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Evaluation of the Homing of Human CD34$^+$ Cells in Mouse Bone Marrow Using Clinical MR Imaging

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Abstract: There have been several reports using various superparamagnetic iron oxide (SPIO) nanoparticles to label mammalian cells for monitoring their temporal and spatial migration *in vivo* by Magnetic Resonance Imaging (MRI). The purpose of this study was to evaluate the efficiency of labeling cells using two commercially available FDA-approved agents: ferumoxide, a suspension of dextran-coated SPIO used as an MRI contrast agent and protamine sulfate used *ex vivo* as a cationic transfection agent to evaluate the use of clinical 1.5 Tesla magnetic resonance imaging equipment in showing the *in vivo* homing of iron oxide-labeled human CD34$^+$ cells in irradiated mouse. After labeling human Hematopoietic (CD34$^+$) stem cells with ferumoxide-protamine sulfate complex (FE-Pro), cellular toxicity, functional capacity and quantitative cellular iron incorporation were determined. FE-Pro labeled cells demonstrated neither short or long-term toxicity nor changes in colonogenic assay of the stem cells and their phenotype when compared with unlabeled cells. Efficient labeling with FE-Pro was observed with iron content per cell varying between 1.91±0.1 pg for CD34$^+$ cells with 100% of cells labeled. After irradiation, Female Balb/c mice underwent MR imaging before and 12, 24, 48 and 72 h after intravenous injection of 0.5-1.5×10^7 labeled CD34$^+$ cells. A significant decrease in MR signal intensity was observed in bone marrow at 24 and 48 h after injection. Our observations confirm that efficient labeling of cells with appropriate contrast agents should facilitate the translation of this method to clinical trials for evaluating the trafficking of infused or transplanted cells by MR Imaging.

Key words: Contrast agent, superparamagnetic iron oxide, stem cell tracking, cord blood CD34$^+$ cells

INTRODUCTION

Cell labeling is finding increasing applications in fields such as cellular biology and medical imaging. Analysis of the distribution and cellular migration or cellular trafficking is essential for many physiological and pathological processes (Wallace *et al*., 1993). Conventional methods based on marker proteins labeled with fluorescence probes or with radioisotopes (^{111}In, ^{123}I, or ^{51}Cr) can be used to study lymphocyte trafficking, but these methods are limited by the isotope half-life, the tracer transfer rate into cells and the toxicity of the labeling process (Wallace *et al*., 1993). MR Imaging can be used to follow labeled cells using an endogenous or exogenous contrast agent. The agent must be specific for a given cell type, must not affect the antigenic properties of the cell, must induce a specific local signal distinguishable from neighboring tissues and must persist on or inside the labeled cell for an adequate time. The best contrast agent for studying cellular migration is superparamagnetic particles (Bulte *et al*., 1996; Weissleder *et al*., 2000), also known as superparamagnetic iron oxide (SPIO) or ultra small super- paramagnetic iron oxide (USPIO). These particles have a remarkable r_2 relaxivity which makes them particularly effective on T_2- weighted imaging. The most widely used product is dextran-coated superparamagnetic particles (Dodd *et al*., 1999; Yeh *et al*., 1995; Jung *et al*., 1995). Cellular uptake by spontaneous endocytosis is not very efficient but can be enhanced by the use of a peptide sequence of HIV-1 transactivator protein (Tat). This protein is internalized by cells when present in the extracellular medium (Vives *et al*., 1997; Rusnati *et al*., 2000). Tat peptide can be conjugated with superparamagnetic particles, resulting in nanoparticles with great stability and cellular permeability (Josephson *et al*., 1999). The conjugate can

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also be labeled with a fluorochrome (FITC), enabling it to be visualized by both flow cytometry and high-resolution MRI (Lewin *et al*., 2000). Other methods used to coat magnetic nanoparticles include monoclonal antibodies (Bulte *et al*., 1999), transfection agents including dendrimers (Bulte *et al*., 2001a) and lipofection agents (Hoehn *et al*., 2002).

Recent progress in the isolation of stem cells from various tissues and organs, along with an improved understanding of their function has extended applications of stem cell therapies not only to hematopoietic diseases but also to cardiovascular and neurological diseases (Orlic *et al*., 2001; Strauer *et al*., 2003; Cai *et al*., 2002). The development of such new stem cell-based therapies requires a quantitative and qualitative assessment of stem cell distribution to target organs (homing), differentiation outcome and engraftment (Strauer *et al*., 2003). Magnetic Resonance (MR) imaging would be well suited for this task, because it can enable both whole-body examinations and subsequent detailed depictions of host organs with near-microscopic anatomic resolution and excellent soft-tissue contrast (Barkhausen *et al*., 2001; Choi *et al*., 1997). In addition, MR imaging allows repetitive investigations without known side effects and without risking radiotoxic damage to the transplanted cells.

