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Migration and homing of bone-marrow mononuclear cells in chronic ischemic stroke after intra-arterial injection

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ABSTRACT

Cell-based treatments have been considered a promising therapy for neurological diseases. However, currently there are no clinically available methods to monitor whether the transplanted cells reach and remain in the brain. In this study we investigated the feasibility of detecting the distribution and homing of autologous bone-marrow mononuclear cells (BMMCs) labeled with Technetium-99 m (^{99m}Tc) in a cell-based therapy clinical study for chronic ischemic stroke. Six male patients (ages 24–65 years) with ischemic cerebral infarcts within the middle cerebral artery (MCA) between 59 and 82 days were included. Cell dose ranged from 1.25×10^8 to 5×10^8. Approximately 2×10^7 cells were labeled with ^{99m}Tc and intra-arterially delivered together with the unlabeled cells via a catheter navigated to the MCA. None of the patients showed any complications on the 120-day follow-up. Whole body scintigraphies indicated cell homing in the brain of all patients at 2 h, while the remaining uptake was mainly distributed to liver, lungs, spleen, kidneys and bladder. Moreover, quantification of uptake in Single-Photon Emission Computed Tomography (SPECT) at 2 h showed preferential accumulation of radioactivity in the hemisphere affected by the ischemic infarct in all patients. However, at 24 h homing could only distinguished in the brains of 2 patients, while in all patients uptake was still seen in the other organs. Taken together, these results indicate that labeling of BMMCs with ^{99m}Tc is a safe and feasible technique that allows monitoring the migration and engraftment of intra-arterially transplanted cells for at least 24 h.

Introduction

Over the last decade, stem-cell-based therapy has been introduced as a promising attempt to improve recovery after stroke (Dobkin, 2007, Kondziolka et al., 2005, 2000, Savitz et al., 2005). Autologous bone marrow-derived stem cells have been used more frequently, both in pre-clinical studies (Chopp and Li, 2002, Liu et al., 2006, Mezey, 2007, Tang et al., 2007) and in a few pilot and ongoing clinical trials (Bang et al., 2005, Mendez-Otero et al., 2007, Mendonca et al., 2006). In experimental models, it has been suggested that possible therapeutic effects could be due to the production of trophic factors by bone marrow-derived cells and lead to an increase in angiogenesis, neurogenesis and synaptogenesis, allowing favorable remodeling of the brain (Chen et al., 2003, Chopp and Li, 2002).

Non-invasive *in-vivo* imaging of the transplanted cells may provide better understanding of many unresolved issues in this field, such as the efficiency of different approaches to cell delivery and the correlation with functional evaluation. Radioisotope cell labeling is a well-established method that allows systemic monitoring of cells, and has been applied in different clinical studies involving cell therapy for myocardial infarction (Goussetis et al., 2006, Hofmann et al., 2005, Kang et al., 2006, Penicka et al., 2007) and acute stroke (Correa et al., 2007).

In this study, we investigated the distribution and homing of intra-arterial-delivered radioactive-labeled bone-marrow mononuclear cells in patients enrolled in a research study to assess the safety of this procedure for chronic ischemic stroke.

Patients and methods

Patients

Six male patients (ages 24–65 years) with ischemic cerebral infarcts within the territory of the middle cerebral artery (MCA) were included in this non-randomized, open-label phase I clinical trial (NCT00473057). The following criteria were required for patient enrollment: (1) age between 18 and 75 years, (2) ischemic stroke in the MCA territory within the last 90 days evidenced by computed tomography (CT) or magnetic-resonance imaging (MRI); (3) recanalization of the involved MCA as assessed by transcranial Doppler

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(TCD) studies; and (4) a score between 4 and 17 according to the National Institutes of Health Stroke Scale (NIHSS). We excluded patients to whom any of the following criteria applied: (1) carotid stenosis (>50%, by Doppler studies) ipsilateral to the stroke, (2) neurological worsening (>4 points in the NIHSS) before injection, due to either edema or intracerebral hemorrhage, (3) thrombophilias or primary hematological diseases, (4) previous stroke with modified Rankin Scale >2, (5) neurodegenerative disorders, (6) auto-immune disorders, (7) intracardiac thrombus, (8) sepsis (according to the 1992 criteria of the Society of Critical Care Medicine and the American College of Chest Physicians), (9) history of neoplasia or other comorbidity that could impact the patient's short-term survival, (10) bone disorders that could increase the risk of the bone-marrow harvesting procedure, (11) renal failure (creatinine >2 mg/ml), (12) liver failure, (13) lacunar stroke, (14) life-support dependence, (15) pregnancy or previous participation in other clinical trials, (16) difficulty in obtaining vascular access for percutaneous procedure, (17) any condition that in the judgment of the investigator would place the patient at undue risk.

