Diet/drug combinatorial approach to target energy metabolism in preclinical glioblastoma multiforme

Zachary M. Augur, Catherine M. Doyle, Minki Hong, Mingyi Li, Purna Mukherjee, and Thomas N. Seyfried

Department of Biology, Boston College, Chestnut Hill, MA, USA

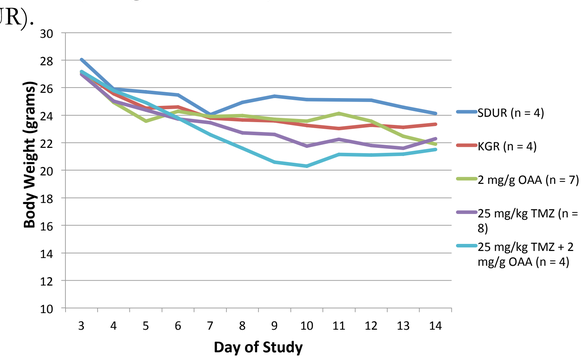
Introduction

Temozolomide (TMZ), a toxic alkylating agent, is the most commonly used chemotherapeutic drug for glioblastoma multiforme (GBM). The clinical significance of TMZ is well studied, but a novel approach is needed to enhance therapeutic efficacy and reduce toxicity. Brain cancer cells are largely dependent on glucose and glutamine for energy metabolism. Therefore, a targeted approach to inhibit these fuels may reduce disease progression. Calorically restricted Ketogenic diet (KD) is a promising alternative in targeting energy metabolism. KD reduces blood glucose and increases ketone levels. While ketones are an alternative to glucose for normal tissue, cancer cells cannot effectively burn ketones due to defects in the mitochondria. We hypothesize that coupling KD with a glutamate scavenger, like oxaloacetate (OAA), could alleviate some negative effects of TMZ. Unfortunately, an excessive level of glutamate in the brain frequently accompanies GBM, which causes excitotoxic neuronal death and an overall more negative prognosis for the patient. Therefore, by scavenging blood glutamate, a product of the glutamine cycle, the glutamine levels will similarly be reduced. We propose that a cocktail therapy could enhance patient survival and reduce GBM growth.

Methods

Tumor Line/Implantation —Tumor tissue fragments (1.0 mm^3) of VM-M3/Fluc luciferin were implanted surgically into the right cerebral cortex of VM/Dk inbred mice. Tumor growth is estimated using bioluminescence imaging.

Feeding — All mice were maintained on a strict KGR diet (12-15% body weight reduction) or an unrestricted standard diet (SDUR).

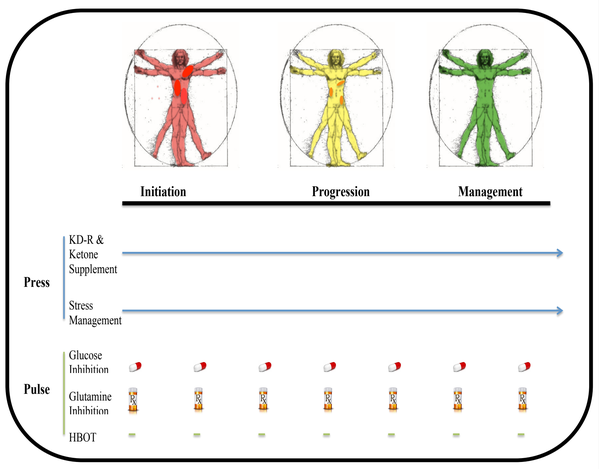


Drug Preparation and Delivery — 2 g of OAA (provided by Terra Biological LLC) per kg body weight was administered in the diet or via intraperitoneal (IP) injections (dissolved in PBS). TMZ was dissolved in a 10% DMSO solution and mice received 15-20 mg of TMZ per kg body weight IP injections daily. Oxygen therapy was delivered every other day for 90 minute sessions.

Background

Oxaloacetate (OAA) is an important intermediate in the citric acid cycle, but has been shown to serve as an effective blood glutamate scavenger and a mitochondrial biogenesis enhancer. At appropriate levels, glutamate serves as a neurotransmitter for normal brain function and its uptake is controlled by astrocytes. In GBM patients, these astrocytes do not remove extracellular glutamate, but instead, release it. The need to reduce excess glutamate in GBM patients is the reasoning behind our proposed cocktail therapy with OAA.

The proposed cocktail therapy is modeled using the press-pulse approach to the metabolic management of cancer as described (Seyfried TN, et al.). By testing appropriate combinations, dosages, timing, and scheduling, the most efficacious and least toxic treatment for GBM can be determined.



