Imaging Gene Expression in Human Mesenchymal Stem Cells: From Small to Large Animals¹

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Purpose:

To evaluate the feasibility of reporter gene imaging in implanted human mesenchymal stem cells (MSCs) in porcine myocardium by using clinical positron emission tomography (PET)-computed tomography (CT) scanning.

Materials and Methods:

Animal protocols were approved by the Institutional Administrative Panel on Laboratory Animal Care. Transduction of human MSCs by using different doses of adenovirus that contained a cytomegalovirus (CMV) promoter driving the mutant herpes simplex virus type 1 thymidine kinase reporter gene (Ad-CMV-HSV1-sr39tk) was characterized in a cell culture. A total of 2.25×10^6 transduced ($n = 5$) and control nontransduced ($n = 5$) human MSCs were injected into the myocardium of 10 rats, and reporter gene expression in human MSCs was visualized with micro-PET by using the radiotracer 9-(4-[fluorine 18]-fluoro-3-hydroxymethylbutyl)-guanine (FHBG). Different numbers of transduced human MSCs suspended in either phosphate-buffered saline (PBS) ($n = 4$) or matrigel ($n = 5$) were injected into the myocardium of nine swine, and gene expression was visualized with a clinical PET-CT. For analysis of cell culture experiments, linear regression analyses combined with a t test were performed. To test differences in radiotracer uptake between injected and remote myocardium in both rats and swine, one-sided paired Wilcoxon tests were performed. In swine experiments, a linear regression of radiotracer uptake ratio on the number of injected transduced human MSCs was performed.

Results:

In cell culture, there was a viral dose-dependent increase of gene expression and FHBG accumulation in human MSCs. Human MSC viability was 96.7% (multiplicity of infection, 250). Cardiac FHBG uptake in rats was significantly elevated ($P < .0001$) after human MSC injection (0.0054% injected dose [ID]/g $\pm$ 0.0007 [standard deviation]) compared with that in the remote myocardium (0.0003% ID/g $\pm$ 0.0001). In swine, myocardial radiotracer uptake was not elevated after injection of up to 100×10^6 human MSCs (PBS group). In the matrigel group, signal-to-background ratio increased to 1.87 after injection of 100×10^6 human MSCs and positively correlated ($R^2 = 0.97$, $P < .001$) with the number of administered human MSCs.

Conclusion:

Reporter gene imaging in human MSCs can be translated to large animals. The study highlights the importance of co-administering a "scaffold" for increasing intramyocardial retention of human MSCs.

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Ault mesenchymal stem cells (MSCs) are bone marrow-derived nonhematopoietic cells, representing approximately 0.01% of the bone marrow mononuclear cell fraction (1). MSCs are an inhomogeneous population of multipotent cells whose true stem cell component is limited (2). Depending on the environmental signals from their niche, these stem cells (also now referred to as multipotent mesenchymal stromal cells) have shown the potential to transdifferentiate in cell culture along various lineages, including bone and cartilage (3), fat, tendon, and muscle (4), as well as cardiomyocytes (5). MSCs can be propagated in large quantities in a cell culture for many passages without substantial functional impairment (4). A reduced immunogenicity of MSCs suggests their use as an allogeneic graft (6,7), and human MSCs do not present hurdles related to ethical and political issues as is the case with other cells, such as human embryonic stem cells. Therefore, transplantation of human MSCs currently holds large promise as a cell-based therapy for repairing damaged

tissues that include the heart (8), and the safety and efficiency of human MSCs in patients are currently evaluated in controlled clinical trials (9).

Numerous potential mechanisms leading to cardiac regeneration after stem cell transplantation have been proposed, including in vivo differentiation of transplanted cells into the different cellular components of the heart; paracrine effects from factors released by the transplanted stem cells; fusion of the administered stem cells with the existing constituents of the heart; and stimulation of endogenous repair with regeneration of stem cell niches in the heart (10). However, the mechanism or combination of mechanisms contributing to cardiac repair and improved cardiac function after cell transplantation still remain obscure. For example, the initial observation that bone marrow-derived stem cells transdifferentiate into cardiomyocytes and vascular structures in mice with acute myocardial infarction as an explanation for functional recovery (11) has been challenged by two independent studies showing no transdifferentiation of bone marrow-derived stem cells into cardiomyocytes after injection into the border zone of acutely infarcted murine myocardium (12,13). Furthermore, conflicting reports on the clinical benefit in patients with coronary artery disease following cell transplantation have raised several questions, including which subpopulation of patients with coronary artery disease may benefit most from which cell type implanted through which delivery approach in the heart (14–17). The development of objective and quantitative noninvasive (imaging) approaches for tracking stem cell fate, including stem cell location, survival, engraft-

ment, and differentiation, may help in better understanding stem cell biology and may lead to better patient stratification and eventual successful implementation of cardiac stem cell therapy into the clinic (18–22).

