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The Cellular Effects of Ketone Body Exposure

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INTRODUCTION

The ketogenic diet is a high fat, low carbohydrate, adequate protein diet that has gained popularity in recent years both as an approved clinical tool and more generally for health maintenance. Through restriction of carbohydrates, molecules called ketone bodies are produced to meet the body's energy demands producing a state known as systemic ketosis. The most abundant ketone body is beta-hydroxybutyrate (β-HB) and is used by cells to produce energy when glucose is unavailable.

The effects of this replacement of glucose with ketone bodies are currently being characterised in different organs and at different stages in development. At present the full effects of ketosis on the developing brain are unclear. Tentative animal-based evidence suggests a ketogenic state during pregnancy has effects on foetal brain and organ development that are evident post-natally (Barry et al., 2018).

This project aims to characterise and investigate some of these effects through determining the effects of β-HB supplementation on the growth of brain cells and systematically reviewing the relevant published literature.

OBJECTIVES

The objective of this project is to assess the effects of glucose deprivation and β-HB supplementation on cells from the NE-4C neuroepithelial cell line. These cells are embryonic neural stem cells that have the potential to differentiate into all types of cells that exist in the human brain, both neurons and glia. Thus, they provide an ideal cell culture model to investigate the effects of β-HB on early neuroepithelial cell development. Glucose deprived cells treated with β-HB will be compared with control glucose deprived cells.

Additionally, the experimental concentrations of other metabolites such as l-glutamine and pyruvate will be varied to investigate whether ketone body metabolism is bypassed by anaplerotic reactions. In the absence of glucose, l-Glutamine and pyruvate may be oxidised to produce α-ketoglutarate and acetyl-CoA, respectively (Yang et al., 2014).

These metabolites can promote cell survival during glucose deprivation without oxidising ketone bodies. Restricting the concentration of these metabolites in the media aids in isolating the role β-HB plays in promoting cell survival and growth.

Figure 1

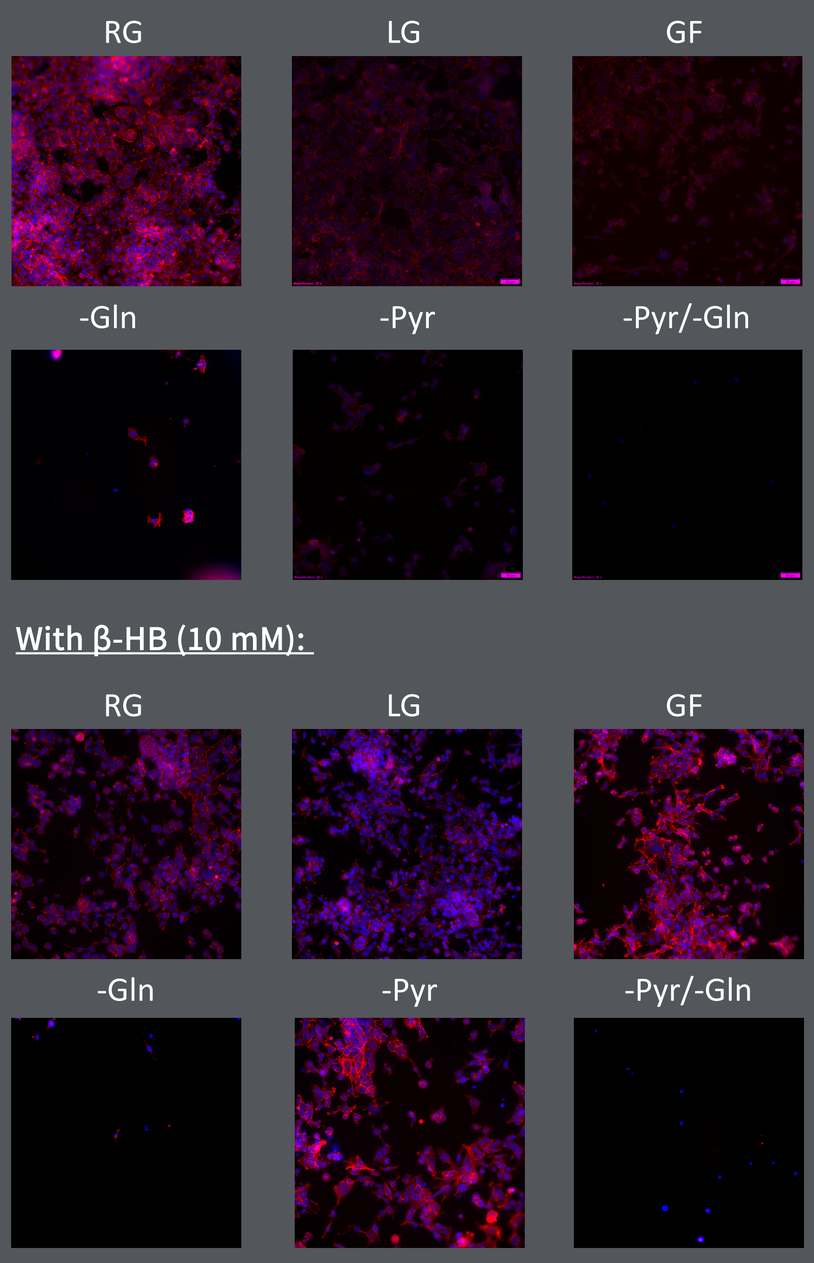

Figure 1: Actin Expression at 72 hours in Regular glucose (RG), low glucose (LG), Glucose Free (GF), GF without l-Glutamine (-Gln), GF without Pyruvate (-Pyr), and GF without L-Glutamine and Pyruvate (-Pyr/-Gln).

