EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

ESRF	**Experiment title:** **Blood brain barrier disruption using focused ultrasound to improve dose-enhancers distribution in a rodent glioma model.**	**Experiment number**: MD1083
Beamline: ID17	**Date of experiment**: from: 12/06/2018 to: 17 /06/2018	**Date of report**: 28/02/2020
Shifts: 12	**Local contact(s)**:	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Note: As we could not obtain the authorization for performing the Blood brain barrier disruption using focused ultrasound at the ESRF, the experiment was modified and we performed studies described hereafter during the allocated beamtime.

In parallel, we've performed an experiment at the Grenoble institute of neurosciences to assess the BBB disruption after application of focused US in glioma bearing rats and we've analysed by MRI the extend of BBB disruption.

Report on the experiments performed at the ESRF:
Synchrotron contrast-enhanced radiation therapy (SSRT) uses heavy elements accumulated in the tumor to enhance the dose upon synchrotron irradiation (1). Iron oxide nanoparticles (ION) are promising elements since they have the ability to accumulate in the tumor cells and have shown a good tolerance profile in preclinical studies (2).

We performed *in silico* simulations of the X-ray dose and *in-vitro* studies to assess the dose-enhancement in presence of iron nanoparticles under synchrotron irradiations at various energies.

A pre-clinical study was then performed in a glioma model in Fischer rats with three aims :

a) To assess using monochromatic X-ray computed tomography the iron nanoparticles biodistribution *in vivo* after either intravenous infusion or direct intracerebral injection *via* convection-enhanced delivery (CED) and the good coverage of the tumor;
b) To evaluate the dose-enhancement, which is directly related to the local iron concentration and the irradiation energy;
c) To evaluate the treatment efficacy.

Protocol:
Tumor inoculation:
Fischer rats were inoculated with 5000 F98 rat glioma cells in the right hemisphere of the brain.

Nine days after tumor inoculation, when the tumor was about 3 mm in diameter several infusion protocols were evaluated:

1. Intravenous injection *via* the tail vein of 1 mL of ION concentrated at: 44 mg/mL, injected 24 h before irradiation or 54 mg/mL, 15 min before irradiation.
2. Injection at the tumor site by CED (speed: 1 µL/min, 32 G Hamilton needle) of ION : 1.78 mg/mL injected 24h before irradiation or 32 mg/mL, 3 h before irradiation.

The radiotherapy treatment consisted of a 20 Gy irradiation delivered in two perpendicular monochromatic beams tuned at 35 keV.

10 groups of animals were studied:
5 non irradiated groups: Controls, 2 intravenous injection protocols, 2 CED injection protocols
and 5 irradiated (x-ray alone, 2 intravenous injection protocols+X, 2 CED injection protocols+X).

To evaluate the tumor's dimensions, the rats were imaged with synchrotron radiation computed tomography above and below the iodine K-edge (31 and 35 keV), after receiving an injection of iodine 400 mg/mL (1 mL *via* the tail vein). The ION's distribution and concentration within the tumor were evaluated using the CT scans. The evolution of the animals' health was followed until the end of survival. Log-rank test was used for studying the statistical significance of the survival results.

Results:
A significant dose-enhancement was obtained *in silico* and *in vitro* in F98 cells after incubation with ION. Computed tomography images showed a large amount of ION within the tumor area after intracerebral injection that could be measured over more than 48h. The concentration of ION was below the detection level of the CT system after intravenous injections. The survivals of the rats that were irradiated was significantly different from all the controls ($p<0.05$); Although a large amount of ION could be measured in the brain of the animals that had received the nanoparticles by CED injections, their survival was not improved, relative to the X-ray alone group. This result might be explained by the imperfect distribution of ION within the tumor volume. Only the rats that had received the ION by intravenous injection 24h before irradiation had their survival improved in comparison of the rats only irradiated (increased survival relative to the controls: ILS% = 73% versus 59%, respectively, $p=0.07$). The delay between the infusion of the nanoparticles and the irradiation time seems of prime importance and warrants further investigations.

1 Article in preparation
Poster « Journées de la recherche médicale 2019 Grenoble University hospital » - award for the best poster : Alexandre Ocadiz et al.