Recently, molecular imaging approaches such as positron emission tomography (PET) using reporter gene imaging and dedicated small-animal micro-PET imaging systems have been used as promising noninvasive, objective, and quantitative techniques to assess location, survival, and engraftment of stem cells after intramyocardial transplantation in small animals (mice and rats) (23–25). In reporter gene imaging, reporter genes such as the mutant form of the gene encoding herpes simplex virus 1 thymidine kinase (*HSV1-sr39tk*) (26–28) are introduced into stem cells via a vector (eg, a recombinant adenovirus) before intramyocardial cell transplantation. In viable stem cells, the *HSV1-sr39tk* is trans-

Advances in Knowledge

- Human mesenchymal stem cells (MSCs) can be transduced to express the mutant thymidine kinase reporter gene, and more than 96% of human MSCs are viable at a viral infection dose of a multiplicity of infection of 250.
- Gene expression in viable human MSCs can be visualized in rat myocardium by using a dedicated small-animal PET imaging system.
- Translation of this imaging approach to large animals (swine) by using a clinically available PET-CT scanner is feasible.
- Biopolymer scaffolds such as matrigel increase intramyocardial retention of human MSCs in the porcine myocardium and may be critical for optimal delivery and noninvasive tracking of human MSCs with current clinical PET-CT scanners.

Implication for Patient Care

- Feasibility of imaging gene expression in human MSCs in large animals by using a clinically available PET-CT scanner lays the foundation for a future clinical translation of the reporter gene imaging approach in cardiac stem cell therapy in patients.

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Abbreviations:

CI = confidence interval

CMV = cytomegalovirus

FHBG = 9-(4-[fluorine 18]-fluoro-3-hydroxymethylbutyl)-guanine

ID = injected dose

MOI = multiplicity of infection

MSC = mesenchymal stem cell

PBS = phosphate-buffered saline

PCR = polymerase chain reaction

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Guarantors of integrity of entire study, J.K.W., S.S.G.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, J.K.W., A.M.L.; experimental studies, J.K.W., R.P., W.S., T.J.B., A.J.C., C.H.N., A.M.L., J.L., F.I., Y.S., R.C.R.; statistical analysis, J.K.W., J.R.; and manuscript editing, J.K.W., R.P., A.M.L., S.S.G.

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lated into HSV1-sr39TK protein, which efficiently phosphorylates derivatives of thymidine and acylguanosine such as 9-(4-[fluorine 18]-fluoro-3-hydroxymethylbutyl)-guanine (FHBG) or other imaging probes (29–32). After phosphorylation, the accumulation of FHBG in stem cells can be visualized with dedicated small-animal PET scanners, thereby reporting indirectly on the presence of viable stem cells after intramyocardial administration.

Dedicated small-animal PET scanners have substantially higher sensitivity and spatial resolution compared with their clinical counterparts, but cannot be used for clinical purposes in patients. To our knowledge, no study has yet addressed the question whether *in vivo* imaging of cardiac reporter gene expression in transplanted human MSCs can be transferred to large animals by using a clinical PET-computed tomographic (CT) scanner, which would be required for an eventual future translation of this imaging approach to patients after stem cell transplantation.

Therefore, the purpose of our study was to evaluate the feasibility of reporter gene imaging in implanted human MSCs in porcine myocardium by using clinical PET-CT scanning.

Materials and Methods

In Vitro Assays and Cell Culture Experiments

Human MSCs.—Human MSCs isolated from healthy adult donors (cryopreserved in passage 2; Lonza, Walkersville, Md) were grown in MSC medium (Poietics; Lonza) supplemented with l-glutamine, penicillin-streptomycin, fetal bovine serum, and other MSC growth supplement as directed by the company. Human MSCs stained positive for CD29, CD44, CD155, and CD166 and negative for CD14, CD34, and CD45. Human MSCs were harvested by means of trypsinization at 80% confluence.

Recombinant adenovirus generation and infection of human MSCs.—Replication-deficient recombinant adenovirus that contained a cytomegalovirus (CMV) promoter driving the mutant herpes

simplex virus type 1 thymidine kinase reporter gene (Ad-CMV-HSV1-sr39tk) was constructed and amplified in HEK-293 cells (ATCC, Manassas, Va) as previously described, and the viral titer was determined with plaque assays (33). Human MSCs at 80% confluence were exposed to Ad-CMV-HSV1-sr39tk in a serum-free medium (Invitrogen, Carlsbad, Calif) for 3 hours at 37°C at various multiplicity of infection (MOI = 0, 2.5, 25, 250, and 2500), were resuspended in complete medium, and were harvested for experiments 36 hours later. In four control experiments, human MSCs were either not incubated with any virus ($n = 2$) or were exposed to the control adenovirus that contained a CMV driving the firefly luciferase reporter gene (Ad-CMV-Fluc, $n = 2$) at MOI of 2.5 and 250.

