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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Monitoring inflammation in stroke using combined high resolution magnetic resonance imaging and synchrotron radiation-phase computed tomography (SR-PCT) of the mouse brain	**Experiment number**: LS2292
Beamline: ID19	**Date of experiment**: from: April 2014 to: June 2014	**Date of report**: June 15, 2015
Shifts: 15	**Local contact(s)**: Vincent Fernandez, Lukas Helfen	*Received at ESRF:*

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Report:

Study#3- K-edge imaging of Gadolinium

Further acquisitions using synchrotron radiations are in needed for publication of this work.

Introduction

Stroke is one of the most common causes of death in the western world. Several lines of evidence suggest that inflammatory processes contribute to the destruction of neural tissue. To better understand and exploit the endogenous mechanisms at work in focal ischemia, molecular events need to be examined in detail in animal models of stroke. We recently demonstrated at ID19 that Synchrotron Radiation X-ray Phase Computed Tomography (SR-PCT) was an unvaluable complement to ultrasmall superparamagnetic particles of iron oxide (USPIO)-enhanced MRI in mouse models of ischemic stroke (1,2). To overcome some limitations of USPIOs, new kinds of nanoparticles have been developed by our collaborators from the Laboratoire de Chimie de l'ENS Lyon (Stéphane Parola). These multimodal nanoparticles consist in a gadolinium (Gd)-based inorganic core (GdF_3) functionalized with a fluorochrome. Another limitation of the approach is the fact that neither MRI nor SR-PCT permits discriminating between nanoparticles and hemorrhagic transformation, a common complication of stroke (Figure 1). Therefore, our current aim is to specifically image gadolinium. To this purpose, we plan to investigate two strategies: K-edge imaging using spectral CT (3) (with the arrival of the first commercial spectral scanner in Lyon in 2015) and K-edge imaging using synchrotron radiation.

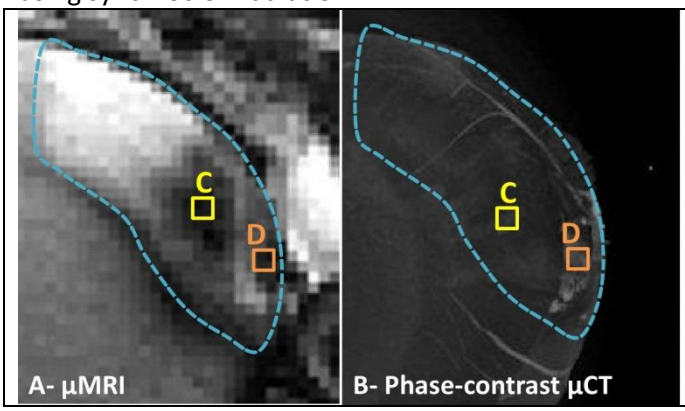

Figure 1- Confounding effects of hemorrhagic transformation in a mouse model of stroke on µMRI and Synchrotron radiation phase-contrast µCT (SR-PCT). The nanoparticles (NP) that were injected to the animal in order to detect macrophages (Mϕ) (C) appear as a hypointense signal on µMRI (A) and hyperintense signal on SR-PCT (B). The hemorrhage (D) has the same hypointense (A) / hyperintense (B) signal and thus cannot be distinguish from NPs without slicing the brain.

Methods

K-edge describes a sudden increase in the attenuation coefficient of photons occurring at a photon energy just above the binding energy of the K shell electron of the atoms interacting with the photons. For this interaction to occur, the photons must have more energy than the binding energy of the K shell electrons. A photon having an energy just above the binding energy of the electron is therefore more likely to be absorbed than a photon having an energy just below this binding energy. To perform K-edge imaging of a given element, two absorption images are respectively acquired just below/above the binding energy of the K shell electrons (4). The subtraction of these two acquisitions produces a specific image of the element. For proof-of-concept of k-edge imaging, we have first prepared tubes with a range of concentration for USPIO and GdF3. We have then used mouse brains (N=3) that had been stereotaxically injected with GdF3.

Results

During a pilot study, we have shown that K-edge imaging of gadolinium was feasible at ID19 thanks to the set-up of a monochromator allowing to acquire images at 50 keV and 50.4 keV (K-edge of Gd = 50.2 keV) (Figure 2).

