Experimental report

11/09/2018

Proposal: 3-07-376 **Council:** 4/2017

Title: Radiobiology in vitro measurementsfor Boron Neutron Capture Therapy

Research area: Other...

This proposal is a continuation of 3-07-369

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Samples: biological cells

Instrument	Requested days	Allocated days	From	To
PF1B	18	18	24/05/2018	29/05/2018
			05/06/2018	18/06/2018

Abstract:

This experiment consists of the irradiation of cell cultures of normal and tumor cells with an intense slow neutron beam for the determination of relative biological effectiveness factors for slow neutrons and for testing the cell response after uptaking boron compounds.

ILL EXPERIMENTAL REPORT

3-07-376 Radiobiology in vitro measurements for Boron Neutron Capture Therapy

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Objective

Some aspects of the radiobiology of Boron Neutron Capture Therapy have been questioned since years. Especially the relative biological effectiveness (RBE) factors corresponding to each dose component. They were systematically taken as constants while they depend on the dose, neutron energy, tissue and endpoint. Also, they were obtained previously at neutron beams with strong gamma contamination.

The aim of this experiment was to establish RBE factors for the thermal component: estimate them properly for different tissues and separately from the epithermal and gamma factors. For that purpose, we irradiated 5 different cell lines (tumor or healthy) in the cold neutron beam at PF1b.

In addition, first tests of the effect of the nitrogen capture, the main reaction occurring at low neutron energies, are done by isotope labeling.

Also, the RBE for the boron component is not well-known for some tumor tissues. We used BPA and new boron nanoparticles to culture the cells 48 hours before the irradiation. Our collaborators from IAB Grenoble, as experts in boron compounds, used new boron compounds at different concentration on these cell lines, using colony assay and γ-H2AX labeling.

Experimental setup

Radiation exposure

The dose rate for the beam at ILL was estimated before the experiment from Monte Carlo simulations with the MCNPX code.

The simulations show a neutron dose rate at the cells of 3.3 cGy/min (1.6 cGy/min for the second quartz cuvette) and a gamma dose rate of 1.05 cGy/min (0.8 cGy/min for the second cuvette). Cells were irradiated between 15-75 min, reaching physical doses around 0.3-3 Gy.

Cells

The tumor cell lines used are: A375 (Malignant melanoma), Cal33 (tongue squamous cell carcinoma), U87 (glioblastoma), SQ20B (squamous cell carcinoma),

The healthy tissue cell lines used are: Hek293 (embryotic kidney), MRC5 (lung fibroblast)

All of them cultured in SFV-enriched DMEM media (except the isotope labeled ones, which were grown in special 15N labeled media).

All the cell culture and sample preparation was done inside the new P2 lab in the guide hall.

Boron compounds

Boron-10 enriched boronophenylalanine (BPA) and nanoparticles with boron encapsulation are used to estimate the boron RBE. Cells are grown 24 hours in the media with boron

compounds. Before the irradiation, the medium is changed to a medium free of boron compounds.

For the other boron compounds, the cells were incubated overnight with different compounds (non-enriched in 10B). After irradiation, the cells were then counted and reseeded for colony assay and DNA double-strand breaks labeling (γ-H2AX). All the other cells were pelleted for protein quantification (which allow to normalize the experiment) and for boron quantification (next step).

Set-up

PF1B was selected due as a well-collimated, intense cold neutron beam with minimum gamma contamination.

Cells were incubated 24 hours before irradiation inside quartz cuvettes (2 mm thick liquid layer). For the irradiation, the cuvettes are placed in the teflon holder. Two cuvettes at once, so the second cuvette receives around half of the dose compared to the first one. After irradiation, the cells are recovered from the cuvettes (with trypsin), counted and prepared for survival assays.

The neutron beam was stopped in a ^{6}LiF sheet 2 cm downstream of the holder. Also laterally and on the top the setup was cladded with lithium rubber to stop scattered neutrons while generating a minimum gamma dose.

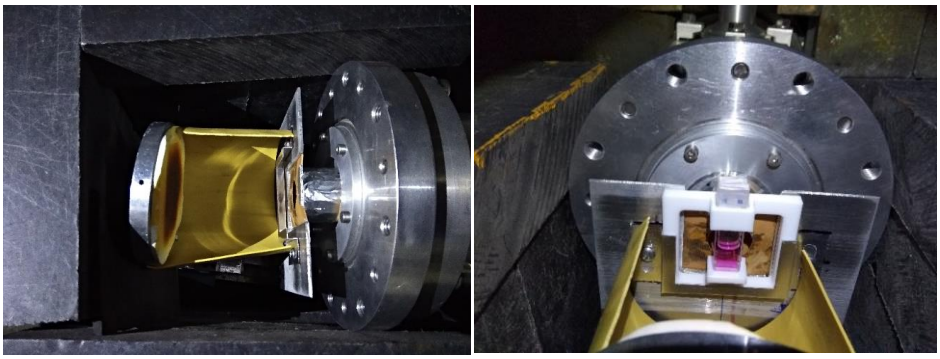

Figure 1: Set-up top view (left). Set-up with a sample placed (right).

