Evaluation of the Biodistribution of Human Dental Pulp Stem Cells Transplanted into Mice

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Abstract

Introduction: Several studies have attempted to use human dental pulp stem cells (hDPSCs) for pulp-dentin complex regeneration *in vitro*. However, the safety of such applications should be first evaluated *in vivo* before their use in clinical trials. The purpose of this study was to investigate the *in vivo* fate of intrapulpally transplanted hDPSCs. **Methods:** hDPSCs were isolated and cultured from impacted third molars. *In vivo* experiments were performed using 7-week-old male BALB/c nude mice. Under deep anesthesia, 1×10^5 hDPSCs were transplanted in mice via the tail vein for intravenous injection or into the pulp chamber for intrapulpal transplantation. A total of 56 mice, 28 per group, were used. Mice were sacrificed at different time points, and the numbers of hDPSCs in the organs were analyzed quantitatively. In addition, qualitative analysis was performed to detect intrapulpally transplanted hDPSCs. **Results:** Intravenously injected hDPSCs were mostly distributed to the lungs and rarely detected in other organs at all observed time points. The hDPSCs transplanted into the pulp chamber rarely migrated to other organs over time. **Conclusions:** These data indicate a differential distribution of transplanted hDPSCs between the intravenous and intrapulpal route and show the safety of pulpal transplantation of hDPSCs. *(J Endod 2017;■:1–7)*

Key Words

Biodistribution, human dental pulp stem cells, mouse, pulp-dentin complex regeneration

Significance

In this study, intrapulpally transplanted hDPSCs exhibited very low distribution in other organs until 48 hours. These data indicate a differential distribution of transplanted hDPSCs between the intravenous and intrapulpal route and show the safety of pulpal transplantation of hDPSCs.

In recent years, various stem cells have been isolated from the oral cavity, including human dental pulp stem cells (hDPSCs) (1). In 2000, Gronthos et al (2, 3) isolated hDPSCs from human third molars for the first time, and these hDPSCs were characterized as highly proliferative cells with self-renewing multidifferentiation properties *in vitro*. They also reported that the hDPSCs generated a dentinlike structure lined with human odontoblastlike cells that surrounded a pulplike interstitial tissue when transplanted into immunocompromised mice (2). Since the first report about the possibility of pulp-dentin complex regeneration using hDPSCs, several groups have studied the role of hDPSCs in pulp-dentin complex regeneration *in vitro* (4–6).

Unlike the controlled environment of *in vitro* systems, transplantation of hDPSCs into animal pulp space requires special techniques and advanced skills. Because of these difficulties, *in vivo* studies on pulp-dentin complex regeneration have generally involved ectopic transplantation of the candidate substance into the subcutaneous tissue, rather than orthotopic transplantation directly into the pulp (7). However, before applying these candidate cells in clinical trials, their treatment efficacy and safety should be evaluated using orthotopic transplantation. It is important to determine the fate of transplanted hDPSCs for safety concerns.

Mesenchymal stem cells (MSCs) possess the capability to differentiate into a variety of cell types. In addition, recent studies indicate that stem cells and tumor cells share many common master regulatory genes (8–10). The interwoven nature of multipotency and tumorigenicity programs is highlighted by the overlap in their molecular machineries, and it is a major challenge to untangle the determinants of multipotency from those of tumorigenicity. Furthermore, the ability of intravenously administered MSCs to migrate to the sites of microscopic tumor lesions was confirmed by micro–positron emission tomography imaging (11). Human immortalized MSCs injected into the tail vein of mice with pre-established colon cancer localized in the capillarylike structures and within the inner layer of small arterioles and in the connective tissue of the tumor and enhanced tumor growth (11). Similarly, human MSCs injected into the tail vein of severe combined

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immunodeficiency mice with human malignant melanoma were incorporated into tumor vessels (12). These findings indicate that MSCs may contribute to the development of the vascular and connective tissue components of the tumor stroma.

Because of these characteristics of MSCs, there is a risk of tumorigenic effects of hDPSCs transplanted into the pulp chamber if the cells migrate to distant organ systems. Thus, it is crucial to not only assess the efficacy of pulp-dentin complex regeneration by hDPSCs transplantation through animal studies but also to verify the *in vivo* fate of hDPSCs transplanted in the pulp. Previous studies showed that most MSCs were entrapped in the lung immediately after intravenous injection, with some MSCs undergoing apoptosis (13, 14). In addition to intravenous injection, researchers have analyzed the systemic distribution and retention of stem cells in the target organ after direct transplantation into the target organ (15, 16). Although *in vivo* fate studies using stem cells are being actively performed in other areas of medicine, the fate of systemically and locally administrated hDPSCs is not fully understood. Therefore, the purpose of this study was to test the *in vivo* fate of intrapulpally transplanted hDPSCs compared with intravenously injected hDPSCs in mice.

Materials and Methods

Isolation, Culture, and Characterization of hDPSCs

Volunteers were recruited from patients who were scheduled for third molar extraction at Yonsei University Dental Hospital, Seoul, South Korea. Informed consent was obtained from each volunteer, and the research protocol was approved by the Institutional Review Board of Yonsei University Dental Hospital (Institutional Review Board number: 2-2015-0055). Normal impacted third molars were collected from adults (19–29 years of age). After extraction, the pulp tissue was gently separated from the crown and root. hDPSCs were isolated and cultured using the method described by Gronthos et al (2).

