Experimental report

28/08/2022

Proposal: 3-07-381 **Council:** 4/2018

Title: Determination of neutron relative biological effectiveness factors for BNCT and test of different boron compounds (Long Term Proposal)

Research area: Biology

This proposal is a continuation of 3-07-369

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Samples: culture

Instrument	Requested days	Allocated days	From	To
PF1B	6	6	26/09/2018	02/10/2018

Abstract:

The measurements proposed in this project pursue the advance in two key research lines for improving Boron neutron capture therapy (BNCT): one is the accurate calculation of the photon equivalent dose in BNCT which is essential for the treatment planning and the second is the search of better boron compounds for the therapy.
In the first problem we want to improve the data on the relative biological effectiveness factors for the main dose contributions: thermal, gamma from H capture and boron dose. The PF1b line at ILL is unique for these measurements due to the high flux and the lack of gamma contamination in the beam.
The second one is the study of compounds that can potentially deliver much more boron atoms to the tumor cells, which would represent a major advance for the therapeutic capability. For this purpose boron-loaded nanostructures are specially promising because they can carry a lot of boron atoms to the tumors via the enhanced permeation and retention effect. We will study different nanoparticles recently synthesized by the groups of Grenoble and Granada by irradiation of the cells previously treated with these compounds and observing the joint effect.

ILL EXPERIMENTAL REPORT

3-07-381. Determination of neutron RBE factors for BNCT

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This is a continuation of experiment 3-07-376 (Radiobiology in vitro measurements for Boron Neutron Capture Therapy), and the initial measurements of a Long-Term Proposal (LTP-3-2: Determination of neutron RBE factors for BNCT and test of different boron compounds)

Objective

Neutron radiobiology data are key for an accurate treatment planning in Boron Neutron Capture Therapy. Current assumed values of the relative biological effectiveness (RBE) of slow neutrons are based on very limited data, often obtained at mixed fields where the dose from gamma contamination exceeded the neutron dose, producing a high uncertainty on the resulting data.

The aim of this experiment was to complete the dose-response data obtained in previous measurements at ILL (3-07-376) in order to achieve sufficient statistics for obtaining more precise RBE data. For that purpose, we irradiated 5 different cell lines (tumor and normal cells) at the cold neutron beam at PF1b, where the gamma contamination is negligible, and only photons produced in the culture media by radiative neutron capture by hydrogen affects the cells, and can be subtracted as they are not the main dose component.
In addition, more tests of the effect of the nitrogen capture, the main reaction occurred at low energies, separated from the hydrogen capture effect, were done by isotope labeling. Also, the RBE for the Boron component is not well-known for some tumor tissues. We are going to use BPA and new Boron nanoparticles to culture the cells 48 hours before the irradiation

Experimental setup

PF1B was selected due as a well-collimated, intense cold neutron beam with minimum gamma contamination. The final setup is shown in Figure 1. As PF1b is a `build your own setup' beamline, there is an empty space where the H113 guide finishes. There we placed a sample holder constructed in Teflon where two quartz cuvettes can be placed for neutron irradiation. Cells are attached in one side of each cuvette (adherent cells) and there are 2 mm of culture medium inside the cuvettes. Before each experiment, the beam was aligned with the holder to make sure that all the cells are irradiated.

A Li6 sheet was situated 2 cm after the holder, to use as a beam stopper. Then, lithium was placed all around the sample holder, surrendered also with lead bricks with a boron layer. The shielding around was lead and concrete.

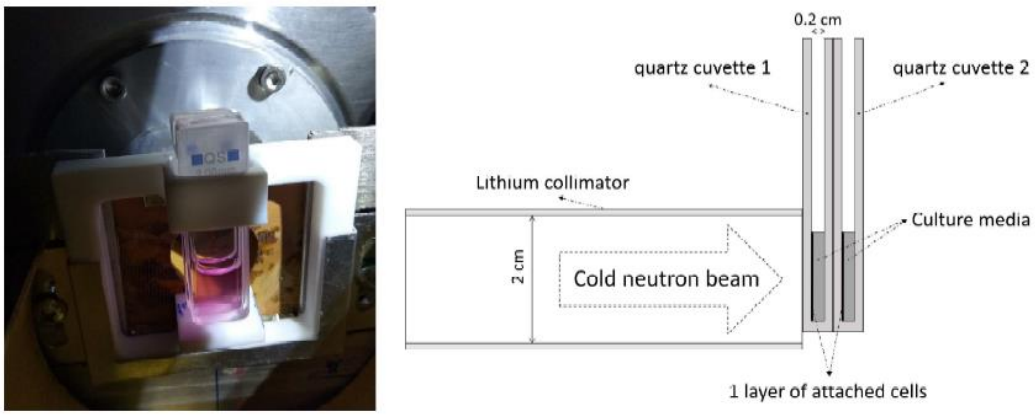

Figure 1. Setup for the cell irradiations.