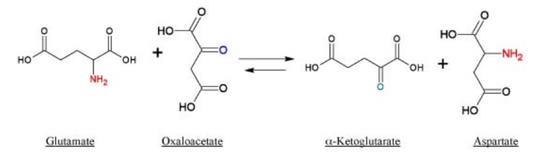
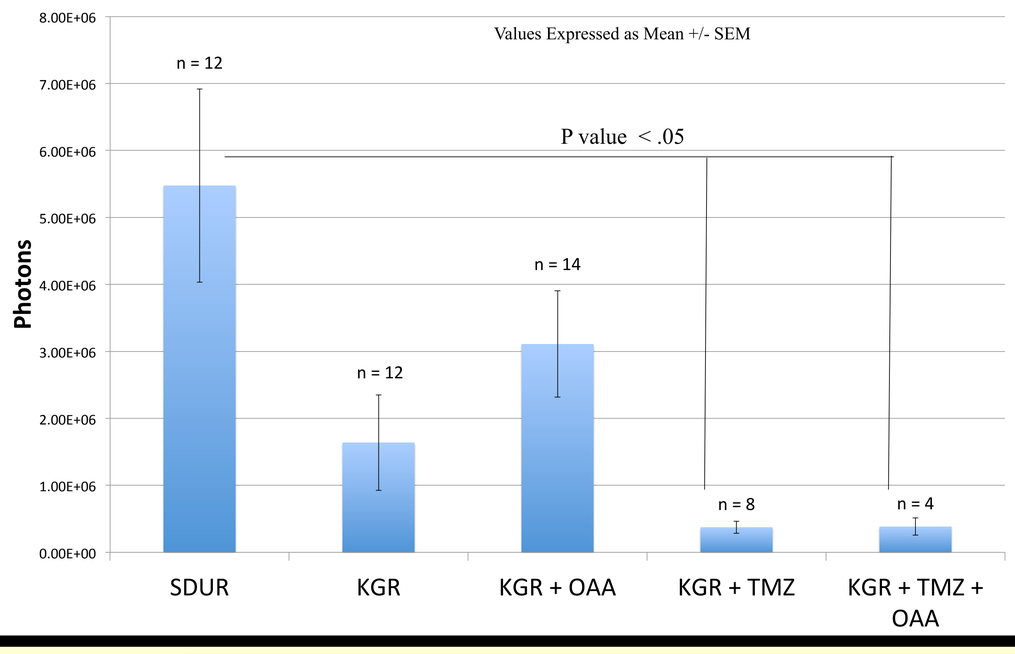
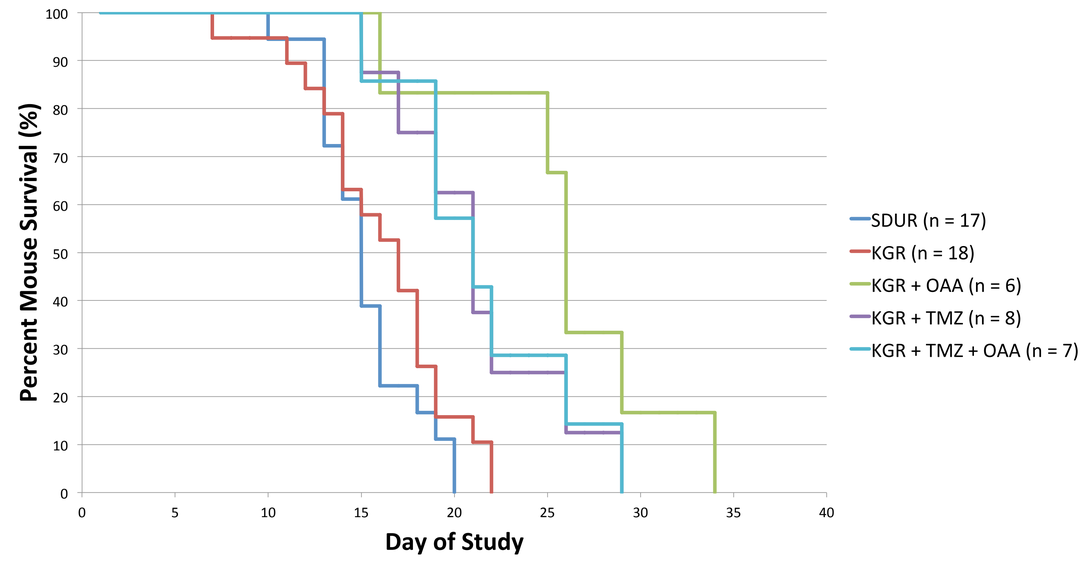


