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ESRF

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 using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now 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

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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:** Characterization of the Gd cellular uptake and radiosensitization with Gd contrast agents	**Experiment number**: MD-1038
Beamline: ID17	**Date of experiment**: from: 30/06/2017 – 8h am to: 04/07/2017 - 8h am	**Date of report**: 29/02/2020
Shifts: 12	**Local contact(s)**: Herwig Requardt	*Received at ESRF:* 01/03/2020

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Report:

Objectifves of the experiment :

This experiment is performed as a complement to the imaging experiment carried out on the ID16-A beamline in February 2017, as part of the MD-1022 proposal. The main objective is to measure the potential radiosensitization of F98 cell after incubation with both gadolinium contrast agents (GdCA) Dotarem (Gd-DOTA cyclic complex) and Magnevist (Gd-DTPA linear complex). It has been previously shown that incubation with 5 mg/mL of both GdCA during 24h lead to an internalization of the gadolinium into the cytoplasm in significant quantities, located mainly in vesicles as lysosomes (see experiment report of MD-1022 experiment performed at ID-16). The irradiation beamline ID17 allow the irradiation at specific monoenergetic X-rays ($\Delta E/E \approx 0.1\%$) from 20 to 90 keV, which can be optimized for each high-Z element of interest to activate Auger electron cascades for example (just above K-edge) or intracellular hot spot of doses to improve radiosensitization. Previous studies suggested that damage caused to lysosomes might be responsible to cell radiosensitization that internalized nanoparticles in it, due to the role of this organelle in the initiation of cell death signaling (Stefancikova *et al.* 2014 & 2016). Furthermore, we previously performed similar experiments on ID17 beamline and reported high-sensitization enhancement ratio (SER) in presence of gadolinium nanoparticles that were incubated or not with cells before irradiation, and compared them to those of performed with Magnevist that was not incubated before irradiation (Taupin *et al.* PMB 2015, 60, 4449–4464). However, simulation studies shows that gadolinium contrast agents, as nanoparticles, should also cause cell damage due to high local doses, as long as the gadolinium is internalized in cells (Delorme *et al.* Phys. Med. 44(11), 5949-5960). The images obtained in the MD-1022 experiment demonstrated that GdCA can be internalized in important quantities in cells after long incubation time, we thus propose in this experiment to determine the SER of cells in incubation conditions with both GdCA and using several irradiation energies.

Experimental protocol

We performed clonogenic assays to determine the SER of F98 rat glioma cells in presence of both Dotarem and Magnevist following irradiation of monoenergetic Xrays. Cells were prepared with 1 million cells in T25 flasks 24h before irradiation, either incubated (at 37 °C in an atmosphere containing 95% air and 5% CO2) with complete culture medium alone, or incubated with complete medium mixted with GdCA at a concentration of 5 mg/mL. In order to separate the contribution of internalized Gd only, with the contribution of Gd present inside and outside the cells, we either irradiated the cells just after having rinsing them ("rinsed" condition), or irradiated in the media-containing Gd ("non-rinsed" condition). Cells irradiated without Gd served as "control" condition. The cells were irradiated in suspension in cryotubes of 2 mL. The dosimetry was determined using a calibrated cylindric ionization chamber (PTW Semiflex ion chamber 31010 – 0.125 cm3) placed in the irradiation condition. Figure 1 shows the irradiation setup and a reprensentation of the beam energies used in the experiment against theoretical SER calculated at different radiation doses. This representation highlight the importance of the x-ray energy and dose choices for the evaluation of the SER. At 1 and 2 Gy, the SER variations would be too small to be measured using clonogenic assays. In the present study, the radiosensitization was evaluated experimentally at 4 Gy for reasons of consistency with our previous studies (Taupin *et al.* 2015, Delorme *et al.* 2017) at 3 characteristic beam energies: 50 keV (low-energy reference below Gd K-edge), 51 keV (just above Gd K-edge, maximum interaction cross section) and 80 keV (less effect expected but interesting for clinical applications).