Quantitative real-time polymerase chain reaction analysis of HSV1-sr39tk mRNA.—Human MSCs exposed to Ad-CMV-HSV1-sr39tk at different MOI (MOI = 0, 2.5, 25, 250, and 2500) along with nontransduced control human MSCs were used for quantitative real-time polymerase chain reaction (PCR) analysis of HSV1-sr39tk mRNA. Total RNA from different samples was extracted 36 hours after viral exposition. After treatment with deoxyribonuclease, 1 μ g of RNA from each sample was used for PCR analysis by using primers designed against thymidine kinase to confirm the absence of any viral DNA contamination. One microgram of RNA was then used for complementary DNA synthesis. The complementary DNA was synthesized by using a complementary DNA synthesis kit (Stratagene, La Jolla, Calif) and the random hexamer as primer in 20- μ L reaction volume. Five microliters of DNA was used for quantitative PCR reaction by using SYBR Green 2 $\times$ Q-PCR master mix (Applied Biosystems, Foster City, Calif). Studies were performed in duplicate.

Western blot analysis of HSV1-sr39TK protein.—Human MSCs exposed to Ad-CMV-HSV1-sr39tk (between 0 and 2500 MOI) were lysed by using a 1 $\times$ cell lysis buffer (Cell Signaling Technology, Danvers, Mass). Twenty micrograms of protein was separated by a 4%–12% bis-tris gradient gel (NuPAGE; Invitrogen),

transferred to a nitrocellulose membrane, and incubated with a polyclonal rabbit anti-HSV1-sr39TK primary antibody (1:20 000 ratio) overnight at 4°C. The washed membrane was then incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (1:10 000 ratio) for 2 hours. For visualization of the immunoreactive bands, the enhanced chemiluminescence detection kit (GE Healthcare, Piscataway, NJ) was used. As an internal loading control, the same membrane was incubated with anti- α -tubulin antibody. Studies were performed in triplicate.

FHBG accumulation assay in human MSCs.—FHBG accumulation assays were performed in human MSCs incubated with varying concentrations of Ad-CMV-HSV1-sr39tk (between 0 and 2500 MOI). Thirty-seven kilobecquerels of FHBG in 500 μ L of normal growth medium was added to the cells and the cells were incubated with the tracer for 1 hour at 37°C. FHBG was then removed and the cells were washed three times with 500- μ L phosphate-buffered saline (PBS) (Invitrogen). The growth medium and washing buffer were collected. After the third washing, the cells were lysed with 150 μ L of 1N NaOH. The collected medium and washing buffer, as well as the cell lysates, were analyzed in a gamma counter (Cobra II; Packard, Meriden, Conn). The protein content of the cell lysates was determined with a Bradford protein assay (BioRad, Hercules, Calif). Data were expressed as disintegrations per minute in the cells divided by those in the medium per micrograms of protein. In addition, human MSCs (incubated at MOI of 250) were exposed (a) with varying FHBG activities (0–370 kBq, at a fixed exposure time of 60 minutes) or (b) for varying time periods (0–240 minutes at a fixed tracer activity of 37 kBq), and FHBG accumulation was assessed as described above. All FHBG accumulation studies were performed in triplicate.

In Vitro Assessment of Human MSC Viability and Metabolic Activity

Human MSCs were exposed to Ad-CMV-HSV1-sr39tk (between 0 and 2500 MOI) and cell viability was assessed by using

the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye-reduction assay. This quantitative colorimetric assay measures the reduction of a tetrazolium component into an insoluble formazan product by cellular mitochondria, thereby detecting minor changes in cell metabolism in response to external factors (34). Assays were performed by using the manufacturer's protocol (ATCC, Manassas, Va). The percentage of viable human MSCs after viral incubation was calculated by dividing MTT assay values by the mean MTT value of the non-treated control human MSCs.

Small-Animal Imaging Experiments

Direct intramyocardial injections of human MSCs in rats.—All animal protocols were approved by the Institutional Administrative Panel on Laboratory Animal Care. Open chest surgery with direct intramyocardial injection of human MSCs into the wall of the left ventricle was performed in 10 female Sprague-Dawley rats (Charles River Laboratories, Wilmington, Mass). The rats (mean weight, 305 g; range, 290–310 g) were intubated and ventilated with 2% isoflurane in oxygen at 2 L/min. After a left lateral thoracotomy, a single injection of 2.25×10^6 Ad-CMV-HSV1-sr39tk (MOI = 250) transduced human MSCs in 50- μ L PBS was performed into the left ventricular myocardium with microscopic guidance in five rats by using a 28-gauge injection needle. Control animals ($n = 5$) received the same number of nontransduced human MSCs. Correct needle placement was verified by the presence of blanching of the myocardium during cell injection. After injection, the chest wall was closed in layers and the pneumothorax was evacuated by means of suction by using a 16-gauge catheter. For postoperative analgesia, buprenorphine (0.05 mg per kilogram of body weight; Hospira, Lake Forest, Ill) and carprofen (5 mg/kg; Pfizer, New York, NY) were administered intramuscularly. All rats recovered uneventfully and were subjected to micro-PET imaging 24 hours after intramyocardial injection of human MSCs.

