^{1}H Magnetic Resonance Spectroscopic Imaging of Phospholipase Activity in Rat Gliomas *In Vivo*

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Introduction: ^{1}H NMR visible lipids can be observed in many cell lines and tissues, especially under pathological conditions, such as cancer (1). The polyunsaturated lipids are particularly interesting, since they accumulate in tumors undergoing apoptosis (2). Evidence indicates that the NMR resonances predominately originate from triglycerides within intracellular inclusions, i.e. lipid bodies (1,3), but very little is known of the processes leading to this triglyceride accumulation. A role for phospholipase A_2 (PLA_2), has been suggested in this process, sinceit is activated during apoptosis (2,4). In this study, we have combined quantitative magnetic resonance spectroscopic imaging (MRSI) of rat BT4C gliomas *in vivo* with biochemical analysis of major cell membrane constituents, phosphatidylethanolamine (PtdEth), -choline (PtdCho), and lysoPtdCho, and their correspoding fatty acyl moieties.

Materials and methods: HSV-thymidine kinase-positive BT4C gliomas (n=4) were inoculated intracranially in BDIX rats, and treated with ganciclovir (GCV) as described before (3). In this study, animals were studied up to 14 days. Ultra short echo-time MRSI data was obtained at 4.7 T on days 0, 2, 4, 6, 8, 11 and 14 of GCV treatment, using pre-localization of the region of intrest with OVS exploiting adiabatic HS pulses under sub-adiabatic conditions, and a STEAM sequence with numerically optimised rf-pulses to prevent contamination from extracerebral lipids (5). This was verified by acquiring MRSI data set from normal animals (n=2). Both water suppressed and water MRSI data were acquired with 16 x 16 phase encoding steps (*TE*=5 ms, *TR*=2.5 s, *FOV*=2 x 2 cm^2 and *NA*=4 for water suppressed and *NA*=1 for water). Anatomic coronal images were acquired with read gradient added MRSI-STEAM (TE = 70 ms, TR = 1 s, NV=64, *NP*=128). Data were Fourier-transformed in spatial dimension and spectral analysis was performed by using jMrui (v. 1.3). Concentration maps were calculated by using the expression $C_l = C_w\ 2/n\ (S_m\ exp(TE/T_{2,l})\ exp(1\text{-}TR/T_{1\_w})\ NA_w)/(S_w\ exp(TE/T_{2,w})\ exp(1\text{-}TR/T_{1\_l})\ NA_l)\ GF$, where C_l and C_w (=40 Mol/kg) are concentrations of lipids and water, respectively, n is number of nuclei in the lipid molecule, S_l *and* S_w are obtained lipid and water signals and *GF* the gain factor. For biochemical analyses, animals were sacrificed prior to treatment, and 4, 8 and 14 days after continuous GCV treatment. At each time point, animals were sacrificed by an overdose of pentobarbital, and the tumors were excised for analysis (n=4). Tumor phospholipids were separated by thin-layer chromatography (6) and the corresponding fatty acids were analyzed by gas liquid chromatography (GLC) as described previously (7).

Results: Lipid resonances were not detected in normal rat brains. Typical MRSI data from a representative tumor-bearing animal are shown in Fig. 1. The concentrations of lipid resonances at 0.9 ppm, 1.3 ppm and 5.4 ppm are shown in Table 1. The results agree well with previous reports, also showing the hallmark increase of polyunsaturated fatty acyl (PUFA) resonances during treatment (2,5). Interestingly, the MRSI detectable significant fatty acyl accumulation was mirrored by an equally dramatic loss of unsaturated fatty acyl moieties [monounsaturated (oleic, C18:1) and polyunsaturated (linoleic acid, 18:2 to docosahexaenoic acid, 22:6n3)] from membrane PtdEth (Fig. 2), corresponding to a maximal 28±3 mMol/kg net release of unsaturated membrane fatty acids (Table 1). The MRSI-derived terminal methyl concentrations correlated well with this biochemical data (R^2=0.99). No significant changes in the fatty acyl composition of PtdCho or lysoPtdCho could be observed. The individual phospholipid concentrations did not change significantly over time (data not shown). By 14 days of treatment the tumors were nearly completely destroyed and replaced by microglial scar formation, reflected also by changes in the lipid MRSI and biochemical analyses.