The Research Ethics Committee of the Institution and the National Committee of Ethics and Scientific Research approved the study protocol. Written informed consent was obtained from all patients. They were informed in detail about the nature of the procedure and the radiolabeling technique.

Bone-marrow aspiration, cell separation and labeling with Technetium-99m

Bone marrow (80 ml) was aspirated under local anesthesia from the posterior iliac crest. BMMCs were isolated by density gradient on Ficoll at 400×*g* for 30 min (Ficoll-Paque Plus 1.077, 1:2, Amersham Biosciences, São Paulo, Brazil). Mononuclear cells were washed in saline containing 5% human serum albumin and filtered through 100-μm nylon mesh to remove cell aggregates. After washing, counting and viability testing, the cells were resuspended in 10 ml of saline solution with 5% autologous serum. For each patient, 2×10^7 cells were labeled with ^{99m}Tc based on previously published protocols (Carvalho et al., 2008, Gutfilen et al., 2006, 1999, Lopes de Souza et al., 2004, Quintanilha et al., 2008). All the procedures for cell preparation and labeling were carried out in a laminar flow. In short, 500 μl of sterile SnCl2 solution was added to the cell suspension in 0.9% NaCl, and the mixture was incubated at room temperature for 10 min. Then, 45 mCi ^{99m}Tc was added and the incubation continued for another 10 min. After centrifugation (500×*g* for 5 min), the supernatant was removed and the cells were washed again in saline solution. The pellet was resuspended in saline solution. Viability of the labeled cells was assessed by the trypan blue exclusion test, and was estimated to be greater than 93% in all cases. Labeling efficiency (%) was calculated by the activity in the pellet divided by the sum of the radioactivity in the pellet plus supernatant, and was estimated to be greater than 90% in all cases.

Flow-cytometry analysis and fibroblast colony-forming assay

Isolated mononuclear bone-marrow cells were characterized by flow-cytometry analysis of specific surface antigens. Cells were incubated for 20 min at room temperature with primary antibodies conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), peridinin chlorophyll protein (PercP) and phycoerythrin cyano 7 (PE-Cy7). The markers tested included: pan-leukocyte: CD45 (Immunostep); pan T cell: CD3 (BD Biosciences Pharmigen); cytotoxic T cell: CD8 (BD Biosciences Pharmigen); helper T cell: CD4 (BD Biosciences Pharmigen); pan B cell: CD19 (BD Biosciences Pharmigen); NK cell: CD56 (BD Biosciences Pharmigen); promonocyte: CD64 (Immunotech); monocyte: CD14 (IQP); hematopoietic progenitor cells: CD117 (BD Biosciences Pharmigen) and CD34 (BD Biosciences Pharmigen); mesenchymal cells: CD105 (Immunostep), CD73 (BD Biosciences Pharmigen) and CD90(BD Biosciences Pharmigen); neutrophils: CD31(BD Biosciences Pharmigen) and CD33 (BD Biosciences Pharmigen); anti HLA-DR (MHC-II, BD Biosciences Pharmigen). After staining, erythrocytes were lysed with the B&D Lysis Buffer Solution. Data acquisition was performed on a BD FACS CANTO cytometer (Beckton & Dickson) and analyzed with the "Paint-a-Gate" software. A fibroblast colony-forming assay was performed to determine the presence of putative progenitor cells of mesenchymal lineages, as previously described (Castro-Malaspina et al., 1980).