Micro-PET imaging of rats.—In all rats, imaging was performed by using a micro-PET system (Concorde R4; Sie-

mens AG, Malvern, Pa), with the rats placed in a supine position. Anesthesia was maintained with 2% isoflurane, and the hearts of the animals were centered in the detector ring during data acquisition. Four hours after intravenous injection of FHBG (mean, 52.17 kBq; range, 50.69–55.5 kBq) via the tail vein, a 15-minute static scan with an approximate resolution of 1.66–1.85 mm (35) in each axial direction was obtained in all animals. With the rats placed in the same position as for the static scan, PET imaging was repeated 1 hour after intravenous administration of fluorine 18 fluorodeoxyglucose (FDG) (mean, 41.44 MBq; range, 41.07–41.44 MBq) for anatomic localization of the heart. Images from both data acquisitions were reconstructed by using the two-dimensional ordered-subset expectation maximization algorithm (without attenuation correction) and were presented as a fusion image by overlaying the FHBG image on the FDG image.

Large-Animal Experiments

Direct intramyocardial injection of human MSCs in swine.—Nine female Yorkshire swine (mean weight, 40 kg; range, 33–42 kg; Pork Power Farms, Turlock, Calif) were anesthetized with tiletamine/zolazepam (5 mg/kg; Webster, Sterling, Mass), tracheally intubated, and mechanically ventilated. Anesthesia was maintained with 2% isoflurane in 100% oxygen, and electrocardiogram, respiration rate, and intraventricular pressure were recorded. All swine underwent a left lateral thoracotomy through the fourth and fifth intercostal spaces. The pericardium was opened and an octopus device (Guidant, Indianapolis, Ind) was placed on the wall of the left ventricle at each injection site to facilitate injections into the heart by reducing the motion of the myocardium. After injections, the chest wall was closed in layers and a pleural tube was placed into the left pleural cavity to drain the remaining free air.

To analyze the difference between the injection of human MSCs with and without matrigel, animals were divided into two groups. In the first group (PBS group, $n = 4$), transduced human MSCs (30×10^6 human MSCs in swine 1, 50×10^6 human MSCs in swine 2, 100×10^6

human MSCs in swine 3, and 200×10^6 human MSCs in swine 4) were suspended in 1-mL PBS and injected into a single area ($1 \times 1 \text{ cm}^2$) of the anterolateral left ventricular myocardium. One (swine 3) of the four swine had died 2 hours after the procedure, before PET-CT scanning was initiated. In the second group of animals (matrigel group, $n = 5$), human MSCs were suspended in 500- μ L PBS mixed with 500- μ L matrigel (BD Bioscience, San Jose, Calif). To limit the total number of animals and to acquire intraindividual data sets in the second group of animals, different numbers of human MSCs (50×10^6 [$n = 2$], 100×10^6 [$n = 2$], 150×10^6 [$n = 3$], 200×10^6 [$n = 2$], 600×10^6 [$n = 1$]) were injected into different injection sites of the left ventricular myocardium (anterolateral, posterolateral, or apical) in four animals. As a control, 50×10^6 ($n = 1$), 100×10^6 ($n = 1$), and 150×10^6 ($n = 1$) nontransduced human MSCs were injected into the anterolateral, posterolateral, and apical left ventricular myocardium, respectively, in one of these five animals.

Clinical PET-CT imaging of the swine.—In all swine, imaging was performed with a clinical PET-CT scanner (Discovery LightSpeed Plus; GE Medical Systems, Waukesha, Wis), with the animals in a supine position. After an initial scout view (30 mAs, 120 kV), a nonenhanced four-detector CT scan of the heart was obtained with the following settings: section thickness of 5 mm, table feed of 38 mm per rotation, 0.8-second gantry rotation time; x-ray tube voltage of 120 kV, and tube current of 150 mA. Eight hours after intramyocardial human MSC injection and 4 hours after intravenous administration of FHBG (mean, 536.5 MBq; range, 370–777 MBq), a 1-hour static PET scan was acquired. Transverse section reconstructions of the CT data sets were performed with a nominal section thickness of 5 mm at an interval of 4.25 mm. All PET images were reconstructed by using an iterative algorithm (ordered-subset expectation maximization, two iterative steps, 28 subsets), with CT-based attenuation

correction applied. No cardiac gating or respiratory motion correction was performed.

Image Analysis of Micro-PET and PET-CT Images

All imaging data were analyzed offline by using nonproprietary PET analysis software developed in our lab (AMIDE, version 0.8.22; <http://amide.sourceforge.net>). Image analysis was performed in random order by one reader (J.K.W.; 2 years of experience in analysis of PET-CT images in swine) who was blinded to all information about the rats and swine (administration of transduced vs nontransduced human MSCs, number of injected cells, and co-administration of matrigel in large animals). One region of interest per injection site encompassing the different injection sites and a remote site of the left ventricular wall were drawn (range, 3–8 mm). Percentage of the injected dose (ID) per gram was calculated by means of a calibration constant obtained from scanning a cylindrical phantom, assuming a tissue density of 1 g/mL, and dividing by the ID that was decay corrected to the time of scanning.

Postmortem Immunofluorescence Staining

After in vivo PET-CT imaging, the swine were sacrificed by means of intravenous administration of saturated potassium chloride. Hearts were excised, rinsed, fixed in 10% neutral buffered formalin overnight, and sectioned in transverse slices. The transverse slices from the level of injection were verified at gross examination, and histologic examination was performed by means of paraffin embedding. Sections were incubated with anti-Vimentin primary antibody (1:200 ratio; Dako, Carpinteria, Calif) and developed with the Envision-Plus secondary antibody-peroxidase kit (Dako) to result in brown staining of the cells of mesenchymal origin.