For transplanted stem cells to be visualized and tracked with MR imaging, they must be labeled with MR imaging contrast agents and the development of these dedicated labeling techniques is currently being broadly investigated (Schoepf *et al*., 1998; Weissleder *et al*., 1997; Hinds *et al*., 2003; Kraitchman *et al*., 2003; Zhao *et al*., 2002; Frank *et al*., 2003; Daldrup-Link *et al*., 2003). Initial cell-labeling techniques were hampered by limited concentration of internalized contrast agent which resulted in a limited sensitivity of MR Imaging to show the labeled cells. To compensate for this limited sensitivity, experimental cell-tracking studies were performed by using MR imagers with very high magnetic field strengths of up to 14 Tesla (Bulte *et al*., 2001b) alternatively, the contrast agent-labeled cells were directly injected into the organ of interest (Orlic *et al*., 2001) in which the cells had a small distribution volume, ensuring that contrast agent concentration remained relatively high in the examined field of view. With clinical 1.5-Tesla MR equipment and clinically applicable contrast agents, it has-to our knowledge-not been possible to trace the *in vivo* distribution of intravenously injected hematopoietic cells to more than one final target organ or to depict the migration of the transplanted cells to several subsequent target organs over time (Schoepf *et al*., 1998). Recently some studies show that it is possible to use 1.5 Tesla MR imaging to monitor the distribution of iron oxide labeled hematopoietic progenitors in different organs (Daldrup-Link *et al*., 2005). Newer and optimized labeling techniques have improved the efficiency of stem cell labeling and allowed the labeling of the cells with contrast agents that have been approved by the US. Food and Drug Administration or are currently being investigated in clinical trials (Frank *et al*., 2003; Daldrup-Link *et al*., 2003). Thus, the purpose of this study was to evaluate the use of clinical 1.5-T MR imaging equipment to depict the *in vivo* distribution of iron oxide-labeled human hematopoietic progenitor cells in irradiated mice. The MR imaging method by which we are exploring this concept is new and has an extensive potential in studying hematopoiesis. The results of this investigation can be later used to mark and follow stem cells *in vivo* and help better understand and monitor stem cell transplantation.

MATERIALS AND METHODS

This study was conducted in the Hematology department of Tarbiat Modares University in Iran.

Isolation of hematopoietic CD34$^+$ stem cells: Suspensions of human hematopoietic progenitor cells were prepared from Umbilical Cord Blood (UCB), which was collected from the umbilical cord vein after normal delivery of a full-term infant after obtaining informed constant. The Medical Ethics Review board of hospital approved the protocol for collecting the UCB for research purpose. The samples were collected in heparin-flushed syringes, stored at 4°C and processed within 8 h of collection. Low-density cells were isolated with density centrifugation (d = 1.077 g mL^{-1}). After centrifugation at 400x for 30 min, the mononuclear cells were collected and washed once in PBS. Subsequently, red cells were lysed with a lysing reagent. After erythrolysis the cell samples were washed twice in the PBS. The CD34$^+$ MNC fraction was directly isolated with superparamagnetic microbead selection using high-gradient magnetic field and mini MACS column (Miltenyl Biotech, Gladbach, Germany). The efficacy of the purification was verified by flow cytometry counter staining with anti-CD34-FITC and anti-CD133-PE antibody. In the cell fraction containing purified cells, CD34$^+$ cells ranged from 80 to 95%.

***Ex vivo* expansion of hematopoietic stem cells:** UCB CD34$^+$ cells were seeded at a concentration of 1×10^5 cells mL^{-1} in hematopoietic stem cell expansion medium (Sigma; S-0189, Stemline™) supplemented with 50 ng mL^{-1} recombinant human thrombopoietin (Sigma®; T-1568) and

stem cell factor (Sigma®; S-7901). Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2. The cell count was monitored daily and cell concentration was readjusted by the addition of fresh medium.

