MD 388 report

In vitro 3D Iodine Dose-Enhancement studies in glioma cell line spheroids.

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Purpose:
Iodine-enhanced synchrotron stereotactic radiotherapy (SSR) takes advantage of the radiation dose-enhancement produced by high Z elements when irradiated with monoenergetic beams of synchrotron X-rays. In the present study, we determined glioma cells response to iodine-enhanced stereotactic radiotherapy (SSR) using a glioma **spheroid model (figure 1)** (1). The radiobiological end point of this study was clonogenic cell survival.

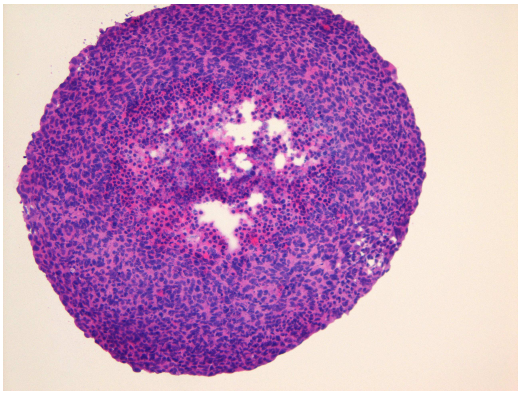

Figure1: Histological slide of a spheroid (Hemalun-Eosine dying).

Materials and Methods:
Spheroids of F98 cells were irradiated in presence of iodine in the culture medium. We evaluated the cellular responses to radiation in the presence of 10 mg/mL of iodinated agents (iomeron) at two different energies: **50keV** which in theory optimal energy for obtaining the maximum dose enhancement and **80keV** which is the optimal energy for treating human brain tumors (2). We performed the same experiments with F98 cells irradiated in suspension in the iodinated medium and without iodine.
Irradiations were carried out at room temperature in aerobic conditions at the ESRF medical beamline. Cells or spheroids were irradiated as suspension in horizontal continuously rotating cryotubes (2 ml, 10mm in diameter), vertically translated up-and-

down through a 500-mm-thick X-ray beam providing a dose rate calculated in water of about 0.5 Gy s 1. Dose calibrations were performed using a cylindrical ion chamber (PTW 31002) coupled with a Unidoss electrometer. They were crosschecked with a high-purity Germanium detector (Eurisys Mesures, Lingolsheim, France). Real-time control of delivered doses was provided by a 10 cm long ion chamber, continuously present in the beam. Three independent experiments have been carried out and averaged. After each irradiation, triplicate low-density subcultures of the cells were established in Petri dishes for colony forming assay. Before pitching, all the cells were dissociated using Trypsin.
Colonies were fixed and stained with violet crystal oxalate (Merck Eurolab) after 15 days of cell growing. Colonies with more than 50 cells were then manually counted. Experimental data were fitted with the linear quadratic model :
$S = \exp (-\alpha D - \beta D^2)$
where S is the survival probability and D the radiation dose (Gy).

Results:
Cells irradiated with iodine exhibited a radiation sensitization enhancement both when irradiated as single cells in suspension or as spheroids in suspension. The radiation sensitization was energy dependent and was more important using cells in suspension. The biological dose enhancement factor (BDEF) was calculated by dividing the doses required to obtain 10% survivals (Dose S10% Control) / (Dose S10% treatment). The BDEF obtained were 1.4 and 2.2 at 50 keV and 1.1 and 1.6 at 80 keV for spheroids and cells in suspension, respectively. Complete results will be soon published.

Conclusions:
The sensitivity of F98 cells to kilo-voltage X-ray beams is enhanced in the presence of iodine, whatever its cellular configuration (single cells or spheroids in suspension). This enhancement depends upon the choice of the X-ray energy beam, as predicted by the theory. We observed that the dose enhancement factor is smaller in the spheroids configuration in comparison with single cells in suspension. The results obtained with the spheroids seems to more closely simulate real tumor response to treatment than single cells in suspension.

(1) Hamer P, Van Tilborg AAG, Eijk PP, *et al.* The genomic profile of human malignant glioma is altered early in primary cell culture and preserved in spheroids. Oncogene 2008;27(14):2091-6.
(2) Boudou C, Balosso J, Esteve F and Elleaume H. Monte Carlo dosimetry for synchrotron stereotactic radiotherapy of brain tumours. Phys. Med. Biol. 2005;50:4841:4851.