MRI tracking of Stem Cells in Multiple Sclerosis

Vincent Dousset, MD, PhD & Thomas Tourdias, MD

Laboratory of Neurobiology of Myelin Diseases

University Victor Segalen Bordeaux

vincent.dousset@u-bordeaux2.fr

For complementary information, please read:

1. Dousset V, Tourdias T, Brochet B, Boiziau C, Petry KG. How to trace stem cells for MRI evaluation? J Neurol Sci. 2008;265(1-2):122-6. Review.
2. In vivo visualization of macrophage infiltration and activity in inflammation using magnetic resonance imaging. Beckmann N, Cannet C, Babin AL, Blé FX, Zurbruegg S, Kneuer R, Dousset V. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009 May;1(3):272-98.
3. Schäfer R, Ayturan M, Bantleon R, Kehlbach R, Siegel G, Pintaske J, Conrad S, Wolburg H, Northoff H, Wiskirchen J, Weissert R. The use of clinically approved small particles of iron oxide (SPIO) for labeling of mesenchymal stem cells aggravates clinical symptoms in experimental autoimmune encephalomyelitis and influences their in vivo distribution. Cell Transplant. 2008;17(8):923-41.
4. Conserved fate and function of ferumoxides-labeled neural precursor cells in vitro and in vivo. Cohen ME, Muja N, Fainstein N, Bulte JW, Ben-Hur T. J Neurosci Res. 2009 Nov 2. . [Epub ahead of print]
5. Serial in vivo MR tracking of magnetically labeled neural spheres transplanted in chronic EAE mice. Ben-Hur T, van Heeswijk RB, Einstein O, Aharonowiz M, Xue R, Frost EE, Mori S, Reubinoff BE, Bulte JW. Magn Reson Med. 2007 Jan;57(1):164-71.
6. Stem cell therapy for myelin diseases. Ben-Hur T, Einstein O, Bulte JW. Curr Drug Targets. 2005 Feb;6(1):3-19. Review.

Labeling stem cells and tracking the cells *in vivo* by imaging techniques is a new challenge that follows the increasing field and perspectives of grafting cells in human to eliminate pathological conditions or to reconstruct a tissue. Progresses in biology have led to distinguish and study cells coming from a tissue. Now, the much more difficult goal is to redo a tissue using cells. Several questions arise when dealing with *in vivo* tracking labeled-cells: why, which contrast agent for which imaging technique, how to label cells *in vitro*, which cells,

what are the risks for cell properties deterioration, which route of cell administration, what is the labeling duration, is it possible to add a therapeutic compound to use cells as delivery vehicle, what are the limitations linked to the concept itself?

OBJECTIVES OF STEM CELLS IMAGING

The primary goal is to assess and follow non invasively cell engraftment. When repeated over time, imaging modalities allow to evaluate *in vivo* cell mobility, viability and in some cases differentiation. Additionally, a lot of new knowledge are drawn by the observation of grafted cells behavior in the living matter. For an example, homing to the bone marrow of human hematopoetic progenitors (CD34+) labeled with MR compatible contrast agent (USPIO ferumoxides) has been assessed *in vivo* in mice at human magnetic resonance field (1.5T) [1]. Homing to the brain is another challenge since few cell types may migrate and traffic through it in physiological or pathological conditions.

Spatial resolution of human compatible *in vivo* imaging modalities is not sufficient to show individual cells and to differentiate targeted cells from others. Increasing the signal of those cells requires a contrast agent with the need of amplification or signal change to be detected. Positron emission tomography (PET), single-photon emission computed tomography and magnetic resonance imaging (MRI) are today the candidates for *in vivo* human CNS cellular imaging. Although sensitivity of PET tracers is much higher at lower concentration than MRI contrast agents, experiments with PET are limited in time due to the short half-life of tracers though impeding the follow-up labeled cells.