Cell labeling with superparamagnetic iron oxide: Different ratios of Fe-Pro complex were examined to determine the optimum concentration of these reagents. The commercially available ferumoxide suspension, ENDOREM® (Guerbet) contains particles approximately 80 to 150 nm in size and has a total iron content of 11.2 mg mL^{-1}. Ferumoxide at a concentration of 100 μg mL^{-1} and protamine sulfate at a concentration of 6 μg mL^{-1} were put into a mixing tube containing serum-free media. Protamine sulfate was prepared as a fresh stock solution of 1 mg mL^{-1} in distilled water at the time of use. The solution was mixed for 10 min with continuous shakings. For cells suspension, FE-Pro complexes were applied directly to the cells and then an equal volume of the respective complete medium was added to the cells in a final concentration of 50 μg ferumoxide and 3 μg protamine sulfate per ml of medium. The cells were then incubated overnight.

Histology: After incubation with FE-Pro, cells were washed three times to remove excess FE-Pro; and then transferred for cytospin slide preparation. Cells were fixed with methanol and washed and incubated for 30 min with 2% potassium Ferrocyanide (Potassium hexacyanoferrate II trihydrate, ACS reagent, 98.5-102.0%, Sigma® P3289) in 3.7% hydrochloric acid. They were washed again and counter stained with nuclear fast red.

Determination of labeling efficiency: FE-Pro labeling efficiency was determined by manual counting of stained and unstained cells on cytospinned slides with Perl staining. The percentage of labeled cells was determined from the average count in 5-10 high-powered fields.

Determination of mean iron concentration per cell: Ferumoxide at a concentration of 50 μg mL^{-1} was mixed with protamine sulfate at a concentration of 3 μg mL^{-1} and incubated with cells overnight to determine the intracellular iron concentration.

After labeling, cells were washed twice with PBS and a specific number of labeled and unlabeled cells were collected for atomic absorption assay.

Histological evaluation of spleen: For histopathologic examination, spleens were taken into paraffin block and sliced with microtome in the thickness of 5 μm for hematoxilin and eosin staining.

In order to reveal homing of $CD34^+$ cells in the spleen of injected mice, spleens of mice were removed after 1-2 days, blocked in paraffin and examined using Prussian blue staining.

Functional and phenotypic analysis of FE-Pro labeled cells: Both labeled and unlabeled $CD34^+$ cells were analyzed with spleen colony assay and histological staining. Labeled cells were injected at a concentration of 1×10^5 to 5×10^6 cells into tail vein in order to obtain the best dose needs for colony assay. Then each group received 6×10^5 $CD34^+$ cells and compared with control group that expected the same number of unlabeled cells. After 10 to 15 days, mice were killed by cervical dislocation and assessed for colony forming unit-spleen (CFU-S) generation. The spleens were put into the methanol for 30 sec and then were counted for colonies.

Animal and animal procedure: 6-8 week-old (20 to 30 g) female Balb/c mice were obtained and housed under pathogen-free conditions and fed with autoclaved food and water. All animal experiments were approved by the Animal Care Committee of Tarbiat Modares University School of Medicine. Mice received a fatal dose of 7.5 Gy total body gamma irradiation using a Co-60 source (Theratron II, 780C, Canada).

Ciprofloxacin (85 mg L^{-1}), Polymixine B (70 mg L^{-1}) and Amphotericin B (80 mg L^{-1}) were added to the drinking water after mice were irradiated.

Human $CD34^+$ cells were counted and resuspended in Stemline media containing 2% FCS and a cell dose of 6×10^5 cells/mouse was transplanted into mice for colony assay. Also a cell dose of 0.5×10^7 to 1.5×10^7 cells/mouse was transplanted into mice for MRI assay by tail vein injection 10 to 12 h after irradiation in a volume of 300 μL medium/ FCS 2%per mouse.

MR imaging: MR imaging of the animals was performed in each group before and 24 (n = 6), 36 (n = 6), 48 (n = 6) and 72 (n = 6) h after injection of labeled cells or contrast agent. Imaging was performed with a 1.5-Tesla MR imaging unit (SN 23344, Symphony, SIEMENS) and a knee coil. Pulse sequences comprised coronal T2-weighted three-dimensional fast field-echo, 32/10 sequences with a flip angle of 25° and an effective section thickness of 650 μm. MR images were acquired with a field of view of 100-75 mm, a 384 × 384 × 16 bit pixel matrix and an in-plane spatial resolution of 200×150 μm. Average signal intensities of bone marrow before and after cell injection were measured by one investigator (H.E.D.), who was blinded to the applied labeling procedure. Signal to noise ratios (SNRs) were calculated by dividing signal intensity

data of the target organ by the image (background) noise (random fluctuations in signal intensity), which was measured in the background anterior to the depicted object. This modification has been designed and conducted for the first time by our department.

