REPORT for MD170 Experiment – 25/11/05

1. Introduction

Since thirty years, radiobiologists and physicians have frequently observed cytotoxic effects on *apparently non-irradiated* cells located at the vicinity of irradiated ones. Despite a number of papers dealing with this so-called "bystander effect", its biological interpretation and the molecular mechanisms implied are still unclear, but might be of high impact on public health if it appears that low radiation doses influence the cells metabolism. In the last proposal, we began to answer the following questions :

1/ Is the vicinity of X-rays tracks able to produce DNA and/or cellular damage in non-irradiated cells?

2/ Which are the early biological sensors involved in bystander effect? Which DNA repair proteins are involved in such effect?

By producing synchrotron X-rays tracks (100 μm large) separated by 500 μm, DNA double-strand breaks (DSBs) in cells situated in the valley were assessed with the anti-pH2AX immunofluorescence technique. We observed the formation of late DNA double-strand breaks (DSBs) that were supposed to be the result of the conversion of base damage (oxidative stress) into single and thereafter double-strand breaks.
In the present proposal, we tried to verify this hypothesis by applying the same irradiation protocols to cells with different culture media supplemented or not by anti-oxidant products, like N-acetyl-cysteine (NAC).

2. Sample preparation and irradiation conditions

Set-up to irradiate cells seeded in slides was already used in previous MD experiments and used routinely at the biomedical facility.

3. Results

Irradiated X-rays tracks are clearly distinguishable by the very large amount of pH2AX foci revealing the presence of induced DSBs 4 h post-irradiation. While the pH2AX signal in cells is very clear by using DMEM and physiologic medium (NaCl 0.9%), it was not observed by using PBS (a medium with phosphates). In addition, the adding of increasing concentration of NAC into the medium resulted in a reduction of the pH2AX signal, as well. Lastly, by using immunofluorescence against XPD, a protein involved in the conversion of base damage into DNA strand breaks, we noticed an increasing yield of XPD foci that parallels observations with pH2AX. Altogether, these findings strongly suggest that bystander effect observed after microirradiation of cells may be caused by an excess of early oxidative stress that can be prevented by phosphates and/or anti-oxydants.

Hence, by-stander effect would not be energetic enough to provide DNA breaks but its biological impact is not negligible. New experiments will be proposed to better understand the real nature of by-stander effect –induced base damage and the genetic status that would be specifically sensitive to by-stander.

4. Work conditions and environment - Conclusions

All along the preparation of this experiment, Thierry Brochard was a precious help for the technical assistance and we want to thank him warmly for his efficiency. A new proposal is in preparation to complete our data and to publish these novel approach of bystander effect with synchrotron.