Statistical Analysis

Data are given as mean $\pm$ standard deviation. In the cell culture experiment on gene expression levels, a linear regression analysis of mRNA expression level on log viral dose (MOI = 0, 2.5, 25, 250, and 2500) was performed, with additional *t*

tests performed between levels. In the cell culture experiments on FHBG accumulation, linear regression analyses of accumulation level on (a) log viral dose (MOI = 0, 2.5, 25, 250, and 2500), (b) incubation time (0, 10, 20, 30, 45, 60, 120, and 240 minutes), and (c) FHBG dose ($0, 3.7 \times 10^{10}, 7.4 \times 10^{10}, 11.1 \times 10^{10}, 14.8 \times 10^{10}, 18.5 \times 10^{10}, 27.75 \times 10^{10}$, and 37.0×10^{10} Bq) were performed, as well as additional *t* tests were performed between levels. In the cell culture experiment on human MSC viability, a linear regression analysis of arcsine-root-transformed viability percentages on log viral dose (0, 2.5, 25, 250, and 2500 MOI) and the virus type (Ad-CMV-HSV1-sr39tk or Ad-CMV-Fluc) was performed. To test differences in radiotracer uptake between the injected and remote myocardium in rats, a one-sided paired Wilcoxon test was performed for both transduced and nontransduced human MSCs. To test differences in the radiotracer uptake between injected and remote myocardium in swine, one-sided paired Wilcoxon tests were performed, stratified by animal. A linear regression of radiotracer uptake ratio on the number of injected transduced human MSCs (10 locations) was performed. Stratified paired Wilcoxon tests were performed (R statistical system, release 2.7.1; <http://www.R-project.org>) by using the “coin” package for conditional inference (<http://www.R-project.org/CRAN/coin>). All other statistical analyses were performed with software (Stata, release 9.2; Stata, College Station, Tex). A *P* value of less than .05 was considered to indicate a significant difference.

Results

Cell Culture Experiments

At an increasing MOI of Ad-CMV-HSV1-sr39tk, human MSCs showed a significant dose-dependent expression of HSV1-sr39tk mRNA and HSV1-sr39TK protein (slope = 4.1; 95% confidence interval [CI]: 3.8, 4.4; *P* < .0001; *R*² = 0.85; Fig 1a, 1b). There were also significant differences between each of the four MOI levels (all with *P* < .001). There was no expression

of HSV1-sr39tk mRNA and HSV1-sr39TK protein in control experiments (Fig 1a, 1b). FHBG accumulation in human MSCs sharply increased between an MOI of 2.5 and 25 (0.002 vs 0.006, *P* < .0001, Fig 1c), but only slightly increased between an MOI of 25 and 250 (0.0059 vs 0.0064, *P* < .08), and remained unchanged between a viral dose of 250 MOI and 2500 MOI (0.0064 vs 0.0065, *P* < .86), suggesting that an MOI of 250 was an appropriate dose for further in vivo experiments. FHBG accumulation in human MSCs was not observed in control human MSCs that were not exposed to Ad-CMV-HSV1-sr39tk or in control human MSCs that were exposed to control Ad-CMV-Fluc virus (*P* < .0001, Fig 1c). As illustrated in Figure 2, FHBG accumulated in human MSCs in a time- and dose-responsive manner. Tracer accumulation significantly increased with both increasing tracer doses (slope = 0.0014; 95% CI: 0.0013, 0.0015; *P* < .0001; *R*² = 0.98) and incubation times (slope = 1.32×10^{-4} ; 95% CI: 1.27×10^{-4} , 1.36×10^{-4} ; *P* < .0001; *R*² = 0.99).

HMSC viability of human MSCs was preserved after incubation with high doses of Ad-CMV-HSV1-sr39tk. There was no significant difference in overall human MSC viability between cells with Ad-CMV-HSV1-sr39tk and the control Ad-CMV-Fluc virus (98.2% vs 94.8%, *P* < .099), while viability for both types significantly decreased with increasing doses of the virus (slope = -0.077; 95% CI: -0.13, -0.02; *P* < .012; *R*² = 0.46; Fig 3). However, at high Ad-CMV-HSV1-sr39tk doses, human MSC viability was still high. Compared with nontransduced control human MSC viability of 100% (95% CI: 99, 100%), 96.7% (95% CI: 93, 100%) of transduced human MSCs were still viable at an MOI of 250 and 94.0% (95% CI: 91, 97%) were viable at an MOI of 2500.

Small-Animal Imaging

Tracer uptake in the myocardial wall after injection of transduced human MSCs was significantly higher compared with the tracer uptake in the remote myocardial wall (0.0054% ID/g vs 0.0003% ID/g, *P* < .043). After injection of nontransduced human MSCs in control rats, radiotracer uptake in the myocardial wall was the same as

the uptake in the remote myocardial wall (0.0003% ID/g vs 0.0003% ID/g, $P < .50$).

