Tumour cells irradiated with gamma rays and proton beams: a 1H MRS study on lipid signals.

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INTRODUCTION

While DNA remains an important molecular target for radiation-induced damage, it is becoming clear that knowledge on the radiation effects on the overall cellular response is necessary. In fact, radiation exposure induces the activation of a cascade of events initiated in the plasma membrane, the cytoplasm and the nucleus, resulting in cytoprotective or cytotoxic responses involved in apoptosis, cell cycle regulation and cell survival after damage repair.

With this respect, 1H MRS may be a valuable tool to study radiation induced alterations in cell metabolism. Some signals have driven the attention of many groups aimed to explore MRS potentialities in the field of cancer treatment [1-3]. Signals from the fatty acid chains of lipids (ML), mostly neutral lipids, have often been studied in order to find a correlation among the spectral modification and the biological outcome [4-5]. In many cell systems, ML signals were found to increase in concomitance with apoptosis [6].The present study aims to exploit MR spectroscopy to compare the effects of gamma rays and proton beams in cancer cells, by means of analysis of ML signals, in view of possible applications in cancer patients.

High energy proton beams are increasingly used in cancer therapy as they possess characteristic that enhance precision in the treatment of some tumours [7], allowing to spare healthy critical organs. Much attention is presently devoted to clarify the question of dependence of the cellular effects on radiation quality. Many studies point to higher values of relative biological efficacy (RBE) of protons with respect to gamma, a topic still controversial, in view of the different linear energy transfer (LET), characteristic of different radiations.

The experiments here described compare the effects of gamma and proton irradiation on cultured cells from human breast carcinoma. Irradiation with proton beams was performed at the first Italian proton therapy facility, at the INFN-LNS (Catania- Italy), adapted for the treatment of tumours of the ocular region.

MATERIALS AND METHODS

Tumour MCF-7 cells from breast human carcinoma were used. Cells were seeded in 175 cm2 flasks, in 50 ml medium. 1H MR spectra at high resolution were run at 400 MHz on WB Bruker spectrometer equipped with a 1mm microprobe (Bruker,Germany). Both 1D and 2D 1HMR experiments were run with water suppression. Cell systems were irradiated with the proton beam available at LNS at different depths along the 62 MeV proton beam, with single doses of 10 and 20 Gy. Gamma irradiation was performed with a 60Co source, with single doses of 5, 10, 20, 40 Gy. Cells were irradiated in the flasks at day 3 after seeding, and measured after different time intervals to detect metabolic mediated effects. Cell killing was measured and cell cycle analysis (FACS) and Annexin-V test for apoptosis were performed.

RESULTS AND DISCUSSION

For irradiation with proton beams, cell samples were irradiated in a home-built collimator that matched the geometrical constrains of the proton beam. The use of a microprobe allowed to recover reliable and well resolved spectra from a relatively small number of cells (about one million).

Intensities of ML signals were determined by integrating both bulk CH2 peak at 1.28 ppm in 1D spectra (not shown) or the CH3-CH2 cross peak in the 2D COSY spectra (peak A) to provide independent measurements of the same molecular species. Figure 1 shows two sets of measurements of ML intensities (calculated as difference [A/(Lys+Ala)Control - A/(Lys+Ala)Irradiated]) from 2D spectra as a function of time after irradiation, in cells irradiated either with proton beams (10 Gy, -□-) at the Bragg peak (high LET), or with ^{60}Co (20 Gy, -■-). Signal intensities were measured with respect to the sum of (lys+ala) amino acids cross peaks as internal reference. A net increase of ML signal intensity was observed irrespective of the radiation quality . The entity of ML increase is comparable in samples treated with a dose of 10 Gy for protons and 20 Gy for gamma rays, pointing to a RBE approximately equal to 2 for these effects. On the contrary, the effects on ML intensity observed for cells exposed in the low LET region of the Bragg peak are comparable with those produced by gamma rays (not shown). These results points to a LET dependence for the observed effects. The variations in ML intensity are related to metabolism-mediated damages induced in irradiated cells, as they become larger as the time interval from the treatment increases.

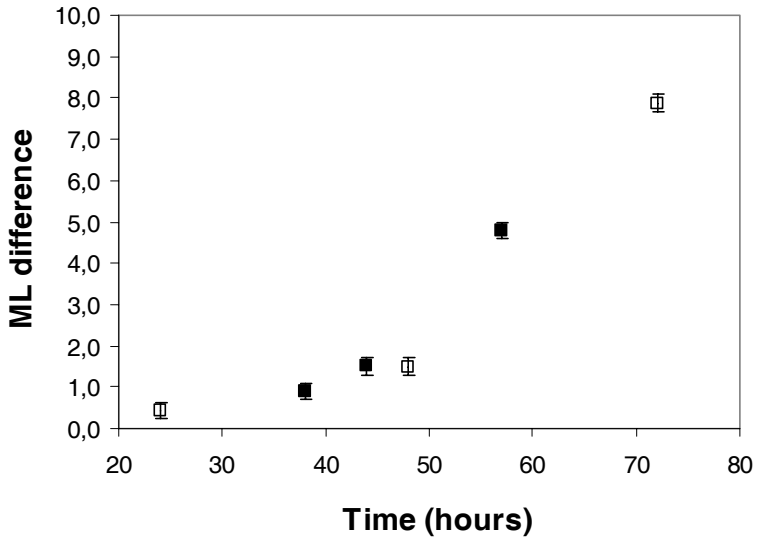

Figure 1 ML difference from 2D COSY spectra calculated as difference [A/(Lys+Ala)Control - A/(Lys+Ala)Irradiated] as a function of time after irradiation for MCF-7 cells. Spectra were run at 400 MHz. Cells were irradiated with 20 Gy of gamma rays (60Co) (-□-) and 10 Gy proton beams (-■-) (62 MeV)

Biological experiments performed on MCF-7 cells after gamma irradiation showed that percentage of apoptotic cells is low (one representative experiment: value of 11% at 48 hours after irradiation, with respect to value of 6% for control sample). The observed increase in ML after irradiation is therefore not related to the onset of apoptosis, at least in the chosen experimental system.

In conclusion the present data point to a relative biological effect of 2 for treatment with proton beams, in the high LET region, with respect to gamma rays.

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