REPORT for MD123 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. Recently, thank to the efforts of U647 INSERM and the support of ESRF, another publication in Cancer Research has been accepted (Biston et al., Cancer Res, 2004, 64, 2317-2323). Finally, in our last accepted proposal (MD90), we performed first experiments by replacing cisplatinum by carboplatinum to increase Pt concentrations into tumors in order to optimise the therapeutic index of the PAT-Plat technique. As specified in this proposal and the previous one, we proposed to apply such technique to human tumors through the injection of cells in nude mice. While feasibility tests have been performed with the collaboration of Boudewijn VanDer Sanden, the use of human tumor xenografted-mice was not possible again this time since the project of L2 facility, required for such experiments (human tumors are classified L2) and foreseen some months ago was not still accepted. We hope that this facility will be available for the next proposals.
Consequently, we deliberately focused the beamtime on:
- the reproducibility of in vitro and in vivo PAT-Plat experiments with carboplatinum like the MD90
- the preparation of nuclear extracts of rodent glioma cells (9L, F98, C6) sumitted to the PAT-Plat conditions in order to examine the activation of DNA repair and stress signalling proteins. This experiment will help us to better determine the genetic statuses preferentially targeted by PAT-Plat and to better choose the human tumor models to be used in the next proposals.

2. Sample preparation and irradiations conditions

Preparation of nuclear extracts, immunoprecipitation and immunoblotting were performed using standard protocols published elsewhere (Foray *et al*., MCB, 22, 4020-4032, 2002). Aliquots of nuclear extracts were stored at -70°C

For the survival of rats bearing F98 gliomas, please see previous reports and proposals

Both in vitro and in vivo set-ups were already developed in routine at ID17 (see previous proposals), irradiation conditions were applied successfully.

3. Results and conclusions

Nuclear extracts performed during these experiments permitted to point out the role of the ATM and BRCA1 protein into PAT-Plat conditions. Interestingly, the response to the three different rodent models of glioma (9L, F98, C6) did not provide the same response, suggesting notably a different genetic status of BRCA1. Further molecular experiments are in progress to establish clearly whether these three models hold BRCA1 mutations.
The second series of in vivo rat survivals in PAT-carboplatinum or PAT-cisplatinum conditions confirmed the previous results obtained with MD90 proposals : the benefit of carboplatinum consists in injecting higher Pt concentrations into brain tumors without increasing the toxicity and permits to gain up to 10 % more survival (33 and 43% with

cisplatin and carboplatinum, respectively). A publication dealing with the comparisons of PAT-Plat therapeutic index with these two drugs will be submitted soon.