Large-Animal Imaging

Anatomic localization and gene expression in human MSCs can be visualized

with clinical PET-CT imaging after intramyocardial administration of a critical number of transduced human MSCs suspended in matrigel. In the PBS group of swine ($n = 3$), between 30×10^6 and 200×10^6 human MSCs were sus-

pended in PBS and injected into the porcine myocardium. On clinical PET-CT images, the location and gene expression in human MSCs could not be delineated. FHBG uptake at human MSC injection sites was the same as that in the

Figure 1

Figure 1: HSV1-sr39tk expression and FHBG accumulation in cultured human MSCs at increasing doses of the replication-deficient recombinant adenovirus Ad-CMV-HSV1-sr39tk (MOI = 2.5, 25, 250, or 2500). Control human MSCs (*Sham*) were incubated with the control Ad-CMV-Fluc at MOI of 2.5 and 250, respectively, or were not exposed to the virus (*w/o*). Expression of (a) HSV1-sr39tk mRNA (b) and HSV1-sr39TK protein in human MSCs was viral-dose dependent. Western blot analyses were performed by using antibodies against HSV1-sr39TK protein and against α -tubulin as an internal loading control. In nontransduced human MSCs and sham-transduced human MSCs there was no expression of both HSV1-sr39tk mRNA and HSV1-sr39TK protein. (c) Graph shows that FHBG accumulation was viral-dose dependent up to an MOI of 250. There was no further significant increase of tracer accumulation in human MSCs at an MOI of 2500. *DPM* = disintegrations per minute, * = statistically significant difference ($P < .05$), error bars = $\pm$ standard deviation.

Figure 2

Figure 2: Characterization of FHBG accumulation in human MSCs incubated at MOI of 250. (a) Graph shows that FHBG accumulation in human MSCs significantly increased with longer tracer incubation times and was highest 4 hours after tracer administration. (b) Graph shows that at fixed incubation time of 60 minutes, FHBG accumulation significantly increased with higher tracer doses. *DPM* = disintegrations per minute, error bars = $\pm$ standard deviation. To convert to Système International units (kilobecquerels), multiply by 37.

remote myocardium (0.001% ID/g vs 0.001% ID/g, $P < .63$). At histologic examination, only few clusters of human MSCs were detected at intramyocardial injection sites (Fig 4a).

In contrast, in the matrigel group of swine ($n = 5$), FHBG uptake at human MSC injection sites was significantly different compared with that in the remote myocardium (0.0021% ID/g vs 0.0007% ID/g, $P < .013$). The signal-to-background ratio increased to 1.87 ± 0.24 at clinical PET-CT after administration of a critical number of approximately 100×10^6 transduced human MSCs, being positively correlated (slope = 0.013; 95% CI: 0.011, 0.014; $P < .0001$; $R^2 = 0.97$) with the number of administered human MSCs (Table, Fig 5). The FHBG uptake after injection of nontransduced control human MSCs was the same as the uptake in the remote myocardium (0.00006% ID/g vs 0.00006% ID/g, $P < .59$). In the matrigel group (Fig 4b), histologic findings showed an increase of intramyocardial retention of human MSCs, with more and larger clusters of human MSCs at intramyocardial injection sites than in the PBS group.

Discussion

In this proof-of-principle study, we have demonstrated that imaging of reporter gene expression in implanted human MSCs can be translated from small to large animals by using a clinical PET-CT scanner. The imaging signal from reporter gene expression increases with the number of implanted human MSCs in porcine myocardium when a “scaffold” strategy is used. Our study highlights the importance of a co-administration of a scaffold along with stem cells to increase retention of transplanted human MSCs within the myocardium and to allow in vivo quantification of human MSC gene expression by using current clinical PET-CT scanners.

Controversies regarding stem cell behavior and biology, as well as conflicting results regarding clinical benefit after intramyocardial administration of stem cells, have underlined the need for quantitative and objective techniques to monitor location, survival, engraftment,

Figure 3

Figure 3: Graph shows viability of human MSCs exposed to increasing doses of both Ad-CMV-HSV1-sr39tk and control Ad-CMV-Fluc (not exposed to the virus [*w/o*] and control human MSCs [*Sham*]) as assessed with the MTT assay. In all subsets of experiments, relative viability in transduced human MSCs was greater than 90% compared with that in nontransduced human MSCs.

Figure 4

Figure 4: Immunohistochemical staining of porcine myocardium. Vimentin staining (brown color) highlights presence of human MSCs within the myocardium after direct open-chest injection. (a) In swine injected with human MSCs that were suspended in PBS, only few clusters of human MSCs can be seen in the myocardium. (b) There are more and larger intramyocardial clusters of human MSCs visible in swine injected with human MSCs that were injected along with the scaffold matrigel. Scale bar = 1 mm. Inset = human MSCs at $\times 400$.