Statistical analysis: For this pilot study, increasing quantities of cells -0.5×10^7, 1×10^7 and 1.5×10^7 cells-were injected into two animals in each contrast agent group. The initial number of cells was chosen on the basis of results from other studies (Daldrup-Link *et al*., 2005). Results of the pilot study revealed that only injections of 1.5×10^7 cells caused visible signal intensity changes in bone marrow.

The MR signals intensities before and after cell injections, quantified as SNR data were presented as means and standard errors of the mean. To compare differences in these quantitative MR data before and after injection of 1.5×10^7 cells, a two-tailed paired Student t-test was used. Differences in SNR data at different time points before and after injection in the same animals were tested for significance with an analysis of variance for repeated measurements. Statistical significance was assigned if $p<0.05$.

RESULTS

CD34$^+$ cells isolated from UCB: CD34$^+$ positive cells were isolated by manual cell separating unit and expanded in the presence of growth factors for more than three weeks. The purity of isolated umbilical cord blood CD34$^+$ cells was determined by flow cytometry. It was about 80 to 95% (Fig. 1 and 2).

Table 1: Colony assay of mouse spleen for human CD34$^+$ cells

Groups No.	No. of CD34$^+$	Mean No. of colony
Group 1 (n = 5)	1×10^5	5±1
Group 2 (n = 5)	2×10^5	7±2.5
Group 3 (n = 5)	3×10^5	13±3.2
Group 4 (n = 10)	5×10^5	24±5.6
Group 5 (n = 10)	6×10^5	26±3.5
Group 6 (n = 5)	8×10^5	23±2.8
Group 7 (n = 5)	1×10^6	8±1.8
Group 8 (n = 5)	2×10^6	5±1.2

*Significant difference between dosage of CD34$^+$: $p<0.05$

Labeling efficiency of FE-Pro and iron content per cell: Cells evaluation demonstrated approximately 100% labeling efficiency with FE-Pro at ratios of ferumoxide 50 μg mL^{-1} to protamine sulfate 3 μg mL^{-1} (Fig. 3). The average iron content per cell following FE-Pro labeling of cells was 1.98±0.03 pg iron cell^{-1} for HSCs. There was also iron in unlabeled HSCs ranging from 0.03 to 0.07 pg cell^{-1}.

Trypan blue dye exclusion test showed no significant increase in cell death compared with control cells at the end of overnight incubation.

Colony forming capacity of labeled and unlabeled CD34$^+$: The spleens of injected mice were counted in different groups. A cell dose of 5 to 6×10^5 cells showed the best result and choiced for final injection (Table 1). There was no different between labeled and unlabeled cells in colony forming properties, as compared with each other (Fig. 4).

In order to show CD34$^+$ cells' engraftment in mouse spleen, the section of spleens stained with Prussian blue for iron-oxide particles (Fig. 5) and Hematoxcilin and Eosin staining for colony assessment (Fig. 6).

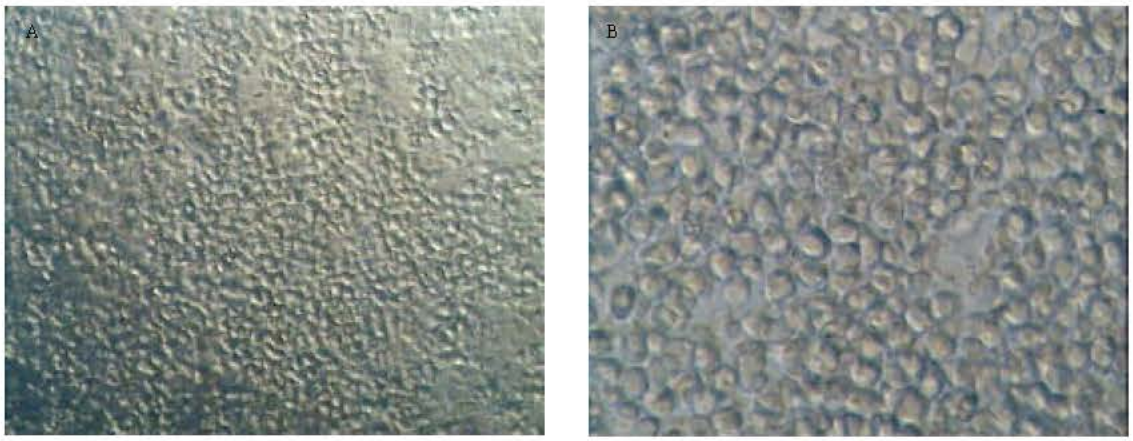

Fig. 1: The CD34+ cells isolated from human umbilical cord blood. Cell culture after one week of the initial seeding of CD34$^+$ cells (A, B). A final magnification for (A) is 20 and for (B) is 40

