REPORT for MD68 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. As specified in our proposal, we investigated further the role of DNA damage repair in cells submitted to PAT-Plat by measuring systematically :
1) DNA breaks induced
2) DNA breaks repaired
by using pulsed field gel electrophoresis technique. Particularly, we focused on the work hypothesis that secondary electrons produced by photoactivation have a short track in matter and therefore may induce at the close vicinity of the cis-platinum molecules additional short DNA fragments.

In parallel, we pursued the series of in vivo PAT-Plat experiments in order to complete data for a publication (See previous Reports).

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.

3. Results and conclusions

An extra-number of more slowly repaired DSB was observed when irradiating CDDP-treated F98 cells at 78.8 keV but not at 40 keV. Interestingly, the PAT-Plat-induced DSBs provides an abnormally large yield of very short DNA fragments (less than 100 kb), confirming our work hypothesis that PAT-Plat emits electrons at the close vicinity of the cis-platinum DNA-adducts. Hence, the production of very short DNA fragments appears to be the signature of PAT-Plat since it odes not occur at any other X-rays energy tested.

In vivo treatments were then performed with different radiation doses and CDDP concentrations. Again, 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.

A publication can now be written.