Effects of β-hydroxybutyrate and Glucose Availability on the Viability and Motility of Human Glioblastoma Cell Line M059J

Kenny Levenson, Jennifer Hurst-Kennedy, and Cindy Achat-Mendes
School of Science and Technology, Georgia Gwinnett College, Lawrenceville, GA 30043

INTRODUCTION

- The Warburg effect is a distinct metabolic phenotype observed in a variety of cancers, including lung, breast, colorectal, and glioblastoma (GBM).[1,4] It is characterized by a high glucose demand while favoring lactic acid fermentation even in the presence of oxygen.[1,4] A normal cell, by contrast, utilizes oxidative phosphorylation for 19 times more ATP production when oxygen is present.
- Many cancer studies have demonstrated that glucose scarcity leads to the inhibition of cancer cell growth[1,3,5,6]. In addition it has been demonstrated that unlike normal glial cells, gliomas are unable to effectively use ketone bodies (KB) as a glucose alternative.[2,3,5] Levels of plasma KB are lowest following meals with high protein or carbohydrate, and increase as a result of an overnight fast, post-exercise, ketogenic diet, prolonged fasting, and starvation.[2]
- This supports the idea that low glucose and high KB blood levels may inhibit cancer cell viability. Thus, it is possible that diet and lifestyle modifications may be a valuable component in the care and management of glioblastoma patients.

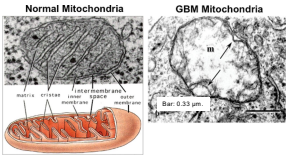


Figure 1. Normal mitochondria (left) contain elaborate cristae, embedded with protein complexes of the electron transport chain - producing ATP via OxPhos. The mitochondrion from the glioblastoma (m) shows a breakdown of cristae. This supports the Warburg effect since lack of cristae in GBM mitochondria indicates that OxPhos would be deficient. Arrows indicate an inner membrane fold.[6]

RESEARCH GOALS

- The overall objective is to describe whether glioma cells can utilize KBs as an alternative to glucose, using the adherent human glioblastoma cell line M059J and the KB, beta-hydroxybutyrate (BHB). We hypothesize that glioma cells will not be able to utilize BHB when deprived of glucose. Therefore, cell viability, quantified using WST-1 assay, and motility, determined by wound healing assay, will be impaired in glioma cells deprived of glucose and treated with BHB compared to glia grown in normal media.

MATERIALS AND METHODS

- M059J glioma cells were maintained in *Normal* media w 10% FBS, 1% Antimicrobial, and 1% NEAA.
- BHB treatments – 0, 1, 2, 5, & 10 mM – were added to either *Zero*, *Low*, or *Normal* growth media with glucose concentrations of 0, 2.5, & 17.5 mM respectively.
- Cell viability was assessed using the WST-1 assay, then quantified with a microplate reader to measure absorbance.

WST-1 Assay: Cell Viability

Seeding	Treatment	WST-1	Measure Absorbance	Analysis
200 ul of M059J cells suspended in normal media were seeded in 96-well plates, excluding outer wells for media-only blanks, or sterile DI water. Incubation time = 72 hrs at 37ºC & 5% CO_2	Wells verified under microscope for even seeding. Growth media was removed from wells then supplemented with 0, 1, 2, 5, or 10 mM BHB in zero, low, or normal media – then incubation for 24 hrs	10 uL of WST-1 solution was added to each well, then placed in incubator. Plate was removed for readings at 0.5, 1.5, & 2.5 hours.	At each time interval, the plate was placed on a shaker for 1 minute prior to placement in a plate reader at 450 nm.	Statistical analysis of absorbance data based on design of 96-well plate

RESULTS

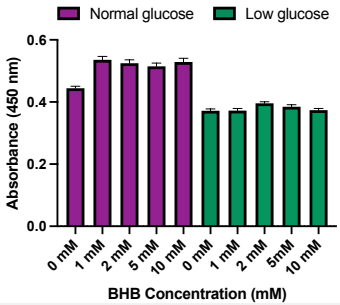


Figure 2. WST-1 data. Data represents absolute absorbance values (no subtraction of blank abs.) at 450 nm. Data were analyzed using a two-way ANOVA showing extremely significant effects of BHB concentration, F (9, 33) = 68.95, p< 0.0001. No significant effect of glucose concentration, F (5, 33) = 0.6431, (p >0.05) on cell viability. This suggests that BHB does not have an effect in the presence of glucose. Bonferroni multiple comparisons t-tests show significant *p< 0.05; ** p< 0.001; *** p<0.0001