Actin stains cytoskeleton red and DAPI stains the nuclei blue.

Figure 2

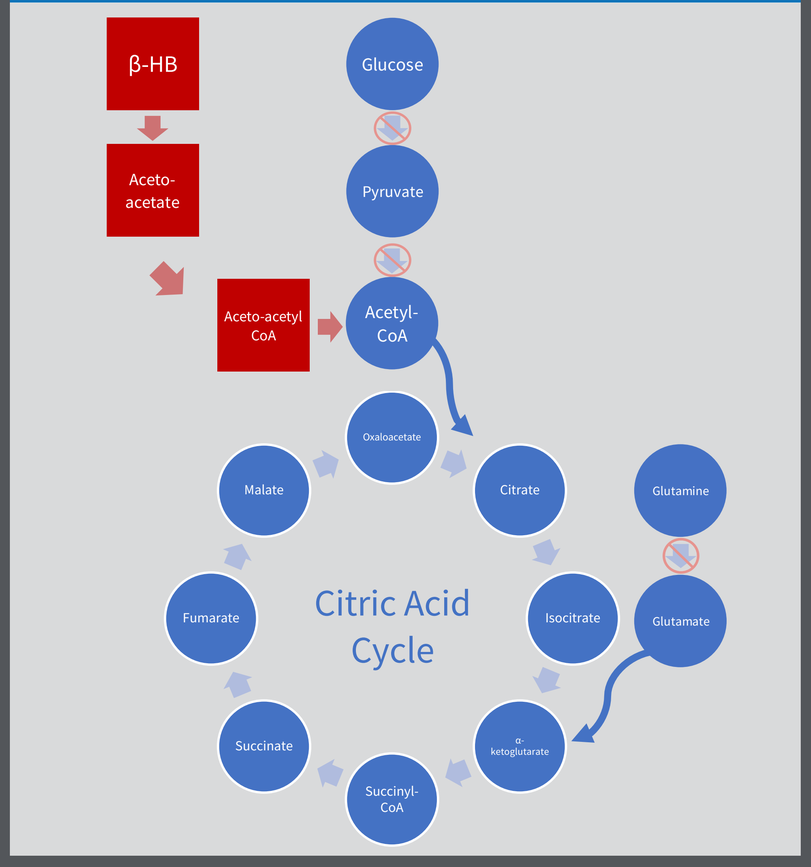

Figure 2: Schematic of the citric acid cycle which provides energy to the cell. Glucose is fed into the cycle by first being broken down to pyruvate and then into Acetyl-CoA. Glutamine can be shuttled into the cycle via glutamate and alpha-ketoglutarate. The red boxes represent the path β-HB takes to enter the cycle. The red circles represent the deprivation of the previous metabolite.

METHODS

The murine neuroectodermal NE-4C cell line was used in all lab based experiments. Cells were sub-cultured in regular media before seeding in the experimental media. 12 different media were prepared for each experiment. Regular glucose (25mM), Low Glucose (5mM), Glucose Free, Glucose free without Pyruvate, Glucose free without l-Glutamine, Glucose free without pyruvate and l-Glutamine.

These were further prepared with and without the addition of beta-hydroxybutyrate (10mM). Using a 96 well plate, three wells were filled with each of the 12 experimental media and were seeded with 5,000 NE-4C cells per well. Four plates were used for each time point studied. Plates were incubated at 37°C, 5% CO2 and fixed using PFA after 48hrs, 72hrs, 96hrs, or 120hrs accordingly.

Cells were viewed under a light microscope and brightfield images were taken. Additionally, cell nuclei were labelled using DAPI and cytoskeleton stained using Actin. This allowed for quantification of cell number and estimation of volume using light microscopy.

A systematic review was performed on the literature using the research question "the effects of exogenous beta-hydroxybutyrate supplementation on cancer cells in vitro".

PRELIMINARY RESULTS

Preliminary results are in line with previous research with growth and survival being impeded by nutrient deprivation.

Supplementation with β-HB did not appear to rescue cells deprived of glutamine or both glutamine and pyruvate. Glutamine deprivation appeared to reduce cell growth more than pyruvate deprivation.

These preliminary findings may be explained by the concept of glutamine addiction proposed in the literature (Rubin, 2019). Further experiments restricting nutrients in the culture media prior to the experiment may help to clarify if the effects observed are due to this phenomenon.

The systematic review of the literature is currently ongoing.

SUMMARY

Supplementation of culture media with β-HB doesn't appear to rescue NE-4C cells from nutrient deprivation in vitro.

This provides a potential explanation for the effects of ketosis on the developing brain observed in animal-based models.

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References:

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