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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

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:** Preliminary experimentation on animal tumor model of Photon Activation Therapy by cisplatin and synchrotron light.	**Experiment number**: LS2100
Beamline: ID17	**Date of experiment**: from: *19/09/2001* to: *25/09/2001*	**Date of report**: 28/02/2002
Shifts: 27	**Local contact(s)**: Tropres Irène (tropres@esrf.fr)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Corde Stéphanie*, Balosso Jacques*, Elleaume Hélène*, Estève François*, Le Bas Jean-François, Charvet Anne-Marie*, Joubert Aurélie*, Adam Jean-Francois*
Equipe d'accueil RSRM, ESRF ,UJF et CHU, Grenoble, France

Foray Nicolas*, *MRC Genome damage and stability centre, university of sussex, Falmer, Brighton BNE9RR, united kingdom*

Report:

Background

Dose enhancement in human radiotherapy is a proven way to improve tumor local control, but it is limited by healthy tissue tolerance. Continuing researches are done to improve tumor lethal damages with respect of surrounding tissues (drug sensitization; contrast mediated dose enhancement…). Two approaches are particularly attractive, the use of high relative biological effectiveness ion beams and tumor targeted irradiation sources as classical brachytherapy or metabolic radiation therapy. The Photon Activation Therapy (PAT) is a combination of these two approaches: a selective excitation of high-Z compound fixed inside DNA tumor should allow radio-toxicity enhancement, thanks to the increase of local dose deposition. Actually, photon-stimulation of these heavy elements induces ejection of an internal electron by photoelectric effect. The following electronic rearrangement may lead to Auger electrons cascades. This phenomenon, predominant with light elements, occurs with lower probabilities with high-Z atoms; nevertheless, energies needed for their resonant excitation are higher and consequently suitable for external radiotherapy. Because of their very short range, these Auger electrons could be very toxic for tumor cells, but only if they are released in the close vicinity of their DNA.

This experiment, that we previously tried but failed due to bacterial contamination of the biological material (see report number LS1698b) aimed at expose cultured cells to different agents chosen as being mildly or not toxic for cells. Actually, in previous studies (LS1392 and LS1698a) we suspected that the chemotherapy (CDDP) toxicity was too high to give chance to Auger effect to be seen with survival assay. Therefore, no animal experimentation has been carried out during these shifts, in spite of the title of the experiment.

Material and methods

We exposed cultured cells to chloro-terpyridine-platinum (a non toxic platinum compound), IudR and Iomeron (non toxic vascular iodinated contrast media). These set of compounds was chosen to allow comparison in-cell and out-cell radiation enhancement effect. A wide range of energies were tuned with the tomography monochromator (among them the different K-edges of the target atoms) to optimize the energy choice. Irradiated cells were sub-cultured after irradiation for colony forming assay.
At the molecular level we wanted to measure once again the level of double strands breaks induced on living cells pre-treated with cisplatin by the resonant absorption of the irradiation just above the K-edge of platinum. DNA damages quantification was done by pulsed-field gel electrophoresis. A direct visualization of the location of the damages and the activation of the proteins involved in their repair was done by immunofluorescence experiment.

Results

1°) Survival assays

We measured dose enhancement factor when irradiation is combined with either external iodine or internal iodine in the cells. External iodine was supplied as radiological contrast agent (Iomeron) and irradiated above and below iodine K-edge (-/+ 33 keV), 50 or 70 keV for a dose range from 0 to 5 Gy. Internal iodine was introduced as iododesoxyuridine (IudR). Irradiated the same way a combination of IudR and Iomeron has also been done. These experimentations have been successful and we established that $DEF_{10\%}$ for Iomeron is 1.1 around iodine K-edge; 2 at 50 keV and 1.5 at 70 keV; for IudR it is 1.4 around iodine K-edge (above or below); 2.6 at 50 keV and 1.35 at 70 keV. And when IudR is combined with Iomeron DEF rises to 1.8 aroud 33 keV and 2.4 at 70 keV, which are unusually high results and show that DNA damages induced by irradiation are cumulative for the two pharmacological agents. It will be very interessant to test this association at 50 keV, the theorical and experimental optimal energy to enhance energy deposit between iodinated and normal tissues.

We made a first attempt of intracellular photoactivation (PAT) with a new platinum compound: PtTC (chloroterpyridine platinum (II)). PtTC is an organic platinum compound able to intercalate in DNA without covalent bound. Therefore it is no toxic and theoretically high level should be achieved in cells. Such levels should enhance the rate of DNA damage occurred by Auger effect. Results are very preliminary and pharmacological steps must be improved. The continuation of these studies was done in the experimentation LS2121 in February.

2) Molecular studies of DNA damage and DNA repair pathways

In this experimentation, human cells were exposed to CDDP at high concentrations (3 and 30 μM) and irradiated above and below platinum K-edge. Cellular preparations were made for pulsed field electrophoresis; immunofluorescence labeling; and western blot. These results confirm previous results: when irradiated above k-edge, cisplatin increased double strand breaks (DSB) rate with reduced repair kinetic. Repair pathways are predominantly homologous recombination, which is usually the minor pathway of DSB repair. These molecular results are consistent with published data. However we would like to confirm them with other cell lines to be more representative of human tumors.