Radiation exposure

The dose rate for the beam at ILL was estimated before the experiment from Monte Carlo simulations with the MCNPX v. 2.5.0 code. The simulations, using the neutron flux value measured by gold foil activation, showed a neutron dose rate at the cells of 1.99 cGy/min (0.93 cGy/min for the second quartz cuvette) and gamma dose rate of 0.78 cGy/min (0.58 cGy/min for the second quartz cuvette). Cells were irradiated between 15-75 min, reaching physical doses from around 0.3-3 Gy.

For the nitrogen labelled cells, those in which nitrogen-14 was replaced by nitrogen-15 were irradiated up to 120 min, as the neutron dose was negligible for them.

Cells

The tumor cell lines used were: A375 (Malignant melanoma), Cal33 (tongue squamous cell carcinoma), U87 (glioblastoma) and SQ20 (squamous cell carcinoma). The normal tissue cell lines used are: Hek293 (embryotic kidney) and MRC5 (lung fibroblast). All of them cultured in SFV-enriched DMEM media (except the isotope labeled ones, which were grown in special N15 labeled media). Cells were detached with trypsin and counted. All the cell culture and sample preparation is done inside the new P2 lab at the guide hall.

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

Results

For all cell lines the dose response curves (survival fraction vs neutron dose) were obtained by subtracting the effect of the secondary photons, thanks to the data obtained from photon irradiation at a hospital LINAC done previously. Results for a tumor and normal cell llines are shown in Figure 2. From these data, the radiobiological coefficients α_n for the different cell lines are obtained.

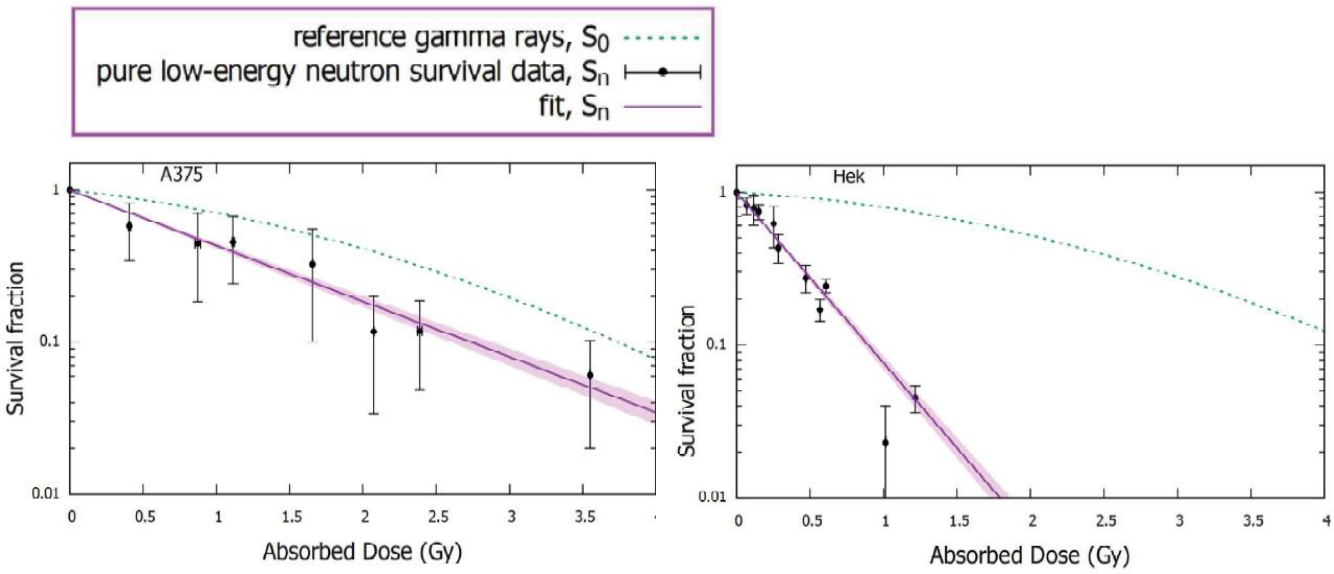

Figure 2. Cell survival data as a function of the dose.

	$\alpha_n(Gy^{-1})$
A375	0.84±0.05
Cal33	1.38±0.05
U87	1.60±0.18
SQ20	1.37±0.12
Hek	2.57±0.10
MRC5	0.98±0.13

Table 1. Neutron radiobiological coefficients measured.

Results for the nitrogen labelled cells are only preliminary. A much stronger survival is found for those cells for which ^{14}N is replaced by ^{15}N. This stimulates new measurements at much higher irradiation times in order to be able to separate the pure neutron effect from the pure secondary gamma one without any subtraction from different experiments.

Conclusions

Neutron RBE (usually assumed the same for different tumors) data obtained shows variation between different cell lines. These measurements provide useful data for more recise treatment planning for the different BNCT clinical applications. This should be complemented with future measurements of the boron compound effectiveness factor for BPA and with measurements of the nitrogen-labelled cells at longer irradiation times.

Papers from the results of these experiments have been submitted to Applied Radiation and Isotopes and to the journal Cells.