CONTRAST AGENTS TO LABEL CELLS IN VITRO

When dealing with this question, the major goal is that the introduction of a contrast agent within a single cell and then within the organism must keep completely safe for the cell life and intrinsic properties, and for the whole recipient organism. The metabolism of the contrast agent should be fully known. Several contrast agents have been used to label cells in experimental studies. It is important to remain that no such studies have been conducted so far on human. The step forward i.e. human use will necessary need safety controls at several levels. With MR imaging, gadolinium chelates and iron oxide particles are currently the best contrast agent candidates to label cells because they are well tolerated when directly injected in the blood stream [4]. Complexes of gadolinium or liposomes carrying gadolinium chelates increase the efficiency of labeling and the stability of gadolinium molecules within the cells [5,6]. Some particles may also carry microscopically detectable fluorescent agents allowing to perform histology more easily in experimental models [5]. Commercially available iron particles such as superparamagnetic iron oxides (SPIO) and ultra-small particle iron oxides

(USPIO) can be used as such to label cells. In several studies using intracellular SPIO or USPIO, it has not been shown deterioration of cell metabolism or animal death [6]. Complex particles of iron oxide, magnetodendrimers, might also been used because their size and relaxivity increase the efficiency of labeling and MR imaging signal change respectively [7]. Some transplanted cells may directly act as therapy like it is expected from stem cells to settle and repair damaged tissues [8-10]. Another challenge is the drug delivery to the brain by cells carrying a therapeutic agent through the so-called "Troie Horse" strategy. New particles may carry MR contrast agent and a therapeutic agent. Thus the transplanted cells might act as delivery vehicles using several expected modalities like changing the cell metabolism or inducing cell apoptosis to activate and liberate the therapeutic agent, etc.

HOW TO LABEL STEM CELLS IN VITRO WITH CONTRAST AGENTS?

Several methods have been described.

Prolonged incubation of the cells in contact to the contrast agent may result in the passive introduction of the contrast agent within the cell. However the efficiency of such method is restricted to cells that express a high degree of phagocytosis or pinocytosis [11].

Using the TAT peptide, an iron-based contrast agent has been successfully taken up within the nucleus of CD34 incubating cells [12].

Transfection of contrast agents in cells may dramatically increase the efficiency of cell labeling. Several transfection agents are commercially available and might be used safely [13,14].

Magneto-electroporation using a magnetic field to increase the membrane permeability transiently results in a quick cytoplasmic accumulation of contrast agent [15].

These methods always require a high level of safety and several analyses to ensure that viability, migration capability and intrinsic properties of cells are not altered.

Other methods use the incorporation of genes in the cell nucleus with the goals either to express a cell membrane receptor to a contrast agent which may accumulate in viable cells, or to induce the production of a MRI detectable contrast agent such as ferritin thus making the cell construct is own contrast agent [16,17].

CELLS ELIGIBLE TO BE LABELED IN VITRO

In the literature, many cell types have been labeled in vitro: embryonic stem cells, human mesenchymal stem cells, neural stem cells, hematopoïetic bone marrow stem cells, oligodendrocyte precursors, schwann cells, olfactory ensheathing cells, monocytes, lymphocytes, microglia, etc. This step does not appear to be challenging. However the control of the amount of contrast agent in the cells is not usually well described since it is quite

difficult to assess it precisely. Heterogeneity from cell to cell must be considered. Figure 1 shows in culture of microglial cells with an uptake of an USPIO-rhodamine contrast agent incorporated with a transfectant.

LABELING-INDUCED RISKS FOR STEMS CELLS

Several properties must be kept after the in vitro management of the cells and the intrusion of the contrast agent: viability, migration capability, differentiation into the appropriate phenotype, controlled proliferation, ability to retain the contrast agent to follow-up the cell course, etc.

For an example, it has been shown that when containing between 9 and 14 pg iron/cell, cells are unaffected in their viability and proliferating capacity, and labeled human neuronal stem cells differentiate normally into neurons [7].

In several experiments, labeled cells with iron particles have been followed up to 8 weeks showing a long lasting duration of the labeling [18,19]. After released out of the cell the behavior of the contrast agent must be known. Commercially available contrast agents probably follow the same catabolism pathway than free particles when injected into the blood stream. This is not yet well established in the literature. Cells like hematopoetic progenitor may get an induction of forced iron elimination after incubation with USPIO or SPIO [20].

ROUTES OF CELLS ADMISNISTRATION

After the in vitro step for labeling using a contrast agent that incorporates into the cell cytoplasm or nucleus, cells must be delivered in the targeted place within the CNS. Two methods can be used: 1) the direct injection of the cells via a stereotactic administration into the brain parenchyma or in the lateral ventricles [5,8,9,21,22]; 2) the injection in the blood stream either in the veins or in the CNS feeding arteries [10,19]. In animals the intraperitoneal administration of cells may also result in cell migration to a targeted organ.