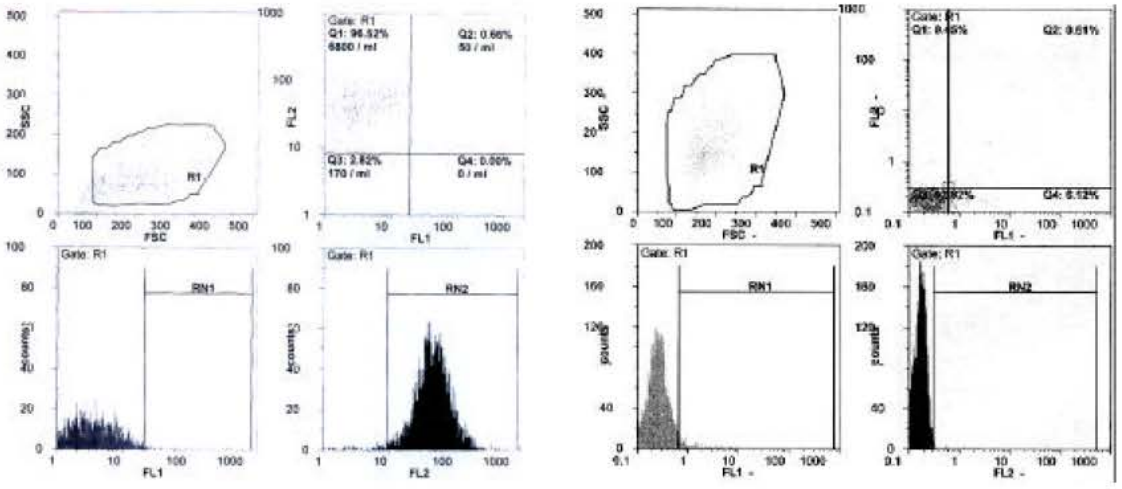

Fig. 2: Flow cytometry histograms show the immunophenotype of UCB CD34$^+$ isolated from the human cord blood. Isolated cells were positive for CD34$^+$ and negative for CD133

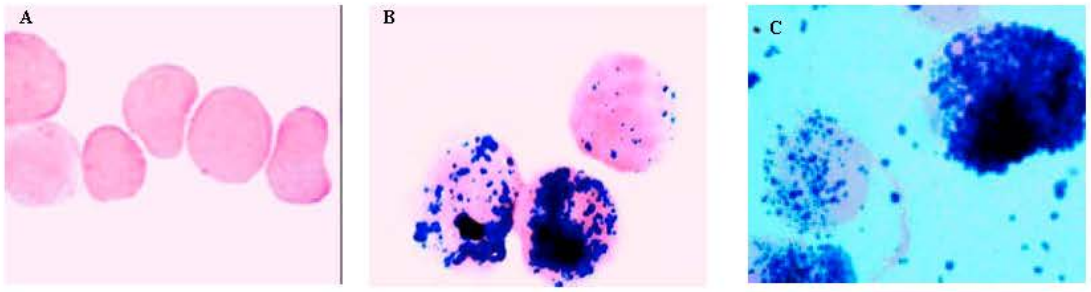

Fig. 3: Stem cells labeled with SPIO and protamine sulfate and stained with Perl`s staining. B and C indicate that stem cells cultured in the presence of SPIO and protamine sulfate absorbed these contrast agents from the medium. A indicates stem cells as a control group