Cell transplantation

Cells labeled as described above were added back to the total mononuclear cell suspension (final volume of 10 ml). Femoral arterial punctures were done in all patients. A 6 Fr. Guiding catheter (Envoy-Cordis, Miami, Florida; or a Guider Soft tip, Boston Scientific, Target Therapeutics, Fremont, California) was used. Patients underwent anticoagulation with intravenous heparin to obtain an activated clotting time of two to three times baseline. A digital cerebral angiography was performed (Angiostar, Siemens Medical Systems, Erlangen, Germany) to allow visualization of the intracranial vasculature before injection and monitoring of flow normality and vessel patency. A large-inner-diameter micro catheter (SL 1018 Boston Scientific, Target Therapeutics, Fremont, California) was navigated to the M1 portion of the middle cerebral artery, and the infusion was done at the rate of approximately 1 ml/min.

Imaging

Whole-body and planar images were captured using a Millennium GE camera (General Electric Medical Systems, Milwaukee, Wisconsin). Acquisition protocols were performed at 2 h and 24 h after cell therapy. Whole-body images were acquired for 20 min in anterior and posterior views, using a dual-head whole-body scanner with high-resolution, low-energy collimator. Planar images were acquired for 10 min, matrix 256×256, in anterior, right and left lateral, and posterior views. Single-Photon-Emission Computed Tomography (SPECT) was performed with two 180° opposed rotating detectors with low-energy high-resolution collimators. The software and hardware for image reconstruction was a Xeleris-GE processing workstation for reconstruction of the SPECT image data. Projections were collected over 24 min, with each detector rotating 180 to make a complete 360 revolution in a circular orbit with the patient placed in the supine position. Image volumes were reconstructed using the OSEM algorithm with axial smoothing and a Butterworth filter with an order of five and a cutoff of 0.45. Image volumes consisted of $64\times64\times64$ voxels each measuring 4.38 mm^3. The detector distance ranged from 14 cm to 20 cm during rotation, resulting in a spatial resolution FWHM (full width at half-max) of approximately 12 mm.

For each patient, regions of interest were drawn for the brain, liver, spleen, lungs, kidneys, bladder, and for the whole body, and the radioactive counts were automatically quantified for these regions in whole body planar images at 2 h after cell injection. Uptake was defined as the percentage of organ-originated number of counts compared to the total number of counts in the whole body. To allow better discrimination of the uptake in the brain, regions of interest corresponding to the left and right hemispheres were drawn in SPECT images and the radioactive counts were automatically quantified. Uptake in hemispheres ipsilateral and contralateral to the lesion was defined as the percentage of hemisphere-originated number of counts compared to the total number of counts in both hemispheres.

CT images were acquired before cell transplantation with a 40–detector row scanner (Brilliance-40, Philips Medical Systems). Volumetric analysis was performed to measure the approximate volumes of the infarcted areas. Volumes were obtained by multiplying the measured area per slice by the section thickness. SPECT and CT

Table 1
Patient characteristics.

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex/age (years)	M/24	M/65	M/47	M/65	M/57	M/47
Infarct location	Left	Right	Left	Left	Right	Right
NIHSS at admission	9	7	4	13	9	13
Time from onset to BMMC infusion (days)	67	82	62	72	59	73
N° of injected cells	5×10^8	1.25×10^8	3.9×10^8	4×10^8	3.2×10^8	1×10^8
Lesion volume (cm^3)	116	47	17	71	181	213

BMMC: Bone-marrow mononuclear cells; NIHSS: National Institutes of Health Stroke Scale.

images were exported by DICOM protocol, and transferred to a Macintosh computer (MacOS X, version 10.4). Images were fused with dedicated image processing software (OsiriX imaging software, version 3.6, Geneva, Switzerland).

Clinical evaluation

Patients underwent neurological examination at admission, on the day of transplantation and at 3, 7, 30, 60, 90 and 120 days after cell infusion. The examination included the Barthel Index, the modified Ranking scale and the National Institutes of Health Stroke Scale. Laboratory tests (complete blood count and biochemical tests for urea, creatinine and electrolytes) were also performed at the same time-points. An electroencephalogram (EEG) was performed within 7 days of cell transplantation.

Statistical analysis

Because of the small sample size, the analysis was mostly descriptive. The descriptive statistics of cell characteristics on FACS are given as mean values with standard deviation. Pearson and Spearman correlation tests were used to assess the relationship between total brain uptake and different variables (age, time from onset to cell therapy, number of injected cells, lesion volume and ipsilateral hemisphere uptake). Probability values of 0.05 or less were considered to indicate statistical significance. Statistical analysis was performed with the SPSS 17 (SPSS Science) statistical software package for Windows.

