MRS SIGNALS CAN MONITOR CELL DAMAGE AS WELL AS APOPTOSIS INDUCED BY GAMMA RAYS AND PROTON BEAMS ON TUMOR CELLS

S. Grande[1], A. M. Luciani[1], A. Palma[1], A. Rosi[1], V. Viti[1], P. A. Lojacono[2], V. I. Patti[2], L. M. Valastro[2], L. Guidoni[1]

[1]Dipartimento Tecnologie e salute, Istituto Superiore di Sanità, Roma, Italy, [2]INFN-Laboratori Nazionali del Sud, Catania, Italy

INTRODUCTION

The analysis of the metabolic events induced by irradiation can be of great help in elucidating different mechanisms of tumor cell death. Several recent studies have indicated that *in vivo* MRS has the potentiality to detect and to evaluate irradiation effects on tumors [1-2] as well as early and delayed adverse metabolic effects on normal tissues of patients after radiation therapy [3-4]. In particular, lipid metabolism, that can be examined through identification and analysis of many individual metabolites [5-6], has deserved much attention as its changes may have different implications for tumor growth *in vivo*. In previous work on tumor cell suspensions, it was demonstrated that many MRS signals are sensitive either to treatment with radiation characterised by low linear energy transfer (LET), such as gamma rays, and high LET, such as proton beams [7-8]. In the present study, we examine aspects of lipid metabolism of cultured tumor MCF-7 cells (mammary carcinoma) that are affected by irradiation. Data from literature indicates that irradiation of MCF-7 cells induces cell death mostly by apoptosis [9]. Identification of MR signals related to metabolic effects or, eventually, to the kind of cell death, may result in spectroscopic markers that, in principle, can be used to detect the effects of radio-therapeutical treatments in vivo.

MATERIALS AND METHODS

MCF-7 cells were grown as adherent cells as described elsewhere [7-8]. To prepare extracts, cells were first treated with perchloric acid to obtain perchloric acid extracts. The cellular material was dissolved in deuterated water. The residual cellular material was washed, frozen and lyophilized. The samples so obtained were then treated with chloroform/methanol 2:1 to extract total lipids. The final material was suspended in deuterated 2:1 chloroform: methanol solution shortly before the MR measurements. Cells were irradiated with a *gammacell* (^{60}Co) and with proton beams of different energy. Cells were detached at different times after irradiation and sample prepared for the MRS measurements. 1D and 2D COSY ^{1}H MR spectra were run at 400, 600 and 700 MHz on WB spectrometers (Bruker, AG, Darmstadt, Germany) by gated irradiating water signal.

RESULTS

The signals of mobile lipids (ML), mostly tryglicerides, in intact MCF-7 cells ^{1}H MR spectra show an increase in intensity 48 hours after gamma irradiation (not shown). In parallel, we observe spectral modification in cell extracts spectra. Figure 1 shows the glycerol region of ^{1}H MR spectra of lipid extracts from irradiated MCF-7 cells 48 hours after irradiation Irradiated cells present more intense triglyceride (TG) signals with respect to controls. Phospholipids signals (PL) can be used as a reference. Very interestingly, the effect appears comparable for samples irradiated with 10 Gy of proton beams and with 20 Gy of gamma rays. Figure 2 shows a region of ^{1}H MR spectra of perchloric acid extracts from gamma and proton irradiated MCF-7 cells 48 hours after irradiation. The spectral region of choline based metabolites relative to two representative experiments for gamma and proton irradiated samples is reported. The glycerophosphorylcholine (GPC) to phosphorylcholine (PCho) intensity ratios is higher in irradiated with respect to controls. This ratio is sensitive to irradiation at a comparable extent for 20 Gy gamma and 10 Gy protons.

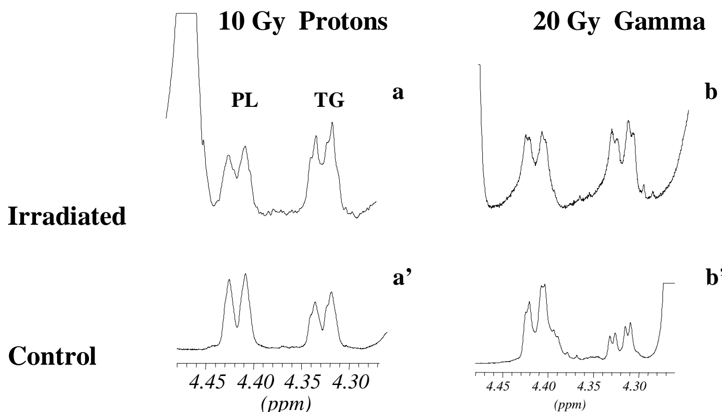

Figure 1. ^{1}H MR spectra (glycerol region) of lipid extracts from irradiated cells prepared 48 hours after irradiation; a'.b'. control, a. proton (10 Gy) gamma (20 Gy) irradiated.

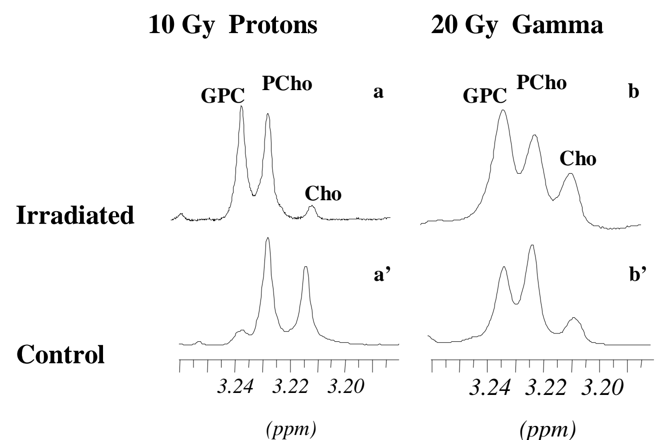

Figure 2. ^{1}H MR spectra (choline based metabolite region) of PCA extracts from cultured MCF-7 cells prepared 48 hours after irradiation. a'.b'. control, a. proton (10 Gy) b. gamma (20 Gy) irradiated

DISCUSSION AND CONCLUSIONS

The most relevant effect observed was the increase of the lipid related metabolite GPC signal intensity in PCA spectra after irradiation. Also TG concentration, measured in the lipid extracts, was affected by irradiation. This behaviour is consistent with the onset of apoptosis. Similar spectral modification were indeed found in other cell systems undergoing apoptosis [10]. The radiation quality affects the entity of the effects on ML signals, TG signals and GPC/PCho ratio in a very sensitive way. Proton beams produced similar effects on the selected molecules at half dose with respect to gamma rays, pointing to a relative biological effect of two.

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