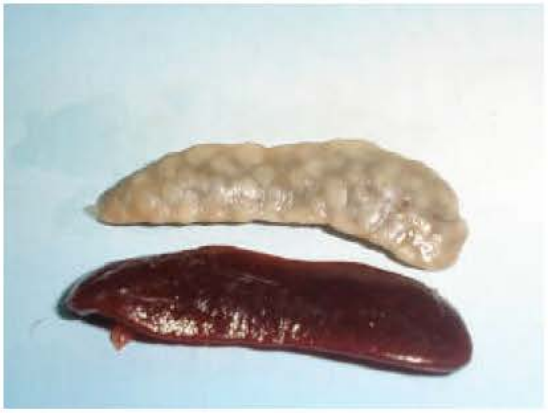

Fig. 4: Comparison of mouse spleen in normal and after transplantation. The spleen above belongs to a mouse that was injected with CD34$^+$ cells (after 14 days) and the picture below shows a normal spleen

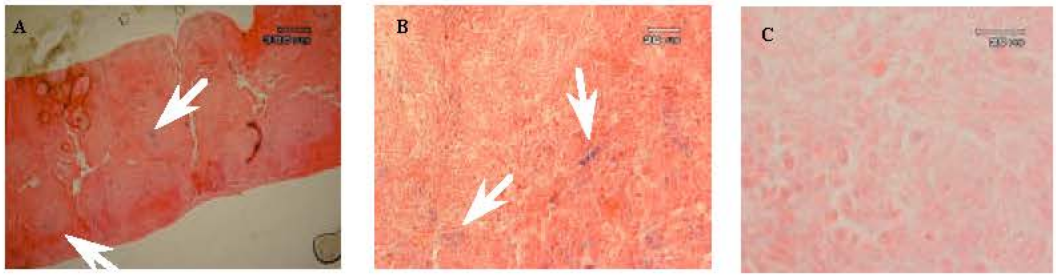

Fig. 5: Prussian blue iron staining of mouse spleen. A and B indicate colonies that include stem cells with iron particles in their cytoplasm after transplantation with labeled $CD34^+$ cells. (C) Shows normal colony with no iron particle in it

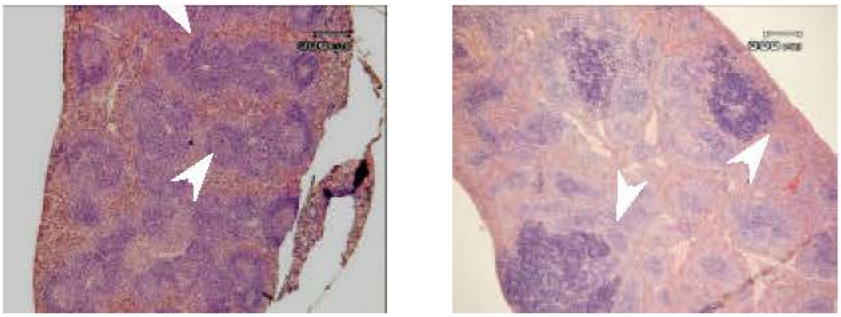

Fig. 6: Hematoxcilin and Eosin staining of mouse spleen. (Left) Shows colonies present in normal spleen. (Right) Indicates CFU-S that formed in spleen of irradiated mouse after transplantation with human UCB $CD34^+$ cells