Results

Patients

The patient characteristics are presented in Table 1. All individuals had cerebral infarcts that involved the MCA territory as documented by neuroimaging exams. Patients received between 1.25×10^8 and 5×10^8 mononuclear cells, and cell transplantation took place between 59 and 82 days after the stroke. Clinical, laboratory and electroencephalographic evaluations showed no adverse effects during the procedure or follow-up, and no patient scored worst on the BI, mRS, or NIHSS tests. The cell immunophenotype is presented in Table 2.

Imaging

Whole-body scans obtained 2 h after transplantation of labeled BMMCs showed uptake in the brains of all patients, which ranged from 0.6% to 5.1 % when compared to the activity in the whole body. The location of cell homing could be better visualized in SPECT images than in whole-body planar images. Transverse views of SPECTs, CTs and SPECT/CT fusion images for two representative patients are shown in Fig. 1. Quantification of cell uptake in SPECT images indicated preferential uptake on the side of the lesion in all patients (Table 3). Nevertheless, these differences were widely variable, ranging from 58% to 98% of total brain uptake (Table 3). The remaining cell uptake was distributed mainly to the liver, lungs, spleen and kidneys in all patients (Table 3). Due to the 6-h half-life of ^{99m}Tc, image resolution was greatly decreased at 24 h after cell transplantation, and regions of interest could not be adequately determined to allow quantification of uptake in different organs (Fig. 2). At 24 h, uptake could only be visualized in the brains of patients 1 and 3, while in all patients uptake was seen in the liver, lungs, spleen, kidneys and bladder. Fig. 2 illustrates the whole-body images from one of the patients in which cells could not be identified in the brain at 24 h; and Fig. 3 shows brain planar views of one patient in which uptake could be identified at 24 h. The presence of free ^{99m}Tc was excluded by thyroid region planar nuclear imaging. Movies 1 and 2 correspond to dynamic three-dimensional SPECT images, demonstrating cell homing in the left MCA territory 2 h after cell

Table 2
Cell immunophenotype.

CD markers	Phenotype	%	No of cells $\times10^7$
$CD45^{low}CD34^{high}SSC\downarrow$	Hematopoietic stem cells	1.60 ± 0.83	11.80 ± 2.17
$CD45^{low}CD34+CD19+$	B-cell progenitors	1.30 ± 0.95	0.37 ± 0.16
CD45+CD34-CD33+CD64+CD14-	Promonocytes	0.78 ± 0.47	0.22 ± 0.08
$CD105+CD90^{high}CD73+CD45\text{-}CD34-$	Mesenchymal stem cells	0.12 ± 0.09	0.03 ± 0.01
$CD105+CD90^{high}CD73+CD45\text{-}CD34+$	Endothelial stem cells	0.04 ± 0.07	0.01 ± 0.01
CD45+CD34-CD64+CD14+	Monocytes	4.45 ± 3.97	1.28 ± 0.68
CD45+CD34-CD3+	T Lymphocytes	8.75 ± 4.80	2.51 ± 0.82
CD45+CD34-CD3+CD4+	Helper T cells	5.84 ± 3.87	1.68 ± 0.67
CD45+CD34-CD3+CD8+	Cytotoxic T cells	2.32 ± 1.57	0.67 ± 0.27
CD45+CD34-CD3-CD19+	B cells	2.32 ± 1.56	0.67 ± 0.27
CD45+CD34-CD56+	NK cells	7.86 ± 3.076	2.26 ± 0.53
Functional assay	No of colonies $\times10^6$ BMMC seeded		
Fibroblast Colony-forming assay	35.75 ± 7.63		

The indicated percentages of immune cell subtypes have been calculated using the Paint-a-Gate PRO software (BD Biosciences), which displays a combination of the morphological characteristics (side scatter and forward scatter) and the specific CD marker expression, thus allowing a multiparametric analysis and identification of the cell subtype percentages.