Figure 1. Influence of OAA, TMZ, and KGR diet on VM-M3/Fluc brain tumor growth



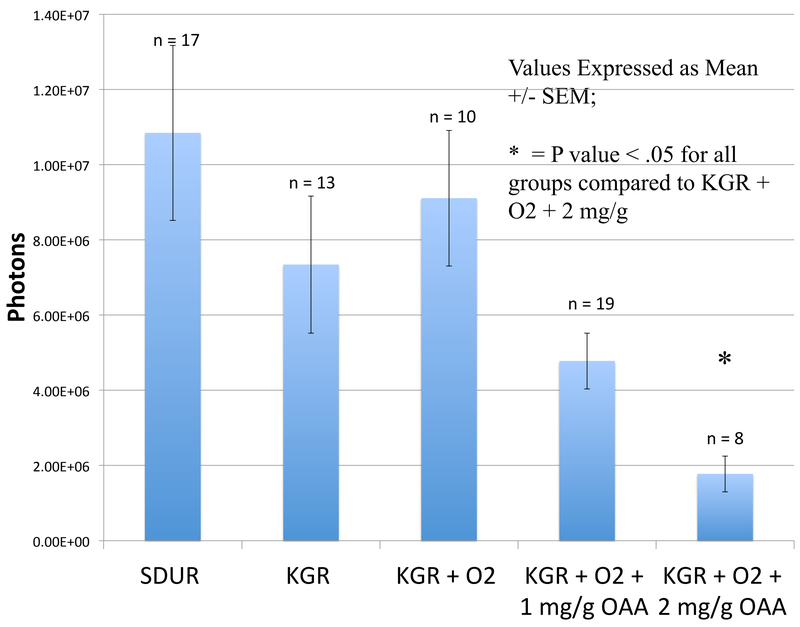
Influence of OAA, TMZ, and KGR on VM-M3/Fluc brain tumor growth: Ex-vivo bioluminescence data showing that mice treated with KGR + TMZ or KGR + TMZ + OAA had a significant therapeutic benefit when compared to the SDUR group. Both KGR + TMZ and KGR + TMZ + OAA showed a trend of reduced tumor growth compared to the KGR diet group alone. No significant therapeutic difference was observed between KGR + TMZ and KGR + TMZ + OAA.

Figure 3. Influence of OAA, TMZ, and KGR diet on survival of tumor-bearing mice



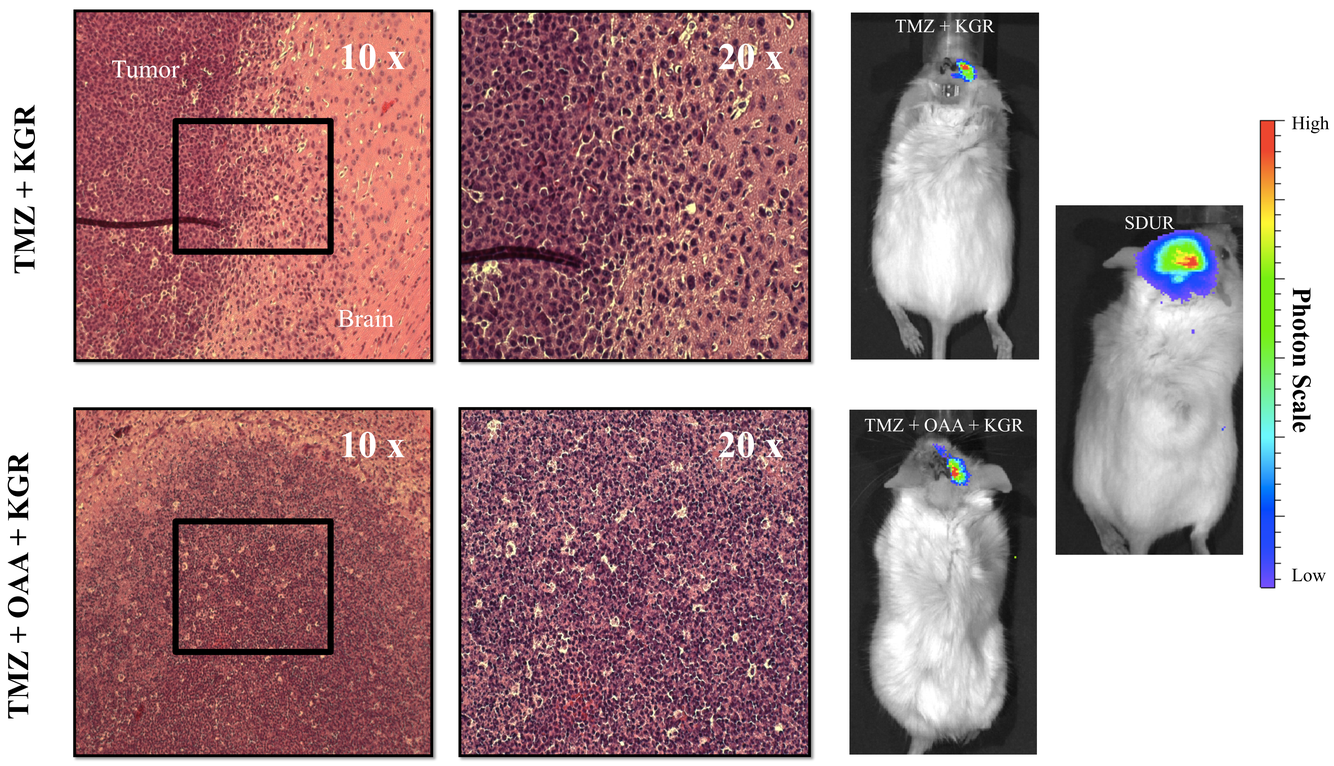
Influence of OAA, TMZ, and KGR diet on survival of tumor-bearing mice: A Kaplan-Meier survival plot shows the percent survival of each study group over a 34 day span. Due to drug toxicity, mice receiving TMZ and TMZ + OAA had a lower survival rate than mice receiving OAA alone. Date of death for all mice was determined once the animals reached morbidity.

