31P NMR-detected phosphocholine and phosphocreatine are markedly reduced sub-acutely in human glioma cells following temozolomide treatment

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Introduction: In current clinical practice, response of gliomas to therapy is generally determined with MRI methods that detect morphological and vascular changes. Such changes often require months to develop (1). Studies with animal models and cultured cells have indicated that ^{31}P MR spectroscopy may be a more sensitive indicator for detecting response. Phosphomonoester (PME) levels, which are commonly elevated in rapidly proliferating cells, often decline in response to therapy (2). In addition, phosphodiester levels often increase as proliferation is slowed by therapy (2). However, the specific response depends on both the therapeutic agent used and the type of cancer being treated. Many therapeutic agents induce apoptosis in tumor cells and studies done with cultured cells have demonstrated that this process frequently causes a reduction in PME compounds (1). We hypothesized that temozolomide (a DNA methylating agent that is known to induce apoptosis) would produce reduced PME levels of human glioma cells of an artificial tumor (3). The tumor was specifically designed to retain apoptotic cells, which are commonly lost in perfusion experiments as they detach from surrounding surfaces early in apoptosis.

Materials and Methods: SF188 cells (grade 4 human glioma, UCSF, Brain Tumor Research Center) were grown in DMEM), which contained 25 mM glucose, 6 mM glutamine, 50 μg/ml gentamicin sulfate and 10% serum. For NMR experiments, the cells were grown inside porous collagen microcarriers (Hyclone, Logan, UT) that had a mean diameter of 200 μm when fully hydrated. For constructing the artificial tumor, they were mixed at a 1:1 volume ratio with non-porous and essentially incompressible polystyrene micro-spheres. The mixture was tightly packed inside a 20-mm NMR tube (3). The polystyrene helped to limited compression of the porous collagen during perfusion. The cells were sustained at physiologic conditions (37 °C, pH = 7.2, dissolved oxygen = 0.2 mM) with a system constructed in our laboratory (3). NMR spectra were acquired with a 9.4T spectrometer (Varian, Palo Alto, CA). ^{31}P spectral parameters were: 60° pulse width, 1000 ms repetition time, 4096 points, and 15000 Hz spectral width. Oxygen consumption was determined continuously with polarographic oxygen probes located upstream and downstream of the tumor (4). Experiments were conducted with either one 165 μg/ml (n=1) or two 130 μg/ml (n=4) doses of TMZ (with a 24-h delay between treatments) In parallel studies, cells grown inside microcarriers were treated with TMZ and examined with a standard TUNEL assay for apoptosis (BD Biosciences, Palo Alto, CA). Endonuclease-cleaved DNA was labeled with fluorescein and detected within the microcarriers with fluorescence confocal microscopy (Biorad, Hercules, CA).

Results: A typical ^{31}P spectrum for a tumor containing ~9 x 10^8 cells is shown in the top of Figure 1. The SNR is very high due to the high cell number and the lack of susceptibility contrast within the tumor. In response to a single dose of TMZ (165 μg/ml), NTP and phosphocreatine levels continued to increase (profiles not shown). However, 40 h after treatment, both began to decline steadily. Phosphocholine (PCh) was the first metabolite to show a marked reduction, which began ~25 hours after treatment (Figure 2). GPC and DPDE-2 increased in parallel with NTP levels, but eventually also decline at the same time as the NTP level. PCr reached undetectable levels ~85 h after treatment, which coincided with cessation of oxygen consumption. PCh also declined to very low levels at approximately the same time. The percentage reductions (from the maximum to the level at 140 hours) were largest for PCr (100%) and PCh (87%); smaller reductions were observed for GPC (39%) and DPDE-2 (57%). Similar patterns were observed when two doses (130 μg/ml) were given on two successive days. The rate of reduction in PCh did not appear to be enhanced by the second dose. TUNEL positive cells were first detected 2 days after the second treatment. By the third day, ~30% of cells were TUNEL positive.

Discussion: The reduction in PCh is consistent with the findings for other cell types and tumors in response to therapy (1). The reduction in NTP could be an indicator of reduced viable cell mass in the tumor. PCr and oxygen consumption were more responsive to therapy than NTP. The slowest response was observed for diphosphodiester resonances. Consistent with our previous work, increases in NTP were observed acutely after therapy (4). TMZ did not markedly increase GPC levels, as has been reported with anti-mitotic agents (5).

Conclusions: The data clearly demonstrate that response of human glioma cells can be monitored with ^{31}P NMR spectroscopy. The most sensitive metabolites were PCH and PCr. In future work, we will conduct more detailed studies to quantify the relationship between specific events in the apoptotic process and phosphorous metabolites.

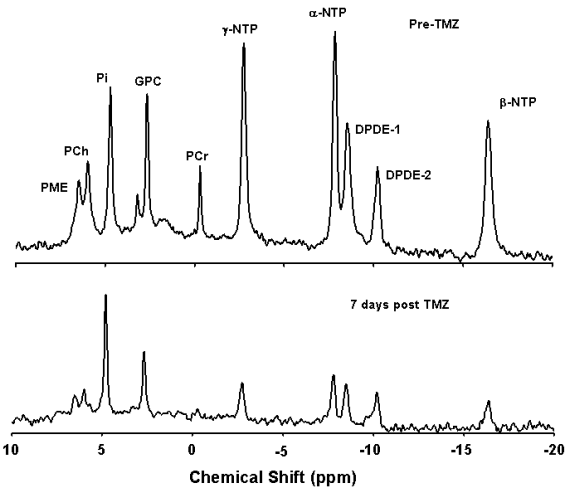

Figure 1. Typical ^{31}P spectra (15 Hz line-broadening) acquired in 20 minutes before, and 7 days after, treatment with TMZ.

References:

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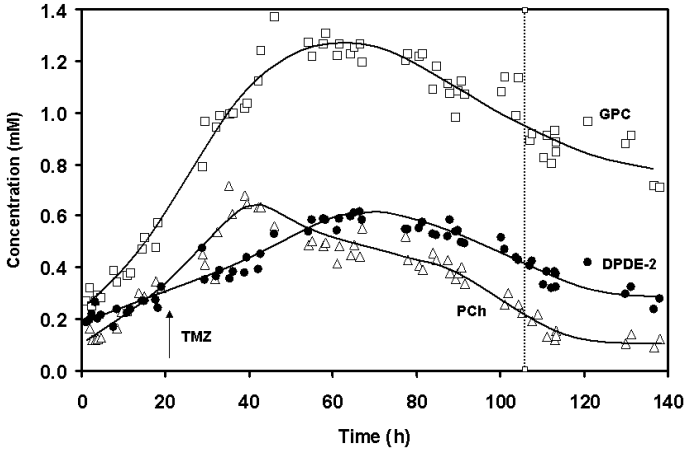

Figure 2. Temporal profiles for changes in ^{31}Pmetabolites following treatment with 160 μg/ml TMZ. The time of treatment is indicated by the arrow. The dashed line indicates the time at which oxygen consumption dropped to undetectable levels (~106 h).

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