REPORT for MD40 Experiment

1. Introduction

Previous experiments (Corde et al. Cancer Res. 2003) have shown that synchrotron photoactivation of cis-platinum (PAT-Plat) consists in an excess of DNA single- and double-strand breaks, probably due to an excess of radiation dose delivered to the close vicinity of DNA. The goal of this experiment was to apply the PAT-Plat conditions to a larger extent (30-85 keV) to human and F98 rodent cells.

2. Sample preparation and irradiations conditions

Equal amounts of human and rodent cells were embedded in agarose plugs (neutral matrix) and submitted to lysis after different treatment to cis-platinum and/or synchrotron radiation (from 30 to 85 keV). After irradiation plugs were washed and kept at 4C in EDTA 0.2M. Yields of DNA double-strand breaks was assessed in pulsed-field gel electrophoresis. Some other plugs will be incubated in nuclear extracts from different cell lines to determine which proteins are involved in the repair of such breaks.
By following a set-up already developed in routine at ID17 (see previous proposals), irradiation conditions were applied successfully. *Our local contact (Thierry Brochard) was a precious help for the technical assistance and we want to thank him warmly for his efficiency.*

3. Results and published paper

The great part of the results obtained from MD40 experiments have been submitted to Cancer Research Journal (**Biston et al. Cure of Fisher rats bearing radioresistant F98 glioma treated with cis-platinum and irradiated with monochromatic synchrotron X-rays). This paper has been accepted without major modifications. Here is the abstract of the paper**:

High-grade gliomas are usually of poor prognostic and conventional radiotherapy, even combined with chemotherapy, still fails to improve survival of patients. Here, we propose an innovative therapeutic approach combining synchrotron radiation with cis-diamminedichloroplatinum (CDDP). As suggested previously, monochromatic synchrotron irradiation of CDDP at 78.8 keV, just above the 78.4 keV platinum absorption K-edge leads to an enhanced photoelectric effect and an increased local toxicity. In order to select a particular radiation energy that could provide supra-additive effect, we used pulsed-field gel electrophoresis to assess yields of DNA double-strand breaks (DSBs) induced in rat F98 glioma cells after CDDP treatment combined with synchrotron X-rays. Thereafter, intra-cerebral CDDP injection combined with synchrotron X-rays was applied to Fisher rats bearing F98 glioma. CDDP concentrations were mapped by synchrotron X-rays micro-fluorescence. An extra-number of more slowly repaired DSB was observed when irradiating CDDP-treated F98 cells at 78.8 keV. *In vivo* treatments were then performed with different radiation doses and CDDP concentrations. All cell inoculations in rat brain resulted in tumor development, and tumor presence was controlled by computed tomography. Among all of the conditions tested, the combination of 3 μg CDDP with 15 Gy resulted in the largest median survival time (206 days). After one year, about 34% of treated rats were still alive. This pre-clinical finding, validated by molecular analysis, represents the most protracted survival reported with this radioresistant glioma model and demonstrates the interest of powerful monochromatic X-ray sources as new tools for cancer treatments.

Data in DNA from human cells confirm the observations with a progressive increase of DNA breaks as far as we reach the 78.4 keV value corresponding to the K edge of cis-platinum.

4. Work conditions and environment - Conclusions

The assessment of the DNA breaks have been done in Institut Curie (Orsay) and at the biomedical facility. While the DNA breaks analysis did cause any practical problem, and despite a good publication from an innovating anti-cancer approach and a very good technical support for the use of the beamline, we had some practical difficulties for protein analysis (molecular biology) since no facility devoted to molecular biology is available at this beamline and since EMBL facilities rooms are too far to perform seriously the expected experiments. Hence, we hope that, in the next future, ID17 will propose appropriate infrastructures for performing molecular biology experiments in better conditions of science and safety. To this aim, a memorandum have been sent to the scientific direction of ESRF. ESRF and ID17 should be aware of the recent developments of biomedical research areas with its specific technology requirements, in order to welcome more users by covering a multidisciplinary spectrum of research approaches. This will make ESRF an unique facility.