Direct administration to the brain or spinal cord

In a pioneer work, Bulte et al. showed the migration of magnetically labeled oligodendrocyte precursors from the site of stereotactic injection in the spinal cord to areas that required myelination in new-born rats [21]. The same group showed the passage in the brain parenchyma of labeled oligodendrocyte precursors directly injected in the lateral ventricle of experimental encephalomyelitis rats [21].

In experimental stroke, implanted USPIO labeled embryonic stem cells in the contralateral hemisphere migrated to the periphery of the stroke area through the corpus callosum and populate the area around the ischaemic infarct [5,8]. It was observed that on arrival in the

lesioned hemisphere several of the cells show neuron-like shapes of cell body with long dendritic- or axon-like extensions [8].

Transplanted iron labeled C17.2 neural stem cells either stayed in the site of injection or migrated along the corpus callosum in normal mouse brains, whereas in neonatal mouse the cells demonstrated a widespread engraftment [22].

SPIO labelled schwann and olfactory ensheathing cells traced in vivo by MRI retain functional properties after transplantation into the CNS promoting axonal regeneration and remyelination [9].

Vascular administration to target the CNS

The injection into the blood stream of stem cell may result in cell destruction in the blood, in the liver or the spleen. Daldrup-Link et al. showed in vivo the homing of SPIO labeled human CD34 hematopoietic progenitor into the femur bone marrow of mice [1]. Targeting the CNS has been achieved by Nakamizo et al. who injected into the contralateral carotid artery human mesenchymal stem cells (hMSC) that engrafted into a U87 glioma implanted in the right hemisphere [10]. Treated animals with hMSC had a significant increased survival [10].

CONCLUSION

Researches in the field of cell labeling and tracking are very tentative. However, it must be stressed that it is a sophisticated approach requiring several steps that we have try to resume here: isolation of cells, phenotype characterization, production/choice of contrast agent, labeling of cells *in vitro*, evaluation of uptake process *in vitro* by staining and/or electron microscopy of cells, MRI of labeled cells *in vitro*, assessment of cell viability, animal model, preparation of cells for transplantation, MRI of transplanted labeled cells *in vivo* and *ex vivo*, histology, verification of the cell type containing the contrast agent, and statistical analysis [9]. The major criticism about some published papers in this field is the weakness arising from statistical analysis that sometime misses to assess reproducibility of experiments or observations. Another limitation is the absence of verification that the labeled cells seen *in vivo* correspond to the cultured cell. Indeed, many labeled cells may dye during the administration and the contrast agent may be taken up by other cells especially macrophages or microglia. Lecture of images or histology are often of single reader thus not ensuring reliability. An effort is needed to go from very interesting isolated observations to strong reproducible methods.

References

1. Daldrup-Link HE, Rudelius M, Piontek G, Metz S, Brauer R, Debus G, Corot C, Schlegel J, Link TM, Peschel C, Rummeny EJ, Oostendorp RA. Migration of iron oxide-labeled human hematopoetic progenitor cells in a mouse model: in vivo monitoring with 1.5-T mr imaging equipment. Radiology 2005;234:197-205.
2. Kraitchman DL, Tatsumi M, Gilson WD, Ishimori T, Kedziorek D, Walczak P, Segars WP, Chen HH, Fritzges D, Izbudak I, Young RG, Marcelino M, Pittenger MF, Solaiyappan M, Boston RC, Tsui BMW, Wahl RL, Bulte JMW. Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction. Circulation 2005;112:1451-61.
3. de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. Nat Biotechnol 2005;23:1407-13.
4. Dousset V, Brochet B, Deloire MS, Lagoarde L, Barroso B, Caille JM, Petry KG. MR imaging of relapsing multiple sclerosis patients using ultra-small-particle iron oxide and compared with gadolinium. AJNR Am J Neuroradiol 2006;27:1000-5.
5. Modo M, Mellodew K, Cash D, Fraser SE, Meade TJ, Price J, Williams SC. Mapping transplanted stem cell migration after a stroke: a serial, in vivo magnetic resonance imaging study. Neuroimage 2004;21:311-7.
6. Bulte JW. Intracellular endosomal magnetic labeling of cells. Methods Mol Med 2006;124:419-39.
7. Bulte JW, Douglas T, Witwer B, Zhang SC, Strable E, Lewis BK, Zywicke H, Miller B, van Gelderen P, Moskowitz BM, Duncan ID, Frank JA. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nat Biotechnol 2001;19:1141-7.
8. Hoehn M, Küstermann E, Blunk J, Wiedermann D, Trapp T, Wecker S, Föcking M, Arnold H, Hescheler J, Fleischmann BK, Schwindt W, Christian B. Monitoring of implanted stem cell migration *in vivo*: A highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat. PNAS 2002;99:16267-72.
9. Dunning MD, Lakatos A, Loizou L, Kettunen M, Charles ffrench-Constant C, Brindle KM, Franklin RJM. Superparamagnetic iron oxide-labeled schwann cells and olfactory ensheathing cells can be traced in vivo by magnetic resonance imaging and retain

