

^1H MRS of Rat Glioma *in vivo* : Role of Polyunsaturated Fatty Acids in Apoptotic Cell Death as Induced by Gene Therapy

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Introduction

We have used ^1H MRS *in vivo* to investigate the metabolic effects of apoptotic cell death as induced by thymidine kinase (tk) mediated gene therapy in a rat BT4C glioma *in vivo*. Tumor cells transfected with the *herpes simplex* thymidine kinase (HSV-tk) gene are eradicated in this model when treated with ganciclovir (GCV) (1,2). The macroscopic features of apoptotic cell death (3) are uniformly preceded by metabolic events, making MRS an attractive approach for studying this phenomenon.

Methods

BT4C gliomas transfected with the HSV-tk gene were induced intracranially in BDIX rats (1). One group was treated with GCV (25 mg/kg x 2, i.p.), while untreated and normal rats served as controls. ^1H MRS was performed on a SMIS console at 9.4T, using a birdcage transmitter and surface coil receiver. Localised STEAM spectra were acquired from a VOI of 43 μl . Diffusion MRS was performed as described earlier (4). Tumour tissue was extracted for lipids and metabolites (5), and analysed by ^1H MRS *in vitro* (n=4-6). Individual lipids classes and phospholipids were separated by TLC followed by GLC analysis of fatty acid content. Tissue ultrastructure was analysed by TEM for apoptotic cell features (n=2-3). ^1H 2D COSY were performed on tissue at different time points *ex vivo* (n=3-4).

Results

Treatment response detected by ^1H MRS is shown in Fig. 1. A significant decrease in choline containing compounds (Cho) could be observed. For the resonances at 0.9 and 1.25 ppm, a significant increase at day 4 was observed. However, this increase was even more pronounced for the unsaturated lipid resonances observed at 5.4 and 2.8 ppm (Fig. 2). No changes in untreated tumours were observed.

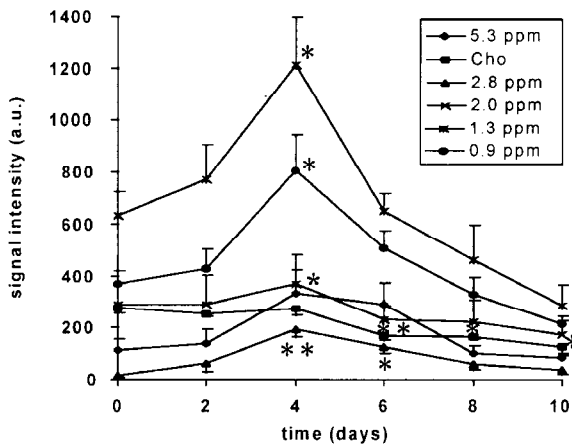


Figure 1. Signal trends relative to tissue water.

* $p < 0.05$, ** $p < 0.01$, Student's paired t-test (n=5-8)

The ADC of the 2.8 ppm peak did not change significantly during the course of study ($0.05\text{-}0.06 \times 10^{-9} \text{m}^2/\text{s}$, day 2 onwards). Tumour COSY revealed cross peaks mostly attributable to protein and lipids (not shown). In these *ex vivo* spectra, treated tumours presented a strong dienyl cross peak (5.4 to 2.8 ppm) with only a low intensity vinyl cross peak (5.4 to 2.0 ppm), suggesting polyunsaturated fatty acids (PUFA).

This connection was also reflected in the stoichiometric increase of these peaks (Fig. 1). Cho depletion was verified by *in vitro* MRS of extracts, which also showed a dramatic 91% depletion in phosphorylcholine on day 4 in treated tumours ($p < 0.01$), compliant with widespread growth arrest. Total lipid extracts showed no significant variation, except for an increased lysophosphatidylcholine (lysoPtdC) to phosphatidylcholine (PtdC) fraction. Tissue free fatty acids and PUFA fraction of triglycerides were also significantly higher on day 4 (Table 1). TEM revealed numerous apoptotic cells in treated tumours and an abundance of intracellular lipid droplets on day 4.

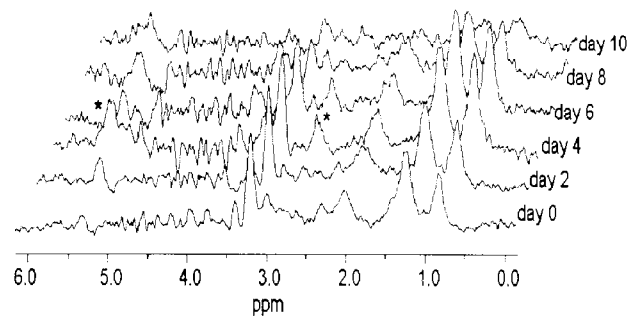


Figure 2. In vivo spectra ($TE=20\text{ms}$, $TM=80\text{ms}$, $TR=2.5\text{s}$, $NS=128$). Residual water removed in time domain. * denotes PUFA.

	normal	day 0	day 4	day 8
lysoPtdC/PtdC (%)	6+1	14+4	24+2*	11+3
FFA ($\mu\text{g/g w.w.}$)	53+4	101+5	194+30*	130+16
- polyunsat. %	13+5	15+2	11+2	17+7
TAG ($\mu\text{g/g w.w.}$)	27+2	162+8	210+69	84+14*
- polyunsat. %	15+2	13+1	28+5*	11+3

Table 1. TLC and GLC analyses of tissue lipids. * $p < 0.05$, compared to day 0, Student's unpaired t-test (n=4-6).

Conclusions

Our data show that GCV induced apoptosis is associated with liberation of membrane fatty acids and a severe accumulation of PUFA in tissue TAG. This is a likely consequence of phospholipase A₂ activity, which catabolically forms lysoPtdC, and is activated during apoptosis (6). The ^1H MRS diffusion data of visible lipids exhibit relatively high translational motion prior to and during treatment, suggesting a nonmembrane origin. Our results show that ^1H MRS can be utilised to monitor apoptotic cell damage during gene therapy *in vivo*.

References

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