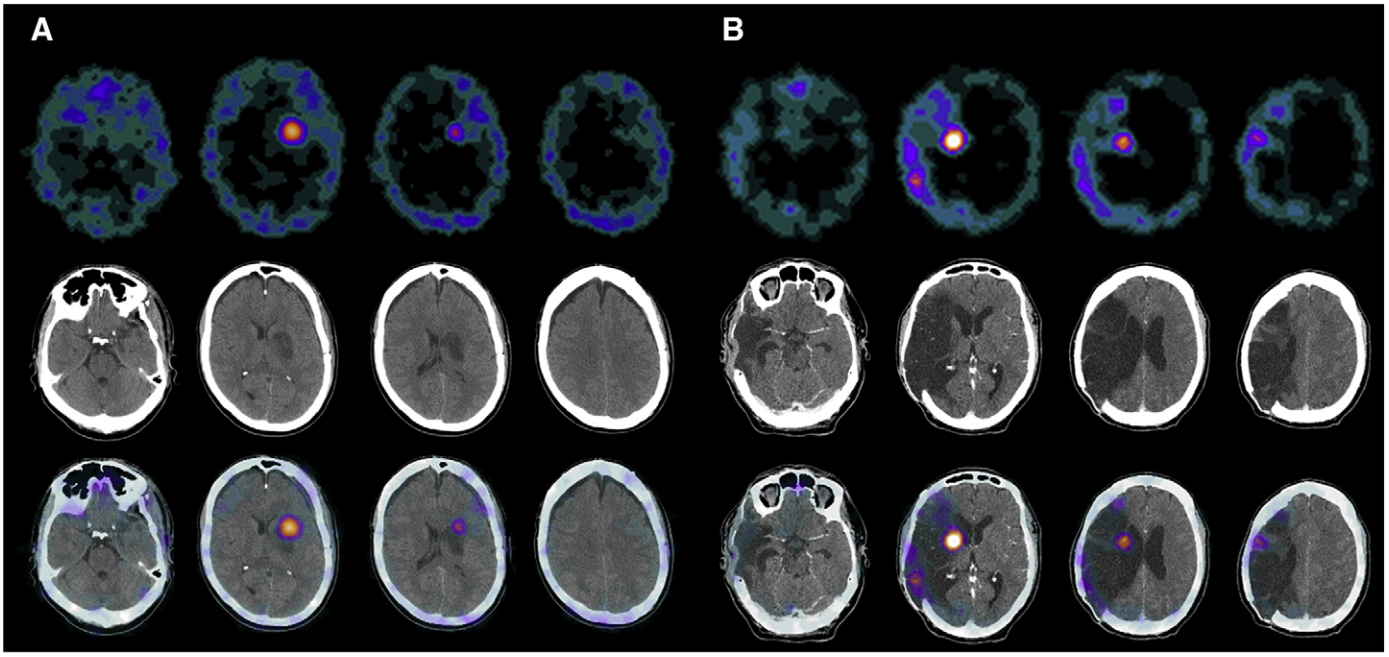

Fig. 1. Transverse views of SPECTs (upper row), CTs (middle row) and SPECT/CT fusion images (lower row) of patients 3 (A) and 6 (B).

transplantation in patients 3 and 4, respectively. Statistical analysis indicated that brain uptake at 2 h seemed to be inversely related to age ($r=-0.883$; $p=0.02$). Also, greater uptake in the brain was related to a preferential uptake in the lesion's hemisphere ($r=0.869$; $p=0.025$). Volume of lesion ($r=0.057$; $p=0.914$), time from onset to infusion ($r=-0.314$; $p=0.544$) and the number of cells injected ($r=0.543$; $p=0.266$) did not seem to be related to brain uptake.

Discussion

Bone-marrow mesenchymal stem cells (MSCs) and mononuclear cells (BMMCs) increase functional outcomes in animal models of stroke when delivered by intravenous, intracerebral and intra-arterial routes (Chen et al., 2001, 2003, Chopp and Li, 2002, Giraldi-Guimaraes et al., 2009, Kamiya et al., 2008, Li et al., 2001, 2000). It has been shown that bone marrow cells target the ischemic lesion, and this is thought to be mediated by injury-induced chemokines (Hill et al., 2004, Shen et al., 2007a,b). Current preclinical evidence indicates that the main mechanism of cell-based therapy is not by direct cell replacement, but by trophic, anti-inflammatory and immunomodulatory effects that have an acute but persistent effect on the brain before these cells die (Barnabe et al., 2009, Bliss et al., 2007, Hess and Borlongan, 2008, Mendez-Otero et al., 2007). Therefore, long-term cell persistence and engraftment in the brain may not be necessary for a therapeutic effect (Bliss et al., 2007, Hess and Borlongan, 2008, Mendez-Otero et al., 2007). Cells may not even need to enter the brain to elicit an effect, but rather act in the periphery to increase trophic-factor expression in the brain, (Borlongan et al., 2004, Sarnowska et al., 2009) and positive effects have been seen even when cell injection takes place 1 month after the stroke (Shen et al., 2007a,b, Yasuhara et al., in press).