The cell surface marker expression and differentiation potential of the hDPSCs were determined. Cell surface marker expression was examined by flow cytometry (FACSverse; BD Biosciences, San Jose, CA) using specific antibodies against CD45, CD73, CD90, and CD105 (BD Biosciences). Flow cytometry data were analyzed using FlowJo (v.10.0; Tree Star, Inc, Ashland, OR). Osteogenic, adipogenic, and chondrogenic differentiation was assessed by alizarin red S, oil red O, and alcian blue staining, respectively. Bone marrow MSCs (Stem Cell Therapy Center, Yonsei University Health System, Seoul, South Korea) were used as the control.

In Vivo Transplantation of hDPSCs

All animal experiments were performed in accordance with the Guideline for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Yonsei University, and were approved by the committee (protocol number 2015-0029). The *in vivo* experiments were performed using hDPSCs at passage 4 and 7-week-old male BALB/c nude mice. A total of 56 mice were used, 28 mice in the intravenous injection group and 28 mice in the intrapulpal transplantation group. Under deep anesthesia induced by intraperitoneal injection of a mixture of 30 mg/kg Zoletil 50 (Virbac, Carros, France) and 10 mg/kg Rompun (Bayer, Leverkusen, Germany), mice were transplanted with 1×10^5 hDPSCs through the tail vein for intravenous injection. For the intrapulpal transplantation, a cavity was prepared with a round carbide bur FG 1/4 (SS White, Lakewood, NJ) (diameter = 0.5 mm) coupled to a high-speed handpiece on the occlusal aspect of the maxillary first right molar until the mesial orifice and the distal orifice of the root canals were exposed. After preparation of the access cavity, pelleted 1×10^5 hDPSCs (centrifugation at 1500 rpm) were inserted into the pulp chamber. Subsequently, the access cavity was sealed with self-adhesive resin cement (RelyX U200; 3M ESPE, St Paul, MN) (Fig. 1*A–F*). After transplantation of hDPSCs into the tail vein or pulp chamber, mice were sacrificed after 15 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, 24 hours, and 48 hours. All procedures were performed under the surgical operating microscope, except for the anesthesia.

Quantitative Analysis of the Injected hDPSCs: Deoxyribonucleic Acid Extraction and Polymerase Chain Reaction Analysis of Human Beta Globin

The mice were sacrificed by carbon dioxide inhalation at various time points after the transplantation of the hDPSCs, and the heart, liver, spleen, kidneys, lungs, brain, and lymph nodes were collected. The biological samples were submitted to genomic deoxyribonucleic acid (gDNA) extraction and quantitative polymerase chain reaction (qPCR) analysis to detect the presence of human cells in the mice recipients. gDNA was extracted from the collected organs and purified using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). Detection of the human beta globin element in the hDPSC gDNA amplifications was performed in accordance with the standard recommended amplification conditions (Applied BioSystems, Foster City, CA) as previously described by Heid et al (17). The qPCR reaction was performed using 100 ng gDNA and the TaqMan Universal master Mix II (Applied BioSystems, Foster City, CA) with primers and TaqMan MGB probes (Hs00758889s1: HBB, Applied BioSystems). All samples were analyzed in duplicate, and qPCR was performed using the StepOnePlus qPCR System (Applied BioSystems). The value of gDNA contained in each somatic cell (diploid) is 6.16 pg with 2 copies of nonrepeated gene. This value was used to calculate the number of human cells (18). Mouse gDNA was isolated from the identical tissues of nontransplanted nude mice and used as the negative control. In addition, human gDNA was isolated from hDPSC culture and used as the positive control.

Immunohistochemistry and Immunofluorescence Staining

Qualitative analysis was performed by immunohistochemistry and immunofluorescence of intrapulpally transplanted hDPSCs to circumvent the difficulties in extracting DNA from a hard tissue such as teeth. The maxilla of mice of the intrapulpal transplantation group were dissected and decalcified for 4 weeks in phosphate-buffered saline containing 5% EDTA and 4% sucrose (pH = 7.4) with agitation at room temperature. The solution was renewed every week. After decalcification, the samples were dehydrated and embedded in a paraffin block. The samples were cut with a microtome in sections of 6 μm. The slides were stained with hematoxylin-eosin and observed under a light microscope for histology. The unstained sections were deparaffinized and rehydrated by xylene and alcohol for immunohistochemistry and immunofluorescence analysis. Monoclonal anti-HLA Class 1 ABC antibody (clone EMR8-5, 1:200 dilution; Abcam Inc, Cambridge, MA) was used as the first antibody. For immunohistochemistry analysis, the Peroxidase Affini-Pure Goat Anti-Mouse IgG (H+L) secondary antibody (Jackson ImmunoResearch Inc, West Grove, PA) was used. For immunofluorescence analysis, the goat antimouse immunoglobulin G, immunoglobulin M secondary antibody (Alexa Fluor 488; Thermo Fisher Scientific, Waltham, MA), and double-stranded nucleic acids (TO-PRO-3; Thermo Fisher Scientific) were used. The slides were observed using a confocal microscope.