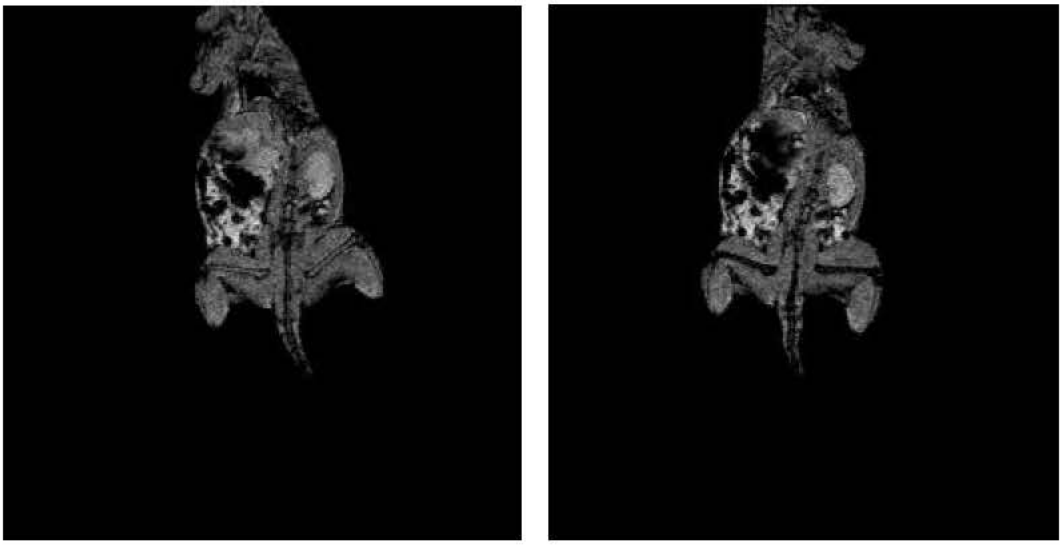

Fig. 7: Follow-up T2-weighted MR images of a Balb/c mouse before (left) and 48 h after (p.i.) intravenous injection of 2×10^7 FE-Pro-labeled progenitor cells (right). MR images show femoral bone marrow. Iron oxide–labeled cells show accumulation and subsequent signal intensity decline in bone marrow 48 h after injection (right). The amount of administered iron was 30 μg through injection of iron oxide-labeled cells

Table 2: MR signal intensities of bone marrow before and after injection of labeled human $CD34^+$ cells

SNR data	Bone marrow
Mice before injection (n = 10)	26.42±4.04
Mice after injection of 0.5×10^7 $CD34^+$ cells (n = 6)	21.17±3.25
Mice after injection of 1.0×10^7 $CD34^+$ cells (n = 6)	15.37±2.65
Mice after injection of 1.5×10^7 $CD34^+$ cells (n = 6)	9.58±2.08

*Significant difference between before and after treatment: $p<0.05$

MR imaging: After injection of ferumoxide-labeled cells, a cell concentration-dependent signal intensity decline was observed in bone marrow on T2- weighted MR images. Injection of 1.5×10^7 cells resulted in a significant signal intensity decline in bone marrow ($p<0.05$) (Table 2 and Fig. 7). A control injection of 1.5×10^7 unlabeled cells did not result in detectable changes in MR signal intensity.

CONCLUSION

In this study we used a method for cell labeling by using contrast agent and transfection agent. After cell labeling, we successfully used clinically applicable 1.5 Tesla MR imaging equipment to monitor homing of labeled cells in bone marrow of lethally irradiated mice. The results showed that by adding 3 μg protamine sulfates and 50 μg ferumoxide per ml culture medium the best effect in labeling process of $CD34^+$ cells was observed. In this concentration, cytoplasmic absorption of iron oxide by $CD34^+$ cells was estimated to be about 1.98 pg $cell^{-1}$. These findings are almost like the findings of other researchers who have used these two agents for cell labeling (Arbab *et al*., 2004a). To make a better labeling, we tried to use another transfection agent. For this purpose we used PLL, a transfection agent, instead of Protamine sulfate. Unfortunately we didn't obtain a reasonable result from this agent contrary to the findings of previous studies (Arbab *et al*., 2004b). In this study about 100% of $CD34^+$ cells were labeled with FE-Pro complex and effectively absorbed iron oxide. But when we used PLL as a transfection agent, the number of labeled cells significantly decreased and moreover the cytoplasmic absorption of the contrast agent was reduced. The advantage of using protamine sulfate and ferumoxide is that both of these agents have FDA-approval and have been used clinically. In a recent study in which the homing of stem cells by MRI was monitored (Daldrup-Link *et al*., 2005), P7228 was used as a contrast agent and liposome as a transfection agent none of which have been approved for clinical use by FDA.

We showed that cell labeling with contrast agent has no effect in the CFU-S formation properties of $CD34^+$ cells. For this purpose labeled and unlabeled $CD34^+$ cells were injected into the tail vein of irradiated mice and compared after two weeks for their CFU-S formation capacity (Table 1 and Fig. 4). The results showed that the labeling process had no effect on the function of labeled cells. In this study we didn't evaluate the effect of labeling on the lineage differentiation in the spleen. Some researches emphasize that the labeling procedure may change the lineage differentiation of labeled stem cells and labeled $CD34^+$ cells tend to differentiate to myeloid and megakaryocytic lineages rather than lymphoid.