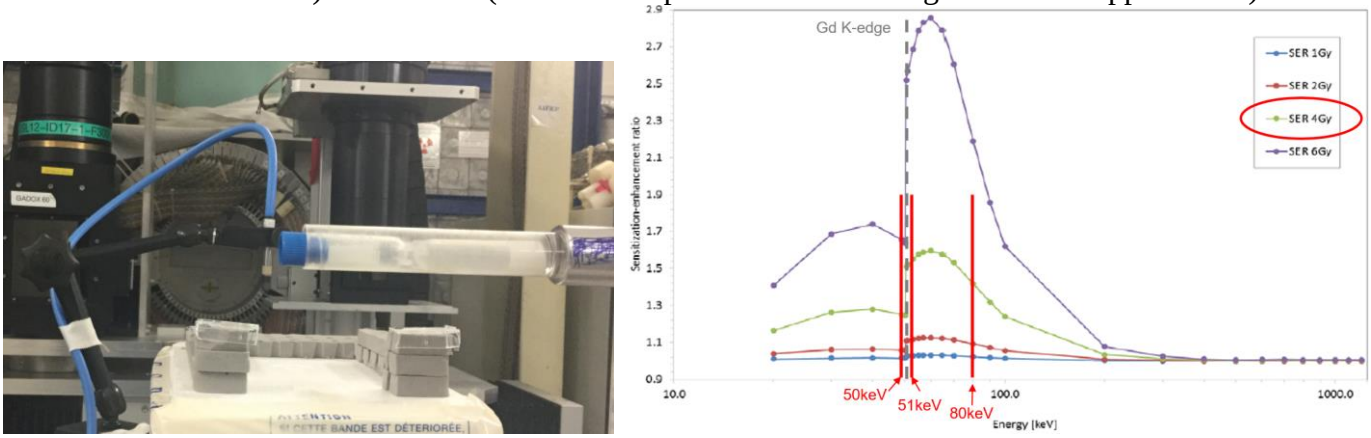

Figure 1: Left) irradiation setup: cells are irradiated in suspension in cryotubes horizontally. The thin synchrotron pencil beam scan the sample until attaining 4 Gy homogeneously distributed in the whole tube. Right) Representation of theoretical SER calculated for different radiation doses as a function of beam-energy, based on macroscopical dose-enhancement simulations with 2.1 mg/mL of Gd (Taupin et al. 2015), and the chosen characteristic beam energies, on either side of the Gd K-edge and at 80 keV, compromise between expected effect and penetration depth. We used here the Gd concentration of 5 mg/mL and the SER-4Gy for reasons of consistency with previous studies.

Following irradiation, three different cell quantities were seeded in triplicate into Petri dishes containing 8 mL of complete culture medium and incubated for 11 days. All experiments were repeated three times. Following staining with crystal violet, colonies of greater than 50 cells were enumerated. The surviving fractions (SF) were determined as the ratio of the number of colonies counted divided by the number of cells plated. The SER-4Gy was determined by the ratio of SF obtained with a Gd-condition with 4 Gy irradiation on SF obtained with the control condition irradiated at 4 Gy.

In addition, the intrinsic toxicity of Gd agents was evaluated from a counting of living cells contained in the flasks after the 24h of incubation with Gd, compared to without Gd.

The Gd uptake by the cells was quantified by means of inductively coupled plasma-mass spectrometry (ICP-MS) for each preparation condition. The measurements were done in triplicate.

Main results:

The ICP-MS measurements showed much higher Gd uptake in cells incubated with Magnevist (0.52±0.05 pg/cell) compared to Dotarem (0.22±0.04 pg/cell), in line to what was observed on image analysis (see MD-1022). It is acompagnied by a much higher toxicity of Magnevist (~30% of cell survival after 24h

incubation) compared to Dotarem (~80% of cell survival), maybe due to this higher uptake, but also due to DTPA linear complex that is believed to be less stable and more toxic than the DOTA complex.
Macroscopical theoretical dose-enhancement factors (DEF) have been calculated for these intracellular concentrations and do not exceed the value of 1.1. For the extracellular Gd concentration of 5 mg/mL (Non-Rinsed conditions), the theoretical DEF reached the values of ~1.37, ~1.65 and ~1.55 for 50 keV, 51 keV and 80 keV respectively.
In terms of SER-4Gy obtained for Dotarem: cells that were rinsed before irradiations shows no additional sensitization, regardless of the beam energy, while significant sensitisation enhancement ratio was observed when Dotarem stay in the media (Non-Rinsed) during irradiation at 51 keV (~1.3) and 80 keV (~1.6). The absence of effect for the "Rinced" condition might suggest that the gadolinium is quickly evacuated outside the cells after rinsing during the transition time between preparation and irradiation (~15-25min). This would be a sign that Dotarem does not induce cellular disturbance, despite strong internalization in presence of extracellular Gd. Compared to theoretical DEF, it could have been expected to have a small SER at 50 keV (not observed), and a higher SER at 51 keV than 80 keV. For the 51 keV energy, the expected Auger and photo-electron cascade produced is of very short range. If no Gd is remaining inside the cells, the external Gd could have very low impact on dose deposition in critical cell targets as cell nucleus that could explain this lower SER. At 80 keV, the longer range of electron produced can compense the lower interaction cross section of X-rays. However, further investigations would be needed to understand the differences in relative behavior of DEF vs SER-4Gy as a function of beam energy.
Magnevist shows very high sensitization-enhacement ratios with both incubation conditions, achieving maximum values for the energy of 80 keV. SER-4Gy obtained ranged from 1.6 to 2 for the "Rinsed" conditions, and from 2.5 to 3 for the "Non-Rinsed" conditions. These results might suggest that combined sensitization effects occurs, one due to physical local dose enhancement caused by Gd retention inside the cells, the other due to intrinsic action of Magnevist that make cells more sensitive to radiation, regardless the beam energy. As for Dotarem, the relative behavior of SER was not consistent with theoretical DEF as a function of beam energy, but care should be taken as the uncertainties was large despite the triplicate conditions of 3 independent series realized for each condition.

Conclusions :

Sensitization-enhancement ratio were evaluated for F98 rat glioma cells incubated 24h with 5 mg/ml with both gadolinium contrast agents Dotarem and Magnevist, after a 4 Gy radiation dose delivered by optimized monoenergetic X-rays. The incubation conditions were choosen following previous imaging studies that shows a significant internalization of Gd in cytoplasm of cells. Promising results have been obtained, although further biological and physical investigations would be needed to understand some of the unexplained behavior, as the inconsistency between theoretical and experimental observed effects as a function of beam energy. Magnevist shows the highest SER, with maximum results obtained up to SER=3 at 80 keV, that would be interesting for a use in photo-activation therapy with high-Z elements. However, strong toxicity were observed and further studies would be needed to evaluate the limits for non-toxic effects with concentration before using it on animals. Dotarem shows also significant SER at 80 keV, up to 1.6, and seems much safer for an *in vivo* use, as it seems better evacuated from cells after the external Gd removal. To obtain an effective therapeutic effect *in vivo*, one could administer Dotarem intra-tumorally with a convection-enhanced-delivery pump for example, to maintain the external Gd concentration the time of irradiation.