* hucMSC exosomes were incubated with the cardiac blood from a healthy rabbit, and hemolysis was observed. For analysis of vascular and muscle stimulation, pyrogen, systemic anaphylaxis and hematology indexes, hucMSC exosomes were given to rabbits, guinea pigs and rats. The histological changes in the vascular and muscle sites of injection in rabbits were analyzed by hematoxylin and eosin staining. Allergy symptoms in guinea pigs and rectal temperature of rabbits were observed and recorded. To study safety *in vivo*, hucMSC exosomes were infused intravenously into rats with acute myocardial infarction. Rats' weight was measured and tail vein blood was collected to evaluate liver and renal function. *Results.* hucMSC exosomes had a protective effect on weight loss and had no adverse effects on liver or renal function. Other detections, such as hemolysis, vascular and muscle stimulation, systemic anaphylaxis, pyrogen and hematology indexes, also showed that hucMSC exosomes were applicable. *Conclusions.* hucMSC exosomes are well tolerated in animal models. This study provides evidence for the safety of intravenous infusion in future clinical therapy.

Key Words: *exosomes, human umbilical cord, mesenchymal stromal cell, safety*

Introduction

Mesenchymal stromal cells (MSCs) represent a promising young-state stem cell source for cell-based therapy. It has been reported that MSC transplantation is beneficial for the treatment of several kinds of diseases [1]. However, transplantation therapy is restrained by several shortcomings. For example, when using viable replicating cells as therapeutic agents, the biological potency of the agents cannot be attenuated when the treatment is already terminated and may be amplified over time when the need for therapy has been eliminated [2]. Although direct endomyocardial transplantation of MSCs is safe [3], intravascular infusion may lead to occlusion in the distal microvasculature because of the large cell size [4]. Moreover, transplantation of human MSCs is reported to promote tumor growth [5]. The potential of MSCs to differentiate into osteocytes and chondrocytes also raises long-term safety concerns regarding tissue ossification or calcification as reported in some animal studies [6]. The possibility of cell fusion at the site of injury also cannot be eliminated [7–9], and it is estimated that <1% of transplanted cells actually reach the target tissue, with most of the cells trapped in the liver, spleen and lungs [10].

With the emergence of the paracrine hypothesis, the therapeutic application of exosomes is more promising. It has been reported that exosomes have functions similar to those of MSCs [11]. By replacing transplantation of MSCs with exosomes, many of the safety concerns and limitations associated with the transplantation of MSCs could be mitigated. Exosomes are one of several groups of secreted vesicles that also include microvesicles, membrane particles, ectosomes, exosome-like vesicles and apoptotic bodies. Among various secreted vesicles, exosomes have better defined biophysical and biochemical properties. They have a diameter of 50–100 nm, with a density in sucrose of 1.13–1.19 g/mL, and can be sedimented at 100 000*g*.

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Their membranes are enriched in cholesterol, ceramide, sphingomyelin and lipid rafts [12]. Exosomes are often used as an alternative for existing delivery vehicles. They contain both proteins and RNAs and can easily pass the contents of cells across the cell membrane and deliver biologically active macromolecules [13–15]. Most exosomes have an evolutionarily conserved set of proteins, including tetraspanins (CD9, CD63 and CD81), Alix and Tsg101, and they also have unique tissue- or cell-type proteins that reflect their cellular sources [16]. Exosomes are increasingly recognized as major players in cell-independent communication. They transfer bioactive signaling molecules through activation of cell surface receptors on a target cell, fusion with the recipient cell or internalization. Exosomes, as natural cell-derived nano-carriers, are also immunologically inert if purified from a compatible cell source [13,14]. Exosomes may overcome the hurdles of immunogenicity, even after repeat administration, particularly because they can be engineered to be immunosuppressive [17]. Exosomes also have the advantage of convenient storage and transportation. They can be stored at −70°C for several months without change in their biochemical activities.

Therefore, the non–cell-based transplantation of exosomes may overcome the deficiencies observed in MSCs and possess a better prospective for clinical use. However, it is still not clear whether it is safe to transplant MSC exosomes into tissues, and there is an urgent need to evaluate this. Because exosomes may be applied to clinical therapy in the future, we focus on exosomes derived from human umbilical cord mesenchymal stem cell (hucMSC exosomes) to evaluate safety.

Methods

Cell culture

Human umbilical cord mesenchymal stem cells were isolated and cultured as described previously [18]. All individuals provided informed consent for the use of their cord in this experimental study, which was approved by the ethical committee of Jiangsu University, China.

Isolation, purification and characterization of hucMSC exosomes

The hucMSC exosomes were isolated as described previously, with minor modifications [19]. Low-glucose Dulbecco's Modified Eagle's Medium (L-DMEM) with 10% fetal bovine serum (FBS) was replaced with exosome-free FBS/L-DMEM when hucMSCs reached 80–90% confluence. The conditioned medium of hucMSC (hucMSC-CM) was collected after cells were cultured with exosome-free FBS L-DMEM for 48 h. hucMSC-CM was centrifuged at 300*g* for 20 min, 2000*g* for 20 min, and 10 000*g* for 30 min to remove cell debris. The hucMSC-CM was concentrated with a 100-kDa molecular weight cutoff hollow fiber membrane (Millipore) and centrifuged at 1000*g* for 30 min. The concentrated hucMSC-CM was loaded onto 5 mL 30% sucrose/D_2O cushions and ultracentrifuged at 100 000*g* for 2 h (Optimal-90k; Beckman Coulter). The bottom of the cushion containing the hucMSC exosomes was collected and washed three times with phosphate-buffered saline (PBS) using a 100-kDa molecular weight cutoff centrifuge tube at 1000*g* for 30 min. The hucMSC exosomes were filtered through a 0.22-μm membrane filter (Millipore) and stored at −70°C for later use. The protein content of hucMSC exosomes was tested by a BCA Protein Assay kit (CWbio), and the number of hucMSC exosomes was quantified using nanoparticle tracking analysis, as described previously [20]. The CD9 (Bioworld) and CD81 (Epitomics) molecules, which were frequently located on the surface of exosomes, were analyzed using Western blotting.

Hemolysis

Approximately 20 mL of blood were collected from a healthy rabbit heart. Glass bead shaking vibration was used to remove the fibrinogen in blood, then it was washed with PBS and centrifuged three times at 1000 rpm for 15 min. Two percent red blood cell (RBC) suspension (2.5 mL) was added to the following three groups: (i) negative control group with 2.5 mL normal saline added, (ii) positive control group with 2.5 mL distilled water added and (iii) hucMSC exosomes group with 400 μg hucMSC exosomes added. The mixed liquids were blended gently and placed in a 37°C incubator. Hemolysis and erythrocyte sedimentation in each group were observed at 3 h.

Vascular stimulation

Three male and three female rabbits were used. hucMSC exosomes (400 μg) diluted with 200 μL PBS were infused slowly via the right ear marginal vein and 200 μL PBS was infused slowly via the left ear marginal vein as a control, once daily for 5 days. The vascular and surrounding tissues were observed, and any abnormal changes were recorded to evaluate the influence of the treatment. The rabbits were sacrificed 96 h after the last treatment. Vascular and surrounding tissues were collected and fixed in 4% paraformaldehyde, embedded in paraffin and cut into 5-μm serial sections, which were then stained with hematoxylin and eosin (H&E). The degree of vascular stimulation was assessed according to macroscopic observation and histopathological examination (Table I). Images were captured using an upright metallurgical

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