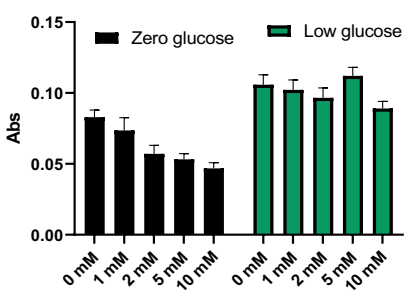


Figure 3. Data were analyzed using a two-way ANOVA showing significant effects of BHB concentration, F (4, 128) = 5.68, p< 0.0001 and glucose availability, F (1, 128) = 94.15, but no significant effect on their interactions (p>0.05) on cell viability. This suggests that BHB does not have an effect at all glucose concentrations. Bonferroni multiple comparisons t-tests show significant *p< 0.05; ** p< 0.001; *** p<0.0001

DISCUSSION

Figure 2. *Cell viability is unaffected by b-hydroxybutyrate when glucose is available*

- Increased glucose availability (17.5 mM) correlates to increased proliferation when compared to cells grown in a low concentrations of glucose (2.5 mM) – regardless of BHB dosage (0-2, 5, or 10 mM).
- BHB concentration showed no significant effect within either glucose concentration groups (2.5 or 17.5 mM), *p< 0.05.
- The effect of glucose concentration on cell viability is significant. *** p<0.0001.

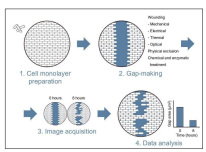
Figure 3. *Cell viability is altered by b-hydroxybutyrate depending on glucose availability*.

- Eliminating glucose significantly decreased cell viability compared to the low dose of glucose.
- BHB (at doses of 1, 2, 5, or 10 mM) significantly decreased cell viability only for the zero glucose condition.
- Compared to 0mM BHB, cell viability decreased only following BHB concentrations of 2, 5, or 10 mM.

Taken together, both cell viability experiments suggest that BHB is non-cytotoxic in the presence of glucose, regardless of glucose conditions tested (2.5 mM & 17.5 mM).

FUTURE DIRECTIONS

- Optimized wound healing assay will be performed to investigate the effects of glucose/BHB concentrations on the motility of M059J cells.
- WST-1 assay will be performed using a wider range of glucose concentrations in media to investigate the role of glucose when BHB is present



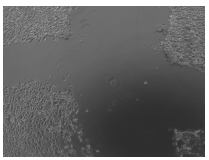


Figure 2. Overview of wound healing assay. Adherent cell lines grow in a monolayer along the bottom of a 24-well culture plate. A wound is created by "scratching" along the bottom of a confluent well, and image of each well is then captured at time zero. Images of each well are captured at various time intervals (1, 3, 12, 24 hrs) for data analysis. The rate of closure will be quantified using ImageJ. The image on the right shows a well M059J monolayer scratched w a p1000 tip prior to treatment at 40X magnification

REFERENCES

1. Gillies RJ, Gatenby RA. Adaptive landscapes and emergent phenotypes: why do cancers have high glycolysis? J Bioenerg Biomembr. 2007 Jun;39(3):251-7. doi: 10.1007/s10863-007-9085-y. PMID: 17624581.
2. Gjedde A, Crone C. Induction processes in blood-brain transfer of ketone bodies during starvation. Am J Physiol. 1975 Nov;229(5):1165-9. doi: 10.1152/ajplegacy.1975.229.5.1165. PMID: 1200135.
3. Mukherjee, P., Augur, Z.M., Li, M. et al. Therapeutic benefit of combining calorie-restricted ketogenic diet and glutamine targeting in late-stage experimental glioblastoma. Commun Biol 2, 200 (2019). https://doi.org/10.1038/s42003-019-0455-x
4. Potter M, Newport E, Morten KJ. The Warburg effect: 80 years on. Biochem Soc Trans. 2016 Oct 15;44(5):1499-1505. doi: 10.1042/BST20160094. PMID: 27911732; PMCID: PMC5095922.
5. Skinner R, Trujillo A, Ma X, Beierle EA. Ketone bodies inhibit the viability of human neuroblastoma cells. J Pediatr Surg. 2009 Jan;44(1):212-6; discussion 216. doi: 10.1016/j.jpedsurg.2008.10.042. PMID: 19159745.
6. Thomas N. Seyfried, Roberto E. Flores, Angela M. Poff, Dominic P. D'Agostino, Cancer as a metabolic disease: implications for novel therapeutics, Carcinogenesis, Volume 35, Issue 3, March 2014, Pages 515–527, https://doi.org/10.1093/carcin/bgt480

ACKNOWLEDGEMENTS
This research project was supported by Georgia Gwinnett College, School of Science and Technology. We appreciate the assistance of Jessica Thompson, Fred Ogalla, and Clark Taylor.