Temozolomide acutely enhances glucose-derived ^{13}C-labeling in metabolic intermediates of an artificial human glioma

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Introduction: Temozolomide (TMZ) is a recently approved chemotherapeutic agent for the treatment of brain tumors. Similar to many chemotherapeutics, TMZ acts by alkylating DNA (1). At physiologic pH, it spontaneously decomposes to produce methyldiazonium ion, which methylates a number of different sites in DNA including N^7 and O^6 groups of guanine and O^3 of adenine (2). Of these, O^6 guanine is believed to be the most important for the cytotoxic effect of TMZ. Human glioma cells can often repair this lesion by removing the methyl group with O^6-alkylguanine DNA alkyltransferase (3). If the lesion is not repaired, O^6-methylated guanine pairs with thymine (instead of cytosine) during DNA replication (4). The thymine can be removed by the DNA mismatch repair system, but because the methylated guanine is not repaired, the mismatch recurs. Repeated futile cycles to correct the mismatch eventually produce DNA strand breaks that subsequently induce apoptosis (5). We previously reported that TMZ caused acute increases in nucleoside triphosphate levels in human glioma cells of an artificial tumor (6). This may have resulted from activation of the mismatch repair system, which is known to halt both DNA replication and RNA transcription (7). In this work, ^{13}C NMR spectroscopy was used to determine if other metabolic perturbations could be detected.

Materials and Methods: SF188 cells (human glioma grade 4, Brain Tumor Research Center, UCSF) were cultured inside porous collagen microcarriers (Hyclone, Logan, UT, USA, mean diameter 200 μm) with supplemented DMEM (10% serum, 50 μg/ml gentamicin). For NMR spectroscopy, the microcarriers were used in an artificial tumor that contained a tightly packed 1:1 mixture of porous collagen and non-porous polystyrene microspheres (6). The system used to sustain the artificial tumor inside the spectrometer was described previously (8). NMR spectra were acquired with a 9.4 Tesla system (Varian, Palo Alto, CA). A 20-mm liquids probe (Varian) was used to acquire ^{13}C spectra with 60° pulses, 1200 ms repetition time, 4096 points, 25000 Hz spectral width and bi-level ^{1}H WALTZ-16 decoupling. Cells were initially fed DMEM with un-enriched glucose while background spectra were acquired. Subsequently, the un-enriched medium was completely replaced with DMEM containing 10 mM [1,6-$^{13}C_2$] glucose (Cambridge Isotopes, Andover, MA). After labeling in intracellular metabolites reached a steady state, a single dose of 130 μg/ml of TMZ was added to the culture medium. This experiment was conducted in duplicate, with independently prepared artificial tumors. Metabolite assignments and concentrations were determined as described previously (8).

Results: Expanded regions of ^{13}C NMR spectra acquired before and 3.5 h after treatment with TMZ are shown in Figure 1. Strong resonances were observed for C-1 and C-6 of glucose and C-3 of lactate and alanine (data not shown). Examination of samples of the perfusate with ^{13}C NMR demonstrated that these compounds were predominantly extracellular. A substantial amount of label was also detected in C-4 of glutamate, which was intracellular. Smaller intracellular resonances were observed for C-3 and C-2 of glutamate. The C-3 resonance was triplet due to the presence of both [3-^{13}C]glutamate and [3,4-$^{13}C_2$]glutamate. Label was also detected in -CH_2- regions of mobile lipids (29.8 and 30.4 ppm) and glycolytic intermediates (63 to 68 ppm). The high ratio of labeling in C-4 glutamate relative to C-3 and C-2 is typically for cultured cancer cells (9). The time courses for the changes ^{13}C metabolites are shown in Figures 2a and 2b. A steady state was established in C-4 glutamate labeling approximately 4.5 hours after the start of the infusion with 10 mM [1,6-$^{13}C_2$]glucose DMEM. Treatment with TMZ caused a marked (44 ± 3%) increase in C-4 glutamate labeling that began 45 minutes after treatment. A similar increase (52 ± 13%) was observed for the total amount of label in C-3 glutamate. A much larger increase (143 ± 14%) was observed for C-2 glutamate. TMZ also caused marked increases in labeling of the 66.0 and 67.3 ppm resonances as shown in Figure 2b. Accumulation of label in the central -CH_2- regions of fatty acids (30.4 ppm) increased at a constant rate throughout the experiment and was not perturbed by TMZ (data not shown). Labeling in the 65.7 ppm resonance was also not perturbed by TMZ.

Figure 1: Expanded regions of ^{13}C spectra acquired (in 30 min) before and 3.5 h after treatment with TMZ. The scale for C-4 glutamate was compressed by a factor of 2.

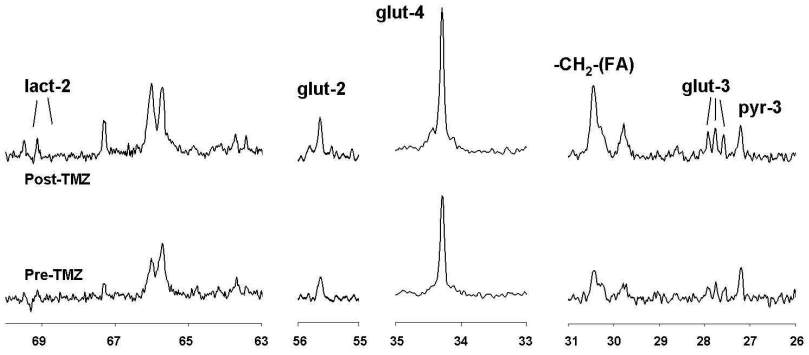
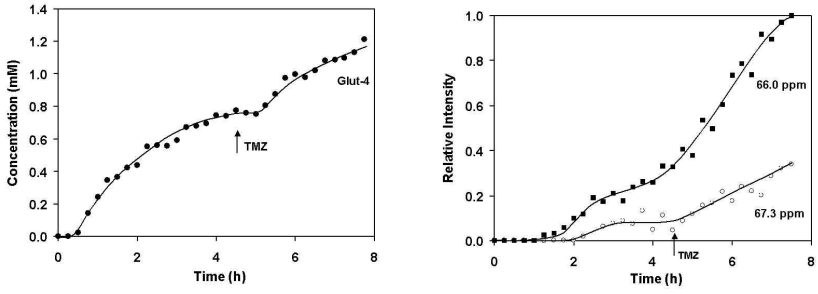


Figure 2: TMZ acutely stimulated ^{13}C labeling in several compounds during [1,6-$^{13}C_2$]glucose infusion (a) C-4 glutamate (34.3 ppm) and (b) 66.0 and 67.3 ppm resonances.



Discussion: The increase in ^{13}C metabolites with treatment is consistent with our previous findings that showed NTP levels were increased by TMZ (6). These perturbations may all be a consequence of activation of the mismatch repair system, which is known to interrupt DNA replication and RNA transcription (7). The large increase in C-2 glutamate labeling relative to C-3 and C-4 suggests that flux into the TCA cycle through pyruvate carboxylase was disproportionately increased relative to that through pyruvate dehydrogenase (9).

Conclusions: These results demonstrate that ^{13}C NMR spectroscopy can detect acute metabolic perturbations in human glioma cells caused by temozolomide. This finding may be useful for designing new clinical methods. For example, by monitoring [1,6-$^{13}C_2$]glucose-derived labeling in C-4 glutamate (with indirect ^{1}H-^{13}C MRS) during treatment with TMZ, it may be possible to detect delivery of the drug to gliomas. It may also be possible to differentiate drug-sensitive and drug-resistant tumors with the same method.

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