The main goal in the development of new stem cell therapies is to achieve and prove the homing of transplanted cells to the particular tissue where they should exert their therapeutic activity (Strauer *et al*., 2003). This is of special importance if the cells are administrated systematically (e.g., after intravenous injection, when cells pass through several intermediate organs before reaching their final destination) rather than directly into the target organ. Data in our xenotransplant model showed that the *in vivo* distribution of systematically injected $CD34^+$ cells to the desired final target organ (bone marrow) can be traced using MR imaging. The observed *in vivo* cell distribution corresponded in general to the distribution of human hematopoietic cells in immunodeficient NOD/SCID mice in previous studies (Van Hennik *et al*., 1999; Kerre *et al*., 2001; Kollet *et al*., 2001). These cells disappeared rapidly from the circulation and accumulated in bone marrow (Kerre *et al*., 2001; Kollet *et al*., 2001). In bone marrow, an increasing accumulation of human cells was observed up to 24 h after injection (Van Hennik *et al*., 1999). This initial cell accretion was followed by cell persistence in marrow (Kerre *et al*., 2001; Kollet *et al*., 2001).

In this study, we first used MR imaging to depict early processes of cell transplantation. And then in order to show the successfully engrafted cells, we evaluated spleens of mice for CFU-S formation. In this context, it is very important to distinguish between homing and engraftment. Homing is characterized by the *in vivo* distribution of transplanted cells to specific target organ. This usually occurs within 24 to 48 h after injection (Oostendorp *et al*., 2000). But cell engraftment is characterized by subsequent proliferation and separation of progenitor cells and which usually occurs over several days or weeks after transplantation (Daldrup-Link *et al*., 2005).

In previous studies mostly NOD/SCID mice have been used as animal models but we have used Balb/c mouse as the animal model to simulate clinical situations. To this end we have also used radiotherapy for complete suppression of the immune system and elimination of Hematopoietic cells in our animal model. In previous studies to evaluate the engraftment of $CD34^+$ cells, a of

radiation dose of 3 to 3.5 Gy has been used for engraftment of human cells in NOD/SCID mouse (Van Hennik *et al*., 1999; Kerre *et al*., 2001; Kollet *et al*., 2001). In order to completely eradicate hematopoietic stem cells, we used a dose of radiation of 7.5 Gy that is lethal for mice and while completely eliminating hematopoietic stem cells it also suppresses the mouse immune system.

We have adjusted the amount of injected cells based on body weight and therefore we have overlooked the dosage of contrast agents and have based our injection upon the number of cells. In clinical situations 2×10^8 to 2×10^9 mononuclear hematopoietic cells per kilogram body weight are injected (Daldrup-Link *et al*., 2005), in this context we have used a dose of $0.5\text{-}1.5\times10^7$ cells per 20 g mouse. We purified $CD34^+$ cells from UCB and expanded them in specific culture medium with growth factors like SCF and TPO; these purified cells were then injected. In former studies mononuclear cells have been totally injected without purification (Daldrup-Link *et al*., 2005). We purified $CD34^+$ cells to eliminate the effect of other mononuclear and stromal cells in the homing of $CD34^+$ cells.

The results of MR imaging showed that injecting 1.5×10^7 cells per mouse causes significant decrease in the intensity of T_2 signal within 48 h (Table 2 and Fig. 7). In the only study performed in this manner the maximum signal reduction was achieved using 3×10^7 mononuclear cells within 24 h (Daldrup-Link *et al*., 2005). We can attribute the time difference between these studies to two factors:

First, we have injected purified $CD34^+$ cells to the animal. This causes the cells effective in homing of $CD34^+$ cells to be eliminated from the sample. Second, we have used Balb/c mice having experienced radiotherapy before engraftment. As some findings emphasize, this can destroy the bone marrow microenvironment ultimately disturbing the homing process of hematopoietic stem cells and causing delay in the reduction of BM signal.

In this study we managed to evaluate the homing of $CD34^+$ cells *in vivo* using the MR imaging method. In this research we showed that using proper contrast and transfection agents the hematopoietic stem cells can be more effectively labeled for MR imaging to monitor homing of $CD34^+$ cells in a noninvasive way. Use of the above agents that have also clinical applications opens a new perspective in the biology of cell therapy to better assess the homing of stem cells after transplantation.

Other potential clinical applications of this technique could be as follows: tracing cells in allogenic and autologous bone marrow transplantation, assessment of specific homing of diverse varieties of stem cell subtypes manipulated using genetic engineering methods and also evaluation of the effect of cell therapy on differentiation of stem cells and knowing the *in vivo* causes of graft rejection in certain diseases.

Nowadays, cells are used in therapy in various medical fields and MR imaging can be used for follow-up of therapy in some disease states. Monitoring of mesenchymal stem cells in injured myocardium (Kraitchman *et al*., 2003) and homing of neurological stem cells in impaired brain tissue (Bulte *et al*., 2001a) are examples of such therapies.

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