

Investigating The Effects of Saccharin and Erythritol on U87 Cell Survival and Proliferation

Natalya Thomas ¹, M. Soliven B.S. ¹, C.Q. Rogers Ph.D. ¹, A. Poff Ph.D. ¹, D. Diamond Ph.D. ², D. D'Agostino Ph.D. ¹
 Department of Molecular Pharmacology and Physiology, University of South Florida, Tampa, FL
 Department of Psychology, University of South Florida, Tampa, FL

BACKGROUND

Glioblastoma (GBM) is known to upregulate aerobic glycolysis for lactate production, commonly known as the Warburg Effect.



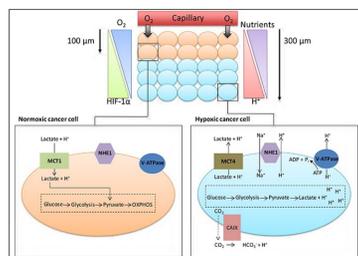
The **Ketogenic Diet (KD)** is currently being investigated as an adjuvant therapy for patients diagnosed with GBM

Reducing extra carbohydrates for energy needs in cancer cells may **decrease cell survival and/or proliferation**.

Sugar Alternatives (SA) are commonly used in KD. U87s, a human-derived glioblastoma cell line, exhibited enhanced growth under physiological concentrations of Erythritol.

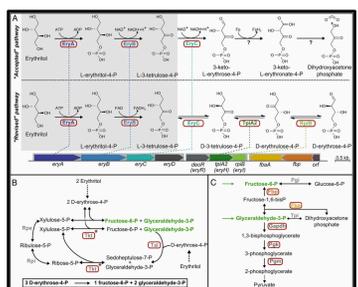
Q: What are the effects of saccharin and erythritol on U87 cell proliferation and survival?

HYPOTHESIZED MECHANISM OF INTERACTIONS



Saccharin --> structure mimics carbonic anhydrase inhibitors

Carbonic Anhydrase IX (CAIX) regulates intracellular pH following hypoxia. **Saccharin** may **decrease** cell survival and proliferation by inhibiting CAIX

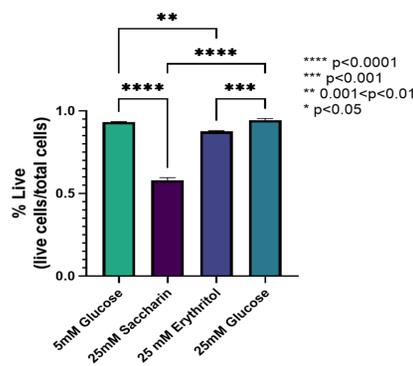


Erythritol --> D-erythrose- 4- Phosphate

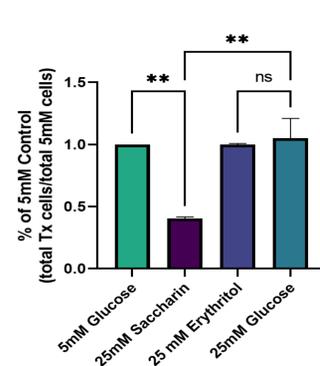
Erythritol is capable of turning into anabolic metabolite that may **enhance** survival and proliferation of U87 cells

RESULTS

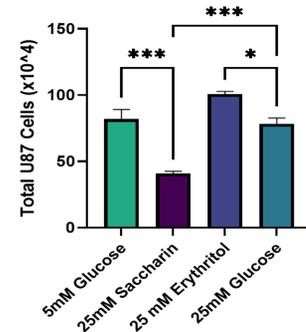
A) U87 Cell Survival Following 48Hrs of 25mM Sugar Alternative Treatment



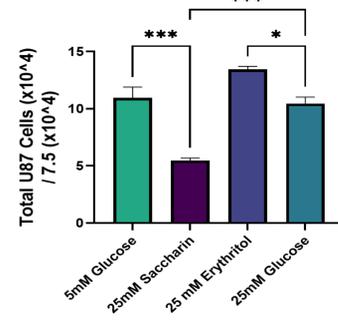
B) U87 Percent of Control Following 48 Hrs of 25mM Sugar Alternative Treatment



C) U87 Total Cell Number Following 48Hrs of 25mM Sugar Alternative Treatment



D) Post- 96 Hour Total U87 Cell Concentration Normalized to Seeding Concentration



The results of cell survival and proliferation following 48 hours of 25mM erythritol or saccharin treatment. Statistical analyses were performed via Prism one-way ANOVA and post-hoc Tukey test.

A) Cell survival measured by **percent live**. There was a significant **decrease** in the saccharin and erythritol groups compared to the 5mM and 25mM glucose controls.

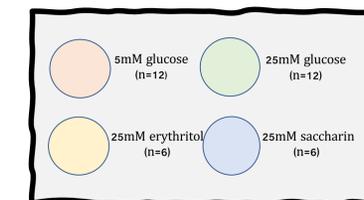
B) Proliferation was measured as **percent of 5mM glucose control**. There was a significant **decrease** in proliferation in the **saccharin** Tx compared to the 5mM and 25mM glucose control, but no significance in erythritol.

C) There was a significant **decrease** in total cell numbers between the 5mM glucose and **saccharin** Tx, and a significant **increase** between 25mM glucose and **erythritol**

D) Proliferation also measured as difference in cell numbers from seeding to post 96-hour experiment. **Saccharin** contained significantly **less** concentration of cells compared to 5mM and 25mM glucose controls. **Erythritol** had significantly **more** cell concentration compared to the 25mM glucose control.

METHODS

- Day -1:** Coat seven 6-Well plates with 50 mM Poly-D-Lysine following Sigma Aldrich protocol
- Day 0:** U87 Cells were seeded at 7.5 x10⁴ cells/well in two 6-well plates, cultured in 2mL of 25mM glucose, 5mM glutamine Dulbecco's Modified Eagle Medium for 48hrs, at 37°C and 5% CO₂
- Day 2:** Replacement of all cell media with 2mL of treatment. Controls were given 2mL of either 5mM glucose, or 25mM glucose. Saccharin and Erythritol were given 2mL of 5mM glucose and 300uL of a saccharin or erythritol stock to achieve 25mM concentration.
- Day 4:** Live/Dead staining with 0.4% Trypan Blue. Cell counting completed via hemocytometer



DISCUSSION AND FUTURE DIRECTIONS

Discussion:

- Saccharin may contribute to some level of cytotoxicity in U87 human-derived glioblastoma
- Erythritol may enhance cell proliferation
- Cell counting to determine proliferation and survival are not the most accurate method of assessment
- The mechanism of how saccharin or erythritol may impact U87 survival or proliferation cannot be determined from this experiment
- There was no significant difference between 5 and 25mM glucose controls, which was not expected

Future Directions:

- Run U87 for 48 hours to analyze growth between seeding and application of treatment
- Inquire why 5mM and 25mM glucose controls were not statistically different
- Potential concentration dependence
- RT-PCR for CAIX
- Mouse model with standard diet (SD), KD, SA+KD and SA+SD Tx