functional properties after transplantation into the CNS. J of Neuroscience 2004;24:9799–810.

10. Nakamizo A, Marini F, Amano T, Khan A, Studeny M, Gumin J, Chen J, Hentschel S, Vecil G, Dembinski J, Andreeff M, Lang FF. Human bone marrow–derived mesenchymal stem cells in the treatment of gliomas. Cancer Res 2005; 65(8): 3307-18.
11. Doche de Laquintane B, Dousset V, Solanilla A, Petry K, Moretto P, Caillé JM, Ripoche J. Iron particle labeling of haematopoetic progenitor cells: an vitro study. Bioscience Report 2002;22:549-54.
12. Lewin M, Carlesso N, Tung CH, Tang WX, Cory D, Scadden DT, Weissleder R. TAT peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol 2000;18:410-14.
13. Frank JA, Miller BR, Arbab AS, Zywicke HA, BS, Jordan EK, Lewis BK, Bryant Jr LH, Bulte JWM. Clinically Applicable Labeling of Mammalian and Stem Cells by Combining Superparamagnetic Iron Oxides and Transfection Agents. Radiology 2003;228:480-487.
14. Miyoshi S, Flexman JA, Cross DJ, Maravilla KR, Kim Y, Anzai Y, Oshima J, Minoshima S. Transfection of neuroprogenitor cells with iron nanoparticles for magnetic resonance imaging tracking: cell viability, differentiation, and intracellular localization. Mol Imaging Biol 2005;7:286-95.
15. Walczak P, Kedziorek DA, Gilad AA, Lin S, Bulte JW. Instant MR labeling of stem cells using magnetoelectroporation. Magn Reson Med 2005;54:769-74.
16. Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Shiocca EA, Basilion JP. In vivo magnetic resonance imaging of transgene expression. Nat Med 2000;6:351-5.
17. Genove G, DeMarco U, Xu H, Goins WF, Ahrens ET. A new transgene reporter for in vivo magnetic resonance imaging. Nat Med 2005;11:450-4.
18. Bulte JW, Duncan ID, Frank JA. In vivo magnetic resonance tracking of magnetically labeled cells after transplantation. J Cereb Blood Flow Metab 2002;22:899-907.
19. Jendelova P, Herynek V, Urdzikova L, Glogarova K, Kroupova J, Andersson B, Bryja V, Burian M, Hajek M, Sykova E. Magnetic resonance tracking of transplanted bone marrow and embryonic stem cells labeled by iron oxide nanoparticles in rat brain and spinal cord. J Neurosci Res 2004;76:232-43.
20. Sun R, Dittrich J, Le-Huu M, Mueller MM, Bedke J, Kartenbeck J, Lehmann WD, Krueger R, Bock M, Huss R, Seliger C, Grone HJ, Misselwitz B, Semmler W, Kiessling F. Physical and biological characterization of superparamagnetic iron oxide- and ultrasmall superparamagnetic iron oxide-labeled cells: a comparison. Invest Radiol

2005;40:504-13.

21. Bulte JWM, Zhang SC, van Gelderen P, Herynek V, Jordan EK, Duncan ID, Frank JA. Neurotransplantation of magnetically labeled oligodendrocyte progenitors: Magnetic resonance tracking of cell migration and myelination. PNAS 1999;96;15256-61.
22. Magnitsky S, Watson DJ, Walton RM, Pickup S, Bulte JW, Wolfe JH, Poptani H. In vivo and ex vivo MRI detection of localized and disseminated neural stem cell grafts in the mouse brain. Neuroimage 2005;26:744-54.