FHBG Uptake Values at Intramyocardial Injection Sites and Myocardial Remote Areas After Injection of Increasing Numbers of Transduced Human MSCs in the Matrigel Group of Swine

No. of Human MSCs	No. of Injection Sites	Injection Site (% ID/g)	Remote Area (% ID/g)	Signal-to-Background Ratio*
50×10^6	2	0.00031 ± 0.00001	0.00033 ± 0.00002	0.94 ± 0.08
100×10^6	2	0.00111 ± 0.00064	0.00062 ± 0.00042	1.87 ± 0.24
150×10^6	3	0.00232 ± 0.00030	0.00087 ± 0.00008	2.66 ± 0.38
200×10^6	2	0.00179 ± 0.00126	0.00055 ± 0.00032	3.11 ± 0.48
600×10^6	1	0.00717	0.00089	8.02

Note.—Unless otherwise indicated, data are the mean $\pm$ standard deviation.

* The signal-to-background ratio was calculated by dividing the percentage of the ID per gram at the level of the intramyocardial injection site by the percentage of the ID per gram at the level of intramyocardial remote area.

and differentiation of implanted stem cells. These parameters, however, cannot be readily obtained in the clinical setting in contrast to preclinical animal studies since extensive histologic analysis of myocardial tissue after cell transplantation cannot be performed in patients. Therefore, the development of noninvasive imaging approaches to monitor the fate of implanted stem cells is needed and may help in successful translation of stem cell therapies to patients (18–22).

Several different imaging approaches for visualizing stem cell fate in the heart are currently under investigation, including magnetic resonance (MR) imaging, optical imaging, and PET. Because of its high spatial resolution and lack of ionizing irradiation, MR imaging is an attractive imaging approach for tracking stem cells. Stem cells can be prelabeled with contrast agents such as iron oxides, and the location of stem cells within the heart after transplantation can be visualized with high spatial resolution (36). However, other cellular events relevant to

stem cell transplantation such as stem cell viability and differentiation cannot be quantified with MR imaging after prelabeling of stem cells. Furthermore, limited sensitivity (10^{-4} mol/L) of MR imaging compared with that of optical imaging (10^{-15} mol/L) and PET (10^{-12} mol/L) precludes detection and quantification of small changes in the imaging signal (37,38). By using optical reporter genes such as the firefly or renilla luciferase gene, optical bioluminescence imaging has been shown to be useful for in vivo tracking of stem cells in preclinical small-animal studies. This technique, however, is only appropriate for visualization of stem cells located in the superficial structures due to light absorption by tissue and it has limited tomographic resolution. Therefore, optical-based imaging approaches cannot easily be translated to large animals and eventually to patients. The advantage of PET-based reporter gene imaging approaches over current optical and MR imaging approaches is that it allows the acquisition of tomographic and

quantitative information with high sensitivity and that cell viability and differentiation (eg, by using cardiac-specific promoters driving PET reporter genes) can be monitored indirectly according to the imaging reporter gene expression levels.

In addition, unlike optical imaging, small-animal reporter gene imaging with micro-PET can potentially be translated to large-animal and, ultimately, to patient imaging with use of clinical PET-CT scanners. However, our initial attempts in translating cardiac PET-based reporter gene imaging of stem cells from small to large animals before an eventual further translation to patients using clinical PET scanners have been challenging. Decreased sensitivity and lower spatial resolution with consecutive partial volume effects challenge the recovery of discrete PET imaging signals originating from limited numbers of implanted stem cells using clinical compared with small-animal PET scanners. Furthermore, radiotracer accumulation and the resulting imaging signal depend on the extent of gene expression, and thus, the presence of a critical number of viable stem cells retained in the heart after transplantation. Therefore, the purpose of our current proof-of-principle study was to assess the feasibility of imaging reporter gene expression in human MSCs transplanted to the heart of swine.

We first optimized reporter gene expression in human MSCs in cell culture experiments. For this purpose, we chose an adenovirus-based gene delivery approach, which is known to have high transduction efficiency. We found that an MOI of 250 resulted in highest reporter gene expression at preserved human MSC viability as evidenced at real-time PCR, Western blotting, cell culture radiotracer uptake studies, and cell viability assays. After injection of transduced human MSCs into the heart of rats, reporter gene expression in human MSCs was successfully imaged by using FHBG and a dedicated small-animal PET scanner. However, gene expression could not be visualized in swine after injection of up to 100×10^6 human MSCs suspended in PBS only, which suggests that the number of via-

Figure 5

Figure 5: PET-CT imaging of intramyocardial reporter gene expression in swine. **(a)** Transverse nonenhanced PET-CT fusion image reconstructed at the level of the left ventricle (LV) after direct open-chest administration of transduced human MSCs. The image was acquired 4 hours after intravenous FHBG administration. A distinct imaging signal (small arrows) can be delineated at the intramyocardial injection site of human MSCs. Note postoperative soft-tissue edema, emphysema, and fluid collection in the left chest wall (large arrows). *T* = beveled part of chest tube. **(b)** Coronal reconstruction of PET data set of the thorax and cranial upper abdomen in the same animal demonstrates high FHBG uptake at the level of intramyocardial injection site (arrows), with low background signal intensity in all other intrathoracic anatomic structures. Note high tracer accumulation in the liver (white *L*) due to biliary FHBG excretion. *R* = right proximal foreleg, *L* = left proximal foreleg.

ble stem cells after plain intramyocardial injection rapidly decreased below the detection threshold of the clinical PET-CT scanner in our study. This hypothesis is corroborated by several studies (39–42) both in swine and humans reporting on rapid wash-out of implanted cells from the myocardial tissue. In those studies, only 0.2%–39% of cells suspended in liquid were retained in the myocardium within the first 1–2 hours, depending on the cell type and the delivery approach (39–42). Therefore, co-administration of cells along with biopolymer scaffolds such as fibrin glue, collagen, alginate, gelatin, assembling peptides, or matrigel has recently been recommended to increase stem cell retention, as well as engraftment and survival after myocardial transplantation (43,44).

