Cellular glutathione detected by ^{1}H NMR can give information on radio-sensitivity in tumour cells *in vitro*

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INTRODUCTION

In a previous study on two cell lines from human carcinoma [1], we associated the cellular level of reduced glutathione (GSH) -1H NMR detected- to radiation resistance to gamma rays. We then investigated whether i) such correlation holds also in tumour cells from human glioma in cell lines with different sensitivity to irradiation, and ii) a different role can be envisaged for GSH in cells from different tumours. Two adenocarcinoma-derived cell lines, MCF-7 and HeLa, were therefore compared to two glioma-derived cell lines, T98G and A172. These cell lines showed different radio resistance, consistent with the observed initial levels of GSH. Radiation resistant cells were then irradiated after Buthionine sulfoximine (BSO) treatment to decrease the concentration of GSH in cells, preventing its radio protective effects.

MATERIALS AND METHODS

All cells were grown as adherent cells as described elsewhere [1]. Cells were irradiated with a gamma cell (^{60}Co) at 20 Gy. To inhibit the activity of γ-glutamylcysteine synthetase, cells were treated with 0.1mM BSO for 18 hours before NMR experiments. 1H NMR spectra were run at 400.14 MHz on a digital Avance spectrometer (Bruker, AG, Darmstadt, Germany) equipped with a 1mm microprobe. Signals were acquired with a 90° RF pulse and a sweep width of 4006.4 Hz. Water suppression was obtained by irradiating water signal.

RESULTS AND DISCUSSION

Fig 1 shows the 1H NMR spectrum of T98G cells from human glioma. All signals related to GSH metabolism are assigned and labelled, according to [1, 2], showing a pattern of intense GSH very similar to that of MCF-7 cells [1]. Irradiation of these two cell lines failed to induce cell killing, when observed after 48 hours (Fig 2). On the contrary, the same treatment induced significant killing in HeLa and A172 cells (not shown), characterised by lower initial GSH, then referred as radiation sensitive cells. In the two resistant cell lines, GSH level, measured by glutamate quantitation in GSH (glu-GSH in Figure 1), was higher than in the two less resistant cell lines, while free glutamate was much less intense. This finding is consistent with the role of GSH in protecting cells from the toxic products of irradiation.

The two radio resistant cell lines were then treated with BSO to decrease GSH level, thus inhibiting its protective function, in order to increase cell killing. Though the initial level of GSH was lowered in both cell lines at the same extent, relevant apoptosis and cell killing were observed only in MCF-7 treated cells, while T98G cells were not affected (Fig. 2).

We then compared the 1H NMR spectra to detect differences in GSH recovery kinetics. (Fig 3). After 2 hours from the end of BSO treatment, both cell lines displayed lower glu-GSH (see arrow), and higher free glu due to GSH synthesis block. (Fig. 3 a, a'). Then, T98G cells quickly recovered GSH , while MCF-7 cells did not. After 24 hours from the end of BSO treatment GSH signals were in fact still low in MCF-7, while almost completely restored in T98G cells (Fig 3 b,b'). The protective effect of GSH is testified by its consumption in the corresponding irradiated samples (Fig 3 c' compared to b'). Fast GSH restoration may be ascribed to highly efficient systems found in glial cells, to protect them against oxidative stress, by producing glutathione.

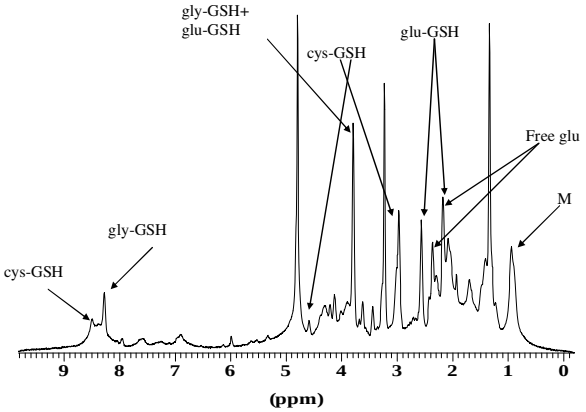

Figure 1 *1H NMR spectrum of T98G cells. Signals from glycine (gly) , cysteine (cys), glutamate (glu) free or in GHS are labelled. M is the .reference signal from methyl groups in polypeptides*

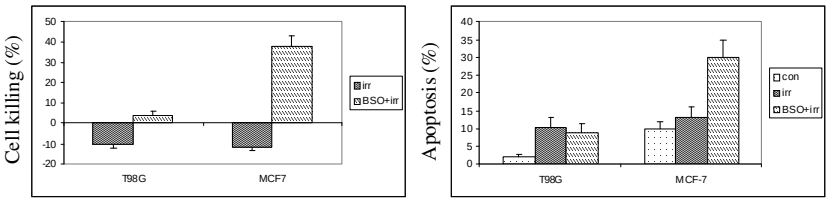

Figure 2 *Percentage of cell killing and apoptosis 48 hours after a single dose (20 Gy) of gamma irradiation, measured in MCF-7 and T98G cells. Before irradiation, cells were treated with BSO for 18 hours. Bars represent the mean + SD. Significant apoptosis was observed only in MCF-7 cells.*

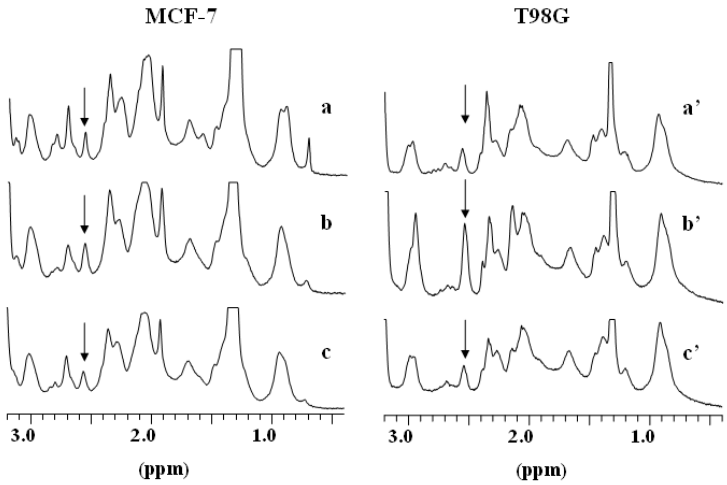

Figure 3 *1H NMR spectra of BSO treated MCF-7 and T98G cells. Traces a,a': 2 hours after BSO treatment; b,b': 24 hours after BSO treatment; c,c': 24 hours after BSO treatment+ irradiation with gamma rays (D=20 Gy).*

CONCLUSIONS

The role of GSH, the most abundant intracellular thiol, as major antioxidant and inhibitor of apoptosis in tumour cells depends, at least in part, on its intracellular level, as shown in cancer cells resistant to apoptosis induced by drugs [3]. Present data show that GSH levels, 1H NMR detected, can provide a method to foresee cell sensitivity to radiation treatment. Moreover, the experimental data also show that GSH metabolism of cell lines from different origin can be differently regulated, thus allowing further hypotheses on cell resistance to possible antitumor treatments.

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