Discussion and conclusions: Our results are the first to establish a meaningful quantitative relationship between membrane lipid catabolism and ^{1}H NMR-visible lipid accumulation *in vivo*. The biochemical analyses of the main glioma membrane phospholipids (~75% of total cell phospholipid) demonstrate that PtdEth loses a significant portion of its PUFA during GCV treatment. This membrane loss appears to be nearly fully reflected in the storage lipid concentrations, as derived from the ^{1}H MRSI data (R^2=0.99). Since PtdCho remains virtually unaffected, our results also provide evidence that the intracellular (and not secretory) form of PLA_2 is involved in these tumors undergoing apoptosis *in vivo*. PLA_2 cleaves fatty acyl moieties from the sn-2 position of phospholipids, which for PtdEth are typically richly unsaturated. PtdEth is also located primarily on the inner (intracellular) leaflet of the plasma membrane, whereas PtdCho resides mostly on the outer leaflet. Earlier single voxel *in vivo* approaches (2,4) have linked PUFA accumulation with apoptosis in response to treatment. Our MRSI data here show that this response in gliomas is not uniform, and that regional differences in lipid accumulation can be accurately visualised and quantified, even in small experimental tumors *in situ*.

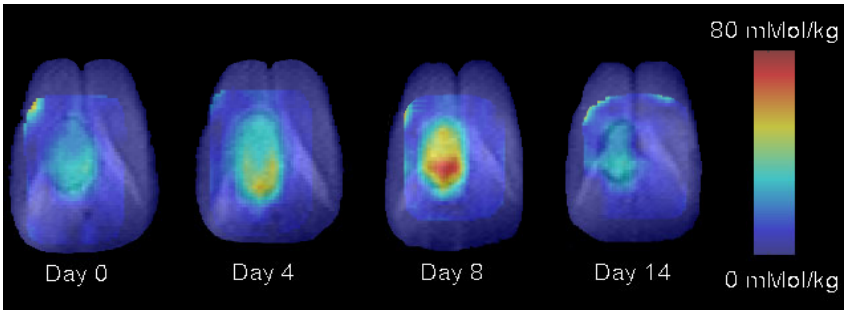

Figure 1. *Representative series of lipid methyl concentration maps during GCV- induced cell death in BT4C rat glioma (data from one animal). MRSI data are superimposed over anatomical MR images.*

Treatment day Source	Day 0 *C* [mMol/kg]	Day 4 *C* [mMol/kg]	Day 8 *C* [mMol/kg]	Day 14 *C* [mMol/kg]
0.9 ppm	24±7	45±6	47±3	30±6
1.3 ppm	87±15	108±14	102±14	56±10
5.4 ppm	34±4	40±7	48±3	31±5
PL	0	25±4	28±3	--

Table 1. *Measured averaged lipid concentrations by MRSI during ganciclovir-induced gene therapy. Data are mean±SEM (n=4). Biochemically analysed release of membrane phospholipid fatty acyl moiteties is indicated by "PL".*

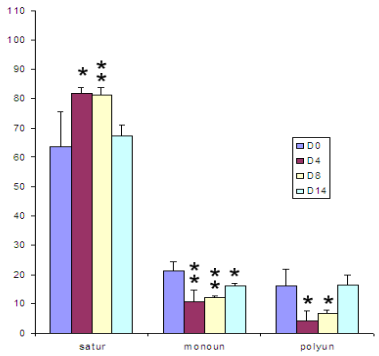

Figure 2. *Result from gas liquid chromatography (GLC) analysis of PtdEth. Percentages of saturated (satur), monounsaturated (monoun) and polyunsaturated (polyun fatty acyl moieties are shown. (*p<0.05, **p<0.01, n=4, Student's unpaired t-test compared to day 0).*

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