Molecular imaging in cell therapy: monocyte tracking and real-time evaluation of angiogenesis in ischemic limb by high resolution MRI

Jelena Kolosnjaj-Tabi, Jose Vilar, Nathalie Lucani, Claire Wilhelm, Gwennhael Autere, Daniel Balvay, Jean-Sebastien Silvestre, Florence Gazeau, and Olivier Clément

1 Paris-Diderot University, Laboratoire Matière et Systèmes Complexes, Paris, Ile de France, France; 2 Paris Cardiovascular Research Center-PARCC / Paris-Descartes University, Paris, Ile de France, France

Purpose: Limb ischemia is a devastating disease with potential tragic outcome (amputation) and high social costs. The effects of cellular therapy, notably the role of monocytes in enhancement of neo-angiogenesis, have been recognized long ago. However it is only recently that advances in molecular imaging have allowed approaches that enable the assessment of distribution and impact of cells in vivo in real time. This is pivotal for the understanding of the outcome of cell therapies and orienting/controlling the treatment with transplanted cells. High-resolution MRI (HR-MRI) appears very promising for the follow-up of cell therapies because it can allow detection of single (labelled) cells and, in case of diseases such as ischemia, it may allow the non-invasive evaluation of post-ischemic neovascularization. The purpose of this study was to evaluate magnetically labelled monocyte infiltration in the ischemic murine hind limb at different time points and to evaluate their therapeutic effect in vivo with a 4.7 T scanner provided with a cryogenic probe that allows a spatial resolution at micrometric scale.

Methods: Murine bone marrow-derived monocytes were isolated and labelled with anionic ultra small iron oxide citrate-coated nanoparticles. The internalization of this contrast agent was evaluated by single cell magnetophoresis and the method of cell visualisation and quantification was optimized in vitro in agarose phantoms. Subsequently, 5x10^7 murine monocytes were intra-venously administered to mice with ischemic hind paw (ischemia was induced by permanent femoral artery ligation). The distribution of labelled monocytes was evaluated by HR-MRI, performed with a cryoprobe-equipped 4.7 T Scanner. A three-dimensional (3D) gradient echo sequence (TR/TE = 20/5 ms, flip angle=50°, resolution: 39 x 500 µm^3) and post mortem by a high definition digital x-ray transducer.

Results: When labelled monocytes are administered to mice, they migrate to the ischemic paw where they can be visualized by HR-MRI (Fig. 1) and consequently quantified, despite a relatively low iron uptake, determined by single cell magnetophoresis (Fig.2). Moreover, labelled cells have a statistically significant pro-angiogenic effect that is quantifiable and comparable to the effect of non-labelled monocytes, as confirmed by post mortem angiography (Fig.3).

Conclusion: High-resolution MRI is an emerging non-invasive method that allows detection and quantification of single cells and can allow the follow-up of the neo-angiogenesis in vivo.

Figure 1: a) 3D gradient echo MR image of the murine paw after treatment with labelled monocytes, sagittal coverage was amplified by overlapping 15 slices with minimum intensity projection (MinIP) volume-rendering technique with OsiriX image processing software; b) magnified view of a zone in fig. 1a; c) image processed by ImageJ software that evidences cells (red arrows); d) angiographic susceptibility-weighted MR image of the paw; e) magnified view of fig. 1d.

Figure 2: Distribution of iron load per monocyte as determined by single cell magnetophoresis

Figure 3: Neo-angiogenesis in control (phosphate buffer saline (PBS)-injected) mice, monocyte ("Mono") and labelled monocyte-injected mice (Np@Mono); N=8 for each group; post mortem angiogenesis was quantified after processing of x-ray images by Primedangio software.