Radioisotope cell labeling is a well-established method that allows systemic monitoring of cells in nuclear-medicine studies (Palestro et al., 2009) , and has already been applied for tracking cells in cell therapy for myocardial infarction (Goussetis et al., 2006, Hofmann et al., 2005, Kang et al., 2006, Penicka et al., 2007) and acute stroke (Correa et al., 2007) in humans. The 6-h half-life of ^{99m}Tc is an important advantage over the half-life of 110 min of 18F-fluorodeoxyglycose (FDG). 111-Indium-oxine, another commonly used radiopharmaceutical, allows cell tracking for up to 96 h, but has disadvantages that include suboptimal photon energies, low resolution images and the 18- to 24-h interval between injection and imaging that is usually required (Banerjee et al., 2005, Palestro et al., 2009). ^{99m}Tc allows imaging for 24–48 h and results in higher image

Table 3
Quantification of uptake in different regions at 2 h after cell transplantation in percentages.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Mean ± SD
Uptake quantified in whole body planar images							
Brain/whole body	5.1	0.7	1.5	0.5	0.9	1.4	1.68 ± 1.71
Liver/whole body	42.4	39.9	47.7	38.5	48.7	44.2	43.56 ± 4.10
Spleen/whole body	5.2	3.1	2.2	4.1	5.6	3.7	3.98 ± 1.27
Kidneys/whole body	3.6	4.1	7.3	2.4	5.9	2.8	4.35 ± 1.89
Lungs/whole body	7.8	6.6	4.7	9.1	8.8	6.2	7.20 ± 1.68
Bladder/whole body	8.7	8.1	10.2	7.9	9.4	9.8	9.01 ± 0.93
Uptake quantified in Brain SPECT images							
Ipsilateral hemisphere/brain	97.8	65.7	82.2	77.4	58.3	79.2	76.76 ± 13.72
Contralateral hemisphere/brain	2.2	34.3	17.8	22.6	41.7	20,8	23.23 ± 13.72

Uptake in the brain, liver, spleen, kidneys, lungs and bladder was defined as the percentage of organ-originated number of counts compared to the total number of counts in whole body planar images 2 h after cell transplantation.
To allow better discrimination of the uptake in the brain than in whole body planar images, uptake in hemispheres ipsilateral and contralateral to the lesion was defined as the percentage of hemisphere-originated number of counts compared to the total number of counts in both hemispheres in SPECT images 2 h after cell transplantation.