Results

-RBE: Cell lines were split into 5 groups of neutron exposure (0, 15, 40, 60 and 75 min), two cuvettes at the same time (reaching two different doses with one time exposure). Most of the irradiation times were repeated more than twice in order to reduce statistical fluctuations inherent to biology experiments. For thermal neutrons we obtain for all cell lines survival curves like black data points in Figure 2.

-The experiment with nitrogen labeling shows that the Hek cells grown in the 15N media have much more survival than the ones grown in 14N media or DMEM.

-Boron compounds: A375, Cal33 and MRC5 were irradiated containing BPA and boron nanoparticles. A clear effect is seen (Figure 2). An additional experiment for quantifying the boron uptake inside the cells will allow us to extract the RBE for BPA.

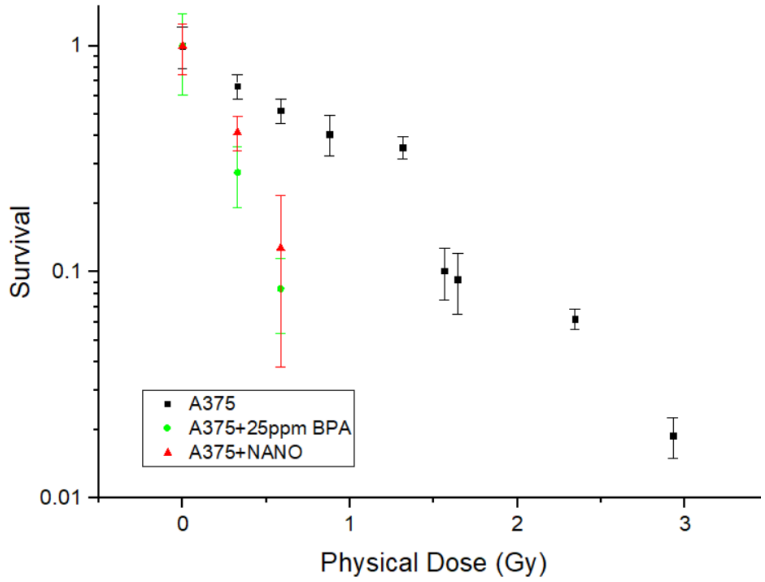

Figure 2: Survival curve of A375 cells irradiated with thermal neutrons (black). Effect of boron compounds on the survival of the melanoma cells (green and red)

Boron atoms were vectorized with cells using a ligand specific recognition, either the hyaluronic acid (at 50µg/ml), the heparonan (at 50µg/ml), or without any specific targeting (BODIPYs at 100µM). We observed that U87MG and A375 have a stronger response to neutrons as compared to CAL33 cells (see figure 3).

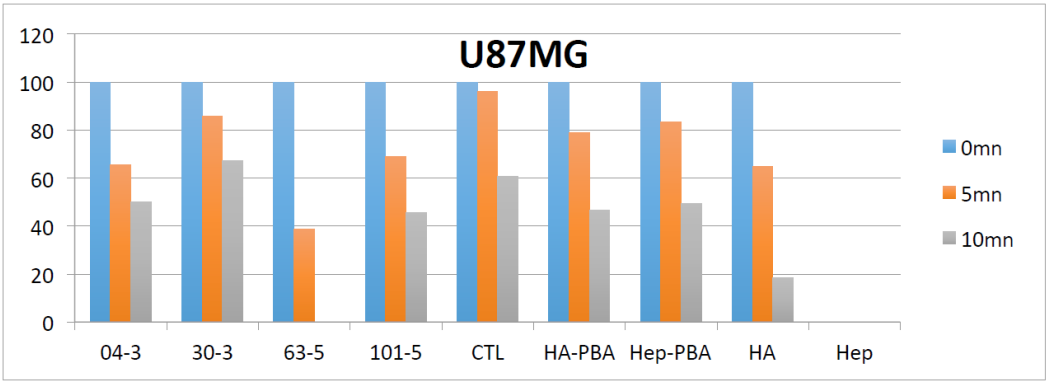

Figure 3: Normalized cell colony assay obtained for U87MG tumor cell lines after neutron exposure, in presence of different boron-containing compounds.

Discussion and conclusions

Thanks to the new biological lab inside the guide hall a lot of different cell lines could be studied in this beam time. Results give us information about RBE in different tissues for thermal neutrons and boron doping (with BPA). Also, the first tests with nitrogen labeling showed promising results.

However, more irradiations are required to have a better statistics in each cell line.

These experiments allowed us to select a mix of 2 compounds (63-5 and Hep-PBA) which will be incorporated into nanoparticles for the next experiment to increase their vectorization and therefore to augment the effects of neutrons. In addition, a compound containing more than 10 atoms of boron will be added to the new synthesis for a better BNCT effect.

By quantifying the boron uptake at the same time of the irradiation will give us more information (this experiment can be done at another instrument like FIPPS)