Matrigel is a solubilized basement membrane preparation that polymerizes at room temperature to produce biologically active matrix material resembling the mammalian cellular basement membrane. When injected along with human embryonic stem cell–derived cardiomyocytes into infarcted hearts of rats, matrigel substantially increased stem cell engraftment and survival in a recent study (45), suggesting that matrigel forms a scaffold capable of reducing the apoptosis (anoikis) induced by the need to detach anchorage-dependent cells from their surrounding matrix before intramyocardial injection. Using non-invasive longitudinal bioluminescence imaging, authors of another recent study (46) have shown that matrigel significantly increases long-term survival (up to 5 months) of bone marrow–derived mesenchymal stem cells injected subcutaneously into mice compared with other biomatrices, such as type 1 collagen and puramatrix, or with PBS only. Our results are in line with those of these studies. We found that co-administration of matrigel along with human MSCs substantially increased acute stem cell retention in the porcine myocardium 8 hours after injection. After injection of 100×10^6 human MSCs along with matrigel, a well-circumscribed imaging signal (signal-to-background ratio of approximately 2:1) could be delineated at the injection site, which

suggests that matrigel can provide a matrix to which human MSCs can attach right after intramyocardial injection thereby decreasing washout of human MSCs from the myocardium. In our study, the imaging signal significantly correlated with the number of administered human MSCs. However, below a critical number of approximately 100×10^6 human MSCs, the PET imaging signal could not be delineated anymore from the background values.

Current clinical PET-CT systems are limited by the poorer spatial resolution and the lower sensitivity compared with those of dedicated small-animal PET scanners (47). The spatial resolution of PET scanners is limited by the detector design (eg, the pitch of the crystal in the axial direction) and the reconstruction process and causes a partial volume effect that makes the PET imaging signal appear substantially lower than it actually is. In particular, when the PET imaging signal originates from a small area (eg, radiotracer uptake in a confined myocardial area after injection of transduced human MSCs), the PET signal from gene expression cannot be recovered and may not be delineated from background values (48). Therefore, future improvements in hardware and post-processing of current clinical PET-CT scanners are warranted to quantify reporter gene expression from even lower numbers of transduced human MSCs than reported in our study (49).

The following limitations of our study need to be addressed. Since we focused on studying the feasibility of reporter gene imaging in an acute swine model within 8 hours of stem cell delivery, we chose an adenovirus-based gene delivery approach with high transduction efficiency but only transient reporter gene expression. Stable integration of a reporter gene(s) into the genome of human MSCs is needed in eventual future imaging studies on longitudinal myocardial stem cell tracking. Furthermore, albeit an acute model, the delivery of human-derived stem cells into swine may have induced an acute immune response in the host, thus negatively affecting

stem cell viability and reporter gene expression in our study. The use of autologous porcine MSC harvested from the bone marrow or the peripheral blood may circumvent the influence of host immunogenicity on stem cell viability and may better reflect the eventual clinical use of stem cells in future studies. Furthermore, for this initial proof-of-principle study we chose injection of human MSCs into healthy myocardium rather than a model of myocardial ischemia to avoid the complexity and interindividual heterogeneity associated with animal models of myocardial ischemia. Retention of stem cells in ischemic tissue may be different from that in a healthy myocardium due to reduced vascular and lymphatic channels and lack of active contraction preventing implanted cells from being squeezed out of the injection site (50). This may influence the magnitude of the PET imaging signal in future studies using animal models of myocardial ischemia. The magnitude of the PET imaging signal may also depend on the volumetric ratio between the administered stem cells and the scaffold, and future studies are needed to optimize this ratio by correlating the PET imaging signal with quantitative histologic assays. Finally, the use of matrigel may not be clinically translatable, and alternative scaffolds may be advantageous when moving toward clinical application.

In conclusion, the results of our study suggest that cardiac reporter gene imaging of human MSCs can be translated from small to large animals by using a clinical PET-CT scanner. This is a further critical step toward future clinical translation of this imaging approach in cardiac cell therapy. Biopolymer scaffolds such as matrigel increase intramyocardial retention of human MSCs within the first hours after administration and may be critical for optimal delivery and noninvasive tracking of stem cells using current clinical PET-CT scanners. Further technical improvements of clinical PET-CT scanners are needed to facilitate clinical translation of noninvasive imaging of reporter gene expression in implanted human MSCs.

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