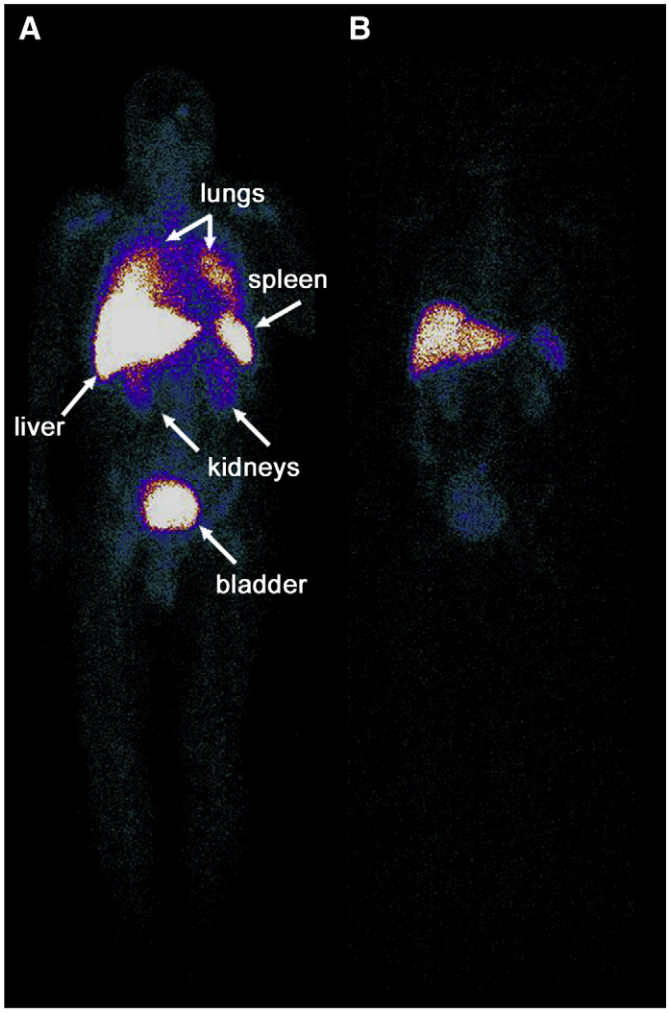

Fig. 2. Anterior whole-body scans performed 2 h (A) and 24 h (B) after cell injection in the territory of the MCA show the distribution of BMMCs labeled with ^{99m}Tc in patient 4. Uptake in the left brain hemisphere could only be distinguished at 2 h. The remaining activity was distributed mainly to the liver and spleen at 2 h and 24 h. Arrows indicate the location of the liver, lungs, spleen, kidneys and bladder.

resolution and a lower radiation burden to the patient (Banerjee et al., 2005, Palestro et al., 2009).

In preclinical models, direct cell labeling with contrast agents such as superparamagnetic iron oxides (SPIO) for MRI allows cell tracking from a few days to several weeks after transplantation (Budde and Frank, 2009, Lee et al., 2008, Modo et al., 2005). Moreover, this technique imaging provides higher spatial resolution and direct anatomical correlation (Budde and Frank, 2009, Lee et al., 2008, Modo et al., 2005). Cell tracking with MRI suffers from common limitations observed with exogenous cell tagging, such as the sensitivity of the imaging technique, dilution of the contrast with cell division, and potential transfer of the magnetic label to tissue macrophages (Budde and Frank, 2009, Lee et al., 2008, Modo et al., 2005). Currently there have been only 4 clinical studies in the literature involving a total of 24 patients using magnetically labeled cells and MRI to monitor the migration of cells to target tissue (Callera and de Melo, 2007, de Vries et al., 2005, Toso et al., 2008, Zhu et al., 2006) and at this time superparamagnetic or paramagnetic agents are not approved by regulatory agencies for cell labeling (Budde and Frank, 2009, Lee et al., 2008, Liu and Frank, 2009, Modo et al., 2005).

In humans, preliminary data from two Phase I clinical trials involving intra-arterial injection of BMMCs for acute ischemic stroke (3–10 days post-ictus) with a total of 25 patients have suggested the procedure is feasible and safe (Correa et al., 2007, Freitas et al., 2006, Friedrich et al., 2006, Mendonca et al., 2006), but the final reports have not been published. In one of these studies, one patient received cells labeled with ^{99m}Tc-HMPAO, and whole-body scintigraphy and SPECT indicated cell accumulation in the brain 8 h after cell transplantation (Correa et al., 2007). Another Phase I study treated 5 patients with a total of 1×10^8 intravenous bone marrow autologous MSCs in two doses at 4–5 and at 7–9 weeks after stroke and found the procedure to be feasible and safe (Bang et al., 2005).

Nonetheless, the safety and feasibility of BMMC injection in chronic stroke, as well as cell labeling, have never been studied in humans. Data from pre-clinical studies with acute ischemic models comparing different administration routes suggest that there are differences in cell retention rate (Lappalainen et al., 2008, Walczak et al., 2008). Intracerebral injection usually leads to poor cell distribution throughout the lesion (Li et al., 2000, Walczak et al., 2008), and intra-arterial injection of BMMCs and MSCs provides greater cell retention when compared to intravenous injection (Kamiya et al., 2008, Lappalainen et al., 2008, Walczak et al., 2008). Nevertheless, there are no reports on the percentage of BMMCs grafted after intra-arterial injection, and only limited reports for MSCs, where cell homing varied from 1% (Lappalainen et al., 2008) to 21% (Li et al., 2001). A recent study suggested that intra-arterial injection of MSCs led to wide variation in cerebral engraftment, and 17% of animals had no cells detectable in the brain. The same study also suggested that high cerebral engraftment rates of MSCs were associated with impeded cerebral blood flow as measured by laser Doppler flow (Walczak et al., 2008). Notwithstanding, there may be significant differences, depending on the ratio between the diameter of cells injected and the capillary size (Lappalainen et al., 2008, Walczak et al., 2008). Furthermore, this effect may have been increased by cerebral edema, because cell injection was performed 30 min after the stroke (Walczak et al., 2008), and therefore further studies are warranted to assess these questions. Early data from a clinical trial investigating the safety and feasibility of BMMCs for acute stroke monitored cerebral blood flow by TCD and EEG, and found no evidence of embolization or electroencephalographic changes (Freitas et al., 2006, Mendonca et al., 2006).