Figure 4. Therapeutic effect of OAA under ketogenic diet and hyperoxia



Combined data showing the therapeutic effect of OAA when administered with O_2 and diet on the VM-M3/Fluc brain tumor growth: Data collected from 3 studies were combined. A statistically significant reduction in bioluminescence was seen for the mice receiving 2 mg/g OAA with O_2 therapy and KGR diet in comparison to the other groups.

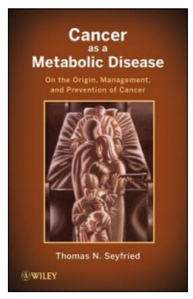
Figure 2. Detection of VM-M3/Fluc brain tumor cells using bioluminescent imaging and histological analysis



Detection of VM-M3/Fluc brain tumor cells using bioluminescent imaging and histological analysis: Samples were fixed in formalin, sectioned at 5µm, stained with haematoxylin and eosin, and then examined by light microscopy. Though there was noticeably less tumor infiltration in both TMZ + KGR and TMZ + OAA + KGR when compared to SDUR or KGR, the number of viable cells in TMZ + OAA + KGR was lower than for TMZ + KGR. Bioluminescent imaging was also used to quantify brain tumors in all mice.

Conclusions

- Therapeutic effects of OAA and TMZ are most noticeable when the drugs are administered in combination under KGR diet (Figure 1). However, survival is not significantly increased due to the toxicity of TMZ (Figure 3).
- There is less tumor infiltration in the brains of mice receiving TMZ or TMZ + OAA under KGR diet (Figure 2).
- There are fewer viable tumor cells in the brains of mice receiving TMZ + OAA under KGR diet when compared to TMZ under KGR diet (Figure 2).
- Administration of OAA under KGR diet increases the percent survival in tumor-bearing mice in comparison to mice receiving KGR diet alone (Figure 3).
- Therapeutic benefit is maximum when OAA is administered with O_2 and KGR diet (Figure 4).



Supported by

SINGLE Cause Cure
Helping to realize the promise of metabolic cancer research

References

- Ye ZC, Sontheimer H (1999) Glioma cells release excitotoxic concentrations of glutamate. Cancer Res 59(17):4383–4391
- Williams DS, Cash A, Hamadani L, Diemer T. Oxaloacetate supplementation increases lifespan in *Caenorhabditis elegans* through an AMPK/FOXO-dependent pathway. Aging Cell. 2009:8:765-768. doi: 10.1111/j. 1474-9726.2009.00527.x.
- Zlotnik A et al (2009) The neuroprotective effects of oxaloacetate in closed head injury in rats is mediated by its blood glutamate scavenging activity: evidence from the use of maleate. J Neurosurg Anesthesiol 21(3):235–241
- Ruban A, Berkutzki T, Cooper I, Mohar B, Teichberg VI. Blood glutamate scavengers prolong the survival of rats and mice with brain implanted gliomas. Invest New Drugs. 2012;30:2226-2235.
- Seyfried T, Yu G, Maroon J, D'Agostino D. Press-Pulse: a novel therapeutic strategy for the metabolic management of cancer. Nutrition and Metabolism. 2017; 14:19
- Shelton, L.M., Mukherjee, P., Huysentruyt, L.C. et al. J Neurooncol (2010) 99: 1655. doi:10.1007/s11060-010-0115-y