Metabolism of glutathione in tumour cells before and after irradiation as evidenced by 1H MR spectra

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INTRODUCTION

Reduced glutathione (GSH), the most abundant intracellular thiol, acts as a major antioxidant in mitochondria. It seems that inhibition of apoptosis in tumour cells depends at least in part on intracellular GSH level, as cancer cells resistant to apoptosis induced by drugs had higher intracellular GSH levels [1].

A deeper insight into the relationship between GSH metabolism and 1H MRS signals should help in clarifying cell capabilities in reacting to chemical and physical insults. In a previous paper [2], we associated the radioresistance of tumour cells to apoptosis with a high level of GSH, as evidenced by 1H MRS. In the present paper, 1H MRS signals of GSH and of free glutamate (glu) of cultured tumour cells, namely MCF-7 from mammary carcinoma and TG98 cells from malignant glioma, were examined. By treating cells with Buthionine sulfoximine (BSO) that inhibits activity of γ-glutamyl cysteine synthetase, intensities of these signals could be related to cell density and proliferation while their ratio appeared dependent on the activity of the enzyme. Intensity changes of the same signals after irradiation could be explained in view of this interpretation.

MATERIALS AND METHODS

MCF-7 and T98G cells were grown as adherent cells as described elsewhere [2]. Cells were irradiated with a gammacell (^{60}Co) at 20 Gy. To inhibit the activity of of γ-glutamylcysteine synthetase, cells were treated with 0.1mM BSO for 18 hours before NMR experiments. 1H MR 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. Peak deconvolution was performed as previously described [2]. We evaluated metabolite relative concentrations by taking the signal at 0.94 ppm from polypetides as intensity reference.

RESULTS AND DISCUSSION

Figures 1a and b show 1H MR spectral regions of T98G cells of glu in GSH and glu (at 72 and 120 h from seeding,respectively). We indicate as "G" the relative concentration of GSH in cells measured by the envelope signal intensity of glu in GSH at 2.55 ppm and as "g" that of free glu at 2.35 ppm while "glutot" indicates the sum G+g.. Cell growth slowed down as cell density increased during progression towards confluent and post confluent state for both MCF-7 and T98G cells. G decreased as a function of the time in culture. The G decrease was accompanied by a parallel decrease of g and glutot while ratios G/g were almost constant up to six days (144 h) after seeding (compare Figures 1a and b). Afterwards, in correspondence with the onset of confluence, g remained almost constant while G further decreased and ratios G/g dropped to lower values.

To clarify to which metabolic steps the GSH and glu changes could be attributed, we monitored the effect of GSH depletion by inhibiting the activity of γ-glutamyl cysteine synthetase by treating cells with BSO. The 0.1 mM BSO concentration produced a strong decrease of G up to 46% of control sample, paralleled by an increase of g, in MCF-7 cells (Table 1). T98G cells were even more sensitive to BSO as G decreased to 16% of control for 0.1 mM BSO treated cells always accompanied by the increase of g (Table 1). As a consequence, G/g ratio markedly decreased in both cell lines (Table 1). The increase of g values did not compensate completely the decrease of G in both cell lines and, consequently, glutot values were lower in treated with respect to control samples (Table 1). This effect was attributed to the lower proliferation in BSO treated samples indicated by the lower number of cells N with respect to control (Table 1). It is therefore confirmed that decrease of G accompanied by increase of g is related to the inhibition of γ-glutamyl cysteine synthetase.

Figures 1aa' show the T98g cell spectrum before and after irradiation (early times). G increase was accompanied by a decrease of g, pointing to an increase of the activity of the γ-glutamyl cysteine synthetase. The up-regulation of GSH may represent a cellular adaptation through a feedback mechanism based on redox-sensitive transcription factors.

On the contrary, at late times after irradiation G decreased without a concomitant change in intensity of g (Figure 1bb'). This result can be attributable to a direct effect of radiation on GSH, probably due to GSH consumption by detoxification reactions of GSH with reactive oxygen species (ROS) generated by radiation.

In conclusion, the examination of 1H MR spectra of irradiated cells has allowed us to evidence the existence of different mechanisms undertaken by tumour cells to react to the radiation insult.

Table 1 Relative concentrations of glu in GSH (G), free glu (g) and glutot=G+g as obtained from spectra of MCF-7 and T98G cells after treatment with BSO.. The number N x10-6 of cells per flask (c/f) is also given.

	MCF-7	MCF-7 0.1 mM BSO tr
N (c/f)	10.5	7.1
G	0.74	0.34
g	0.71	0.98
glutot	1.45	1.27
	T98G	**T98G 0.1 mM BSO tr**
N (c/f)	2.5	2.2
G	0.49	0.09
g	0.43	0.66
glutot	0.92	0.75

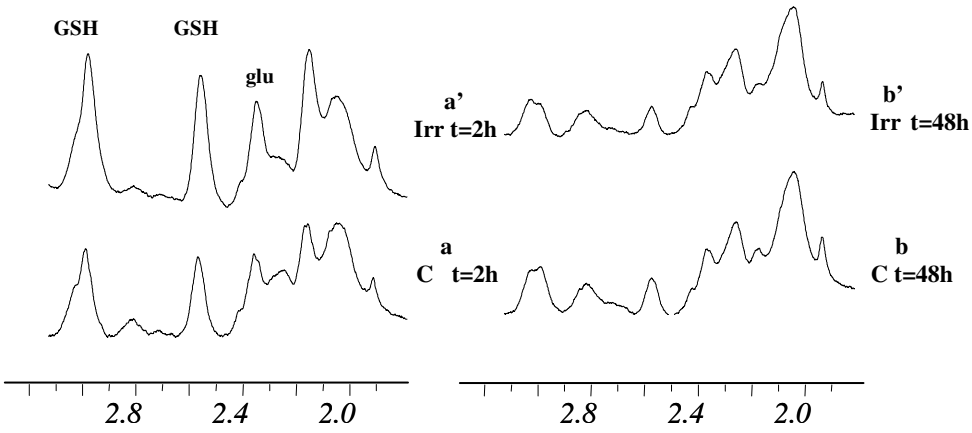

Figure 1 – 1H MR spectra (region 3.1-1.8 ppm) of T98G before and after irradiation: c control, irr irradiated at the indicated times (in hours h).

REFERENCES

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