Repeated Doses of Temozolomide Cause Repeated Increases in Nucleoside Triphosphates in Cultured Human Glioma Cells

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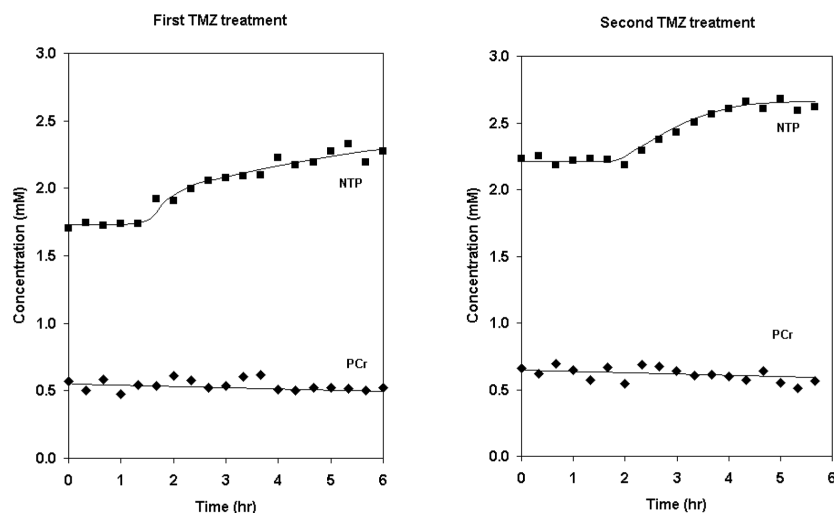
Introduction: Detecting the delivery of chemotherapeutics to brain tumors could be clinically useful for managing therapeutic regiments for individual patients. However, for most drugs, direct detection is impractical with magnetic resonance methods due its low sensitivity and the low dosages used. An alternative approach to detecting drug delivery is to monitor the acute metabolic responses they cause. Temozolomide (TMZ) is a newly approved alkylating agent for the treatment of malignant gliomas. It is a DNA methylating agent and is generally used at doses of 150-200 mg/m^2, which are administered orally once daily for five successive days. It crosses the blood-brain barrier and undergoes pH-dependent activation to 5-(3-methyltriazen-1-yl)-imidazo-4-carboxamide, MTIC (1), which methylates O^6 and N^7 groups of guanine-rich regions of DNA (2). Many mammalian cell types possess alkyltransferase activity, which can remove the methyl group and defeat the therapeutic effect of TMZ (3). Other mechanisms may also be important in protecting cells from alkylating agents (4). These processes are likely energy consuming and may alter the energetics of glioma cells. In this work, we examined the effects of TMZ on cultured human glioma cells with ^{31}P NMR spectroscopy.

Materials and Methods: SF188 human glioma cells (Brain Tumor Research Center, UCSF, San Francisco, CA) were cultured inside porous collagen microcarriers (Cultisphere-S, Hyclone, Logan, UT, USA) that had a mean diameter of 200 microns. After approximately 10 days of incubation with DMEM and 10% serum (changed daily), cell growth was observed throughout the microcarriers (by light microscopy), but at a low density. For NMR spectroscopy, the microcarriers were used in an artificial tumor. It was constructed by mixing the collagen microcarriers with sterile polystyrene beads of similar size in a 1:1 volume ratio (to prevent compression of the microcarrier bed during perfusion) and packing the mixture tightly into a 20-ml NMR tube. Polyethylene filters above and below the NMR-detectable window were used to retain the microcarriers inside the NMR tube during continuous perfusion with 12 ml/min of DMEM/serum. Polarographic probes in the inlet and outlet lines were used to continuously monitor the oxygen consumption rate. pH was detected with a probe in the outlet medium line. A gas exchange bottle was continuously flushed with He, O_2 and CO_2 to control the dissolved oxygen and pH of the medium. Temperature was monitored with a thermocouple and maintained with a microstate controller. All environmental parameters were continuously recorded with a laptop computer controlled with a LabVIEW (National Instruments) program written in our laboratory. All medium-containing components were steam sterilized prior to use. NMR spectra were acquired with a 9.4 Tesla system (Varian Inova), which was equipped with an 8.9 cm vertical bore magnet. A 20-mm liquids probe (Varian) was used to acquire ^{31}P spectra (60° excitation, TR=1000 ms, NP=8192, SW=15,000 Hz, 1200 excitations). Spectra were quantified by comparing resonance areas to that for a phenol phosponic acid reference capillary. The results are expressed as mmol/L of total NMR volume. The S/N on the γ-NTP resonance with 15 Hz exponential apodization exceeded 50:1 for 20-min spectra. For untreated cells, the NTP and oxygen consumption levels increased linearly, indicating that they grew well inside the microcarriers during the NMR experiments. After acquisition of a series of baseline ^{31}P spectra, the perfusion system was spiked with a bolus of TMZ, to give a final concentration of 100 mg/L. Although the dosage used clinically is substantially lower (400 mg/70kg), this value is comparable to the value that is actually delivered to brain tumors, since TMZ is lipophilic and accumulates to high levels in gliomas (5).

Results: With SF188 cells grown on tissue culture plastic, the IC_{50} (level for 50% death) for a single dose of TMZ was 120 mg/L. In the presence of O6-benzylguanine, an alkyltransferase inhibitor, the IC_{50} was markedly reduced to 60 mg/L, which indicates that these cells possess significant demethylation capacity. Cells grown to a total level of approximately 1×10^9 (based on calculations in ref. 6) were treated twice with 100 mg/L TMZ, with 24 hours between the treatments. The responses in the γ-NTP and PCr resonances are shown in the figure below. For both additions, the NTP concentration increased within 15 minutes of the time of TMZ addition. The final NTP levels were significantly higher than the initial values: 1st dose 1.73 ± 0.02 vs. 2.25 ± 0.06 mM, $p<0.005$; 2nd dose 2.22 ± 0.03 vs. 2.61 ± 0.08 mM; $p<0.005$. Similar increases were observed for the α and β-NTP resonances. The PCr concentration did not change significantly, although a slight downward trend was observed. No significant changes were observed for any other ^{31}P metabolite. The findings of this experiment were confirmed in a duplicate experiment.

Discussion: The results show a clear and marked increase in NTP levels with TMZ therapy. Increased NTP has also been observed with treatment of other types of cultured cells. Doxorubicin, actinomycin-D and daunomycin all caused substantial increases in NTP with human T47D breast cancer cells (7). In contrast, cytotoxic doses of cytosine arabinofuranoside or cis-platin did not have any acute effect on NTP levels (7). However, increased NTP was observed when human ovarian carcinoma or rat lymphoma cells were exposed to cis-platin (8). The increase was as high as 70% for wild-type cells and was significantly less with cis-platin resistant cells. These finding are in contrast to the findings for cis-platin with T47D cells and indicate that the high-energy phosphate response to therapy is cell type dependent.

Conclusions: These results demonstrate that ^{31}P NMR is sensitive to acute changes caused by therapy and may be useful for clinical detection of delivery of drugs to brain tumors. It may also be possible to distinguish drug-sensitive and drug-resistance in tumors, which could develop with extended use.



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