The total number of cells injected was the maximum obtained from 80 ml of bone-marrow aspiration, and varied according to each patient (1.25×10^8 to 5×10^8). These differences in cell quantity were in accordance with the interval approved for this protocol. This cell dose was consistent with other studies involving intra-arterial BMMC injection in ischemic myocardial infarction (Huikuri et al., 2008,

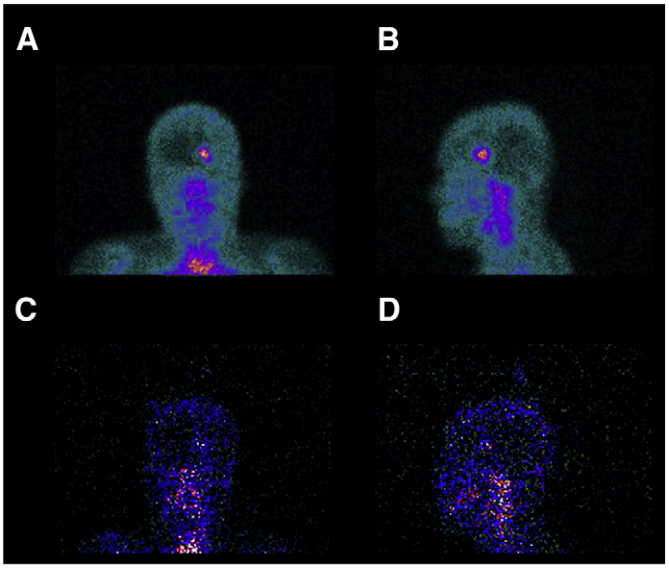

Fig. 3. Anterior and left lateral views of brain planar scans indicate homing of BMMCs at 2 h (A and B, respectively) and 24 h (C and D, respectively) after cell injection in patient 3.

Lunde et al., 2006, Meyer et al., 2006, Penicka et al., 2007, Strauer et al., 2005, Tendera et al., 2009) and cerebral infarction (Correa et al., 2007, Mendonca et al., 2006). The transplanted cell population was characterized using a well-defined set of phenotypic markers, and we observed many phenotypes that included different progenitor cells. Moreover, the functional characterization of fibroblastic like cells confirmed the presence of putative progenitor cells of mesenchymal lineages.

We have previously reported preliminary data from this study that indicated that the procedure was safe (Battistella et al., 2008) and that the cells labeled with ^{99m}Tc migrated to the brain of the first patient enrolled (Barbosa da Fonseca et al., 2009). Here, we provide further evidence that BMMC homing occurs in the brain after intra-arterial injection in chronic stroke. Furthermore, we provide for the first time the quantification of cell uptake in the brain and in other organs after cell therapy for stroke. A study with an animal model of stroke has suggested that the abundance and responsiveness of bone marrow progenitor cells to gradients of factors such as Stromal Derived Factor-1 may decrease with age and reduce the chemoattraction of such cells to the damaged tissue (Kucia et al., 2006). However, it is not currently possible to explain the wide variability of homing between patients, and there are probably multiple variables involved that remain to be investigated in future studies.

In summary, we would like to suggest that non-invasive imaging modalities such as the one described in this study can be used to monitor the delivery and tracking of cells, and may improve understanding of possible functional responses in the setting of chronic stroke. Moreover, the advantages offered by other imaging modalities, such as cell labeling with SPIO for MRI, could be used in the future in conjunction with SPECT to provide further information on cell homing.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.expneurol.2009.10.010.

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