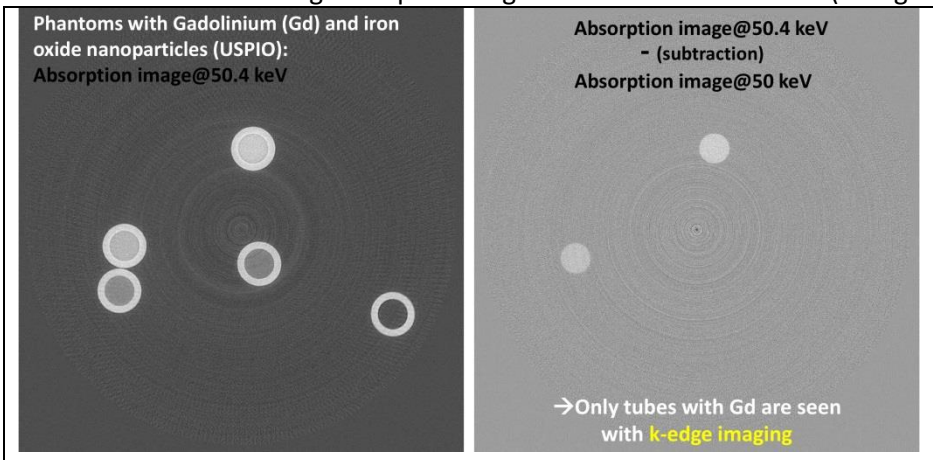

Figure 2- Proof of concept of specific imaging of gadolinium with K-edge imaging. Phantoms were prepared with a range of concentrations of iron oxide nanoparticles (USPIO) on the one hand (N=2: 500 and 250 mM), and a range of concentrations of Gadolinium-based nanoparticles on the other hand (N=2: 500 and 250 mM) prepared in PBS (N=1 phantom with PBS only). Absorption images were acquired at 50 keV and at 50.4 keV (left image). Subtraction of the two acquisitions depicts the tubes with Gadolinium only (right image).

Moreover, while scanning a mouse brain sample stereotaxically injected with the Gd-based nanoparticles, we realized that bleeding had occurred on one side of the brain because K-edge imaging did not produce any signal on this side (Figure 3). Therefore K-edge imaging with synchrotron radiation appears as a promising approach to distinguish nanoparticles from hemorrhage.

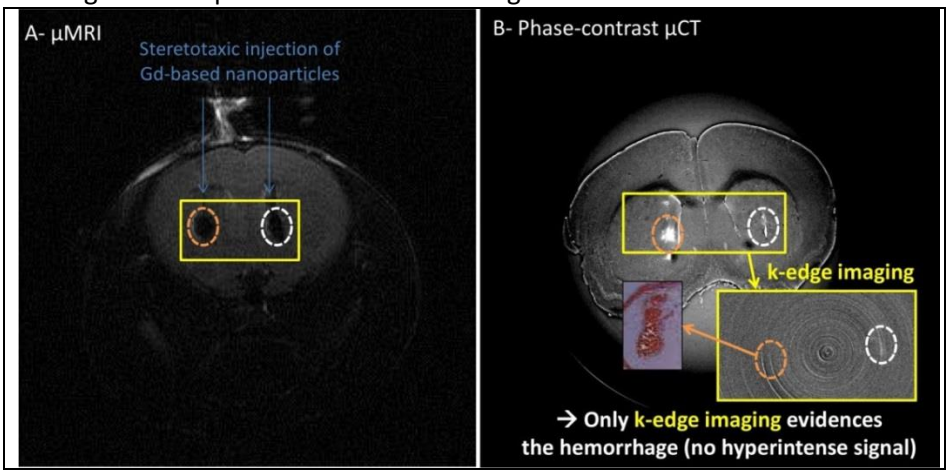

Figure 3- Proof of concept of discrimination between Gadolinium and hemorrhage with K-edge imaging. One mouse was stereotaxically injected with Gadolinium-based nanoparticles in the left and right hemisphere, resulting in hypointense signal on MRI (A) and hyperintense signal on SR-PCT (B). K-edge imaging showed a hyperintense signal in the right hemisphere. The injection in the left hemisphere had inadvertently provoked a hemorrhage. This was evidenced with K-edge imaging without having to slice the brain.

Conclusion & perspectives

The next step is to evaluate the sensitivity of the technique and to test it in a mouse model of stroke (proposal to be submitted in September). In a first set of experiment, phantoms will be prepared with a range of hybrid nanoparticles concentrations to assess the sensitivity of detection. In a second set of experiment, the same range of concentrations will be stereotaxically injected into the brain of C57/Bl6 adult male mice (N=6) to perform the same tests *ex vivo*. In a last set of experiment, mice (N=6) will undergo middle cerebral artery occlusion followed by MRI at Day 0 (before injection) and Day 1 (24h after injection) according to our well-established protocol. After the last imaging exam, mouse heads (brain within the skull) will be collected and prepared for K-edge imaging. After K-edge imaging, brains will be extracted from the skull, sliced and observed under the microscope to detect hemorraghic events as well nanoparticles thanks to their fluorescence. These experiments will provide a reference frame for K-edge imaging with spectral CT, a new technology with high potential for in-vivo molecular imaging with CT.

Acknowledgments

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