

Effect of Cisplatin, Cetuximab, and BGT226 on SCC25 and SCC61 Squamous Cell Carcinoma Metabolic Rates

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KEYWORDS. Oral cancer, metabolism, glucose, lactate

BRIEF. This study examined the impact of cancer treatments on cellular glucose consumption and lactate secretion.

ABSTRACT. Cancer cells rely on glycolysis and aerobic fermentation for the majority of their energy, rather than oxidative phosphorylation. This phenomenon, known as the Warburg Effect, increases glucose uptake and lactate secretion. The purpose of our research was to determine the impact of three cancer drugs (cisplatin, cetuximab, and BGT226) on the metabolism of two oral squamous cell carcinoma lines (SCC25 and SCC61) by examining the effect of the treatments on cellular glucose consumption and lactate secretion. Cellular metabolism provides an earlier measure of drug efficacy than current methods, which include tumor growth measurements and measurement of glucose metabolism by computed tomography (CT) and positron emission tomography (PET). Treatment with cetuximab yielded no significant changes in either glucose or lactate levels. However, our research successfully determined that cells treated with cisplatin and cells treated with BGT226 exhibited substantially reduced lactate production and reduced glucose consumption when compared with untreated cells, correlating to slowed metabolism.

INTRODUCTION.

Each year, over 11,000 individuals die from head and neck cancers [1]. Despite medical and technological advances, five year survival rates for oral cancers remain at a low 53% [2].

When cells begin to divide and reproduce excessively and abnormally, bypassing the standard enzymatic checkpoints that regulate the reproductive cell cycle, this is known as cancer. While most healthy, normally functioning cells obtain energy by breaking down glucose into ATP through both the processes of glycolysis and oxidative phosphorylation, cancer cells tend to rely primarily on glycolysis, a phenomenon known as the Warburg Effect [3].

There are a number of treatment methods available for cancer. One approach is the use of chemotherapy medication such as cisplatin, which is designed to kill rapidly dividing cells. Another method of treatment is the use of targeted therapies such as cetuximab and BGT226, designed to block the normal cell signaling pathways that promote cell division, thereby impeding the growth of cancer cells and tumors.

Currently, it is difficult to quickly assess the efficacy of certain treatment methods. Currently employed methods include tumor growth measurements, and measurement of glucose metabolism by computed tomography (CT) and positron emission tomography (PET). One potential approach is to determine the effect of a treatment on the metabolic rate of cancer cells, measured directly through glucose and lactate levels. Glucose is one molecule that can be broken down by cells to generate energy (Figure 1). Through the process of glycolysis, it is split into molecules of pyruvate. Due to the Warburg Effect, cancer cells then tend to reduce pyruvate to form lactic acid, or lactate, through fermentation. By examining the concentrations of these two molecules in the growth media of *in vitro* cells, the relative metabolic rates can be established, as glucose is consumed and lactate produced by the metabolic activity of the cells.

Understanding the specific impacts of treatments on the metabolic rates of head and neck cancers, most commonly squamous cell carcinomas, can provide insight into drug efficacy. The effect of three different medications, cisplatin, cetuximab, and BGT226, on the glucose and lactate levels of squamous cell carcinomas has not yet been well defined, and is the focus of this study. This knowl-

edge could be significant in both reinforcing and stimulating further research into cancer treatment efficacy measured through metabolic rates.

MATERIALS AND METHODS.

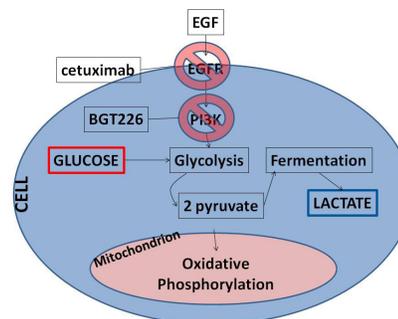


Figure 1. Simplified signaling pathway highlighting the role of glucose, lactate, and cancer treatments.

Materials.

We used SCC25 (overexpressed epidermal growth factor receptor (EGFR)) and SCC61 (overexpressed EGFR and upregulated phosphoinositide-3-kinase (PI3K)) squamous cell carcinoma cell lines. The cells were cultured using DMEM/F12 media supplemented with 10% fetal bovine serum (FBS) and 0.4 $\mu\text{g/ml}$ hydrocortisone.

The treatments included cisplatin, a standard of care chemotherapy drug; cetuximab, a standard of care epidermal growth factor receptor (EGFR) inhibitor; and BGT226, an experimental phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) inhibitor.

To test for glucose and lactate levels in cell media, two commercially available kits were used. For lactate the L-Lactate Assay Kit from EtonBio was utilized, which reacts lactate with lactate dehydrogenase in the presence of NAD^+ to form pyruvate and NADH. The tetrazolium salt INT is then reduced in an NADH coupled reaction to form formazan, which is absorbent at 490nm. Since the amount of lactate present in the media is directly related to the amount of formazan produced, decreased absorbance indicates decreased lactate concentration in the media, reflecting decreased cellular lactate secretion. For glucose analysis the Amplex[®] Red Glucose/Glucose Oxidase Assay Kit-Invitrogen was used, which functions by reacting glucose oxidase with glucose to form hydrogen peroxide and gluconolactone. The hydrogen peroxide then reacts with Amplex[®] Red Reagent to form resorufin, fluorescent at a wavelength of 545nm. Since the amount of glucose present in the media is directly related to the amount of resorufin produced, increased fluorescence indicates increased glucose concentration in the media, reflecting decreased cellular glucose consumption.

Relative absorbance and fluorescence were measured using a Tecan Infinite M1000 PRO plate reader, San Jose, California. Statistical significance between control and treatment groups was determined using a rank sum test with $p < 0.05$ indicating significance.

Methods.

Cells were plated with 10^5 cells per 35-mm dish and incubated for a period of 24 hours. Four aliquots of each cell line were then fed with treatment medias,

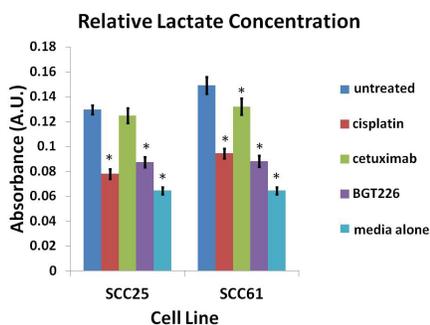
(untreated media as a control, cisplatin-176 μ M, cetuximab-13nM, BGT226-300nM), and then allowed to incubate for another 24 hours. A condition of media with no cells was also included as a negative control. At this point, the assays were conducted on the collected media, diluted with deionized water in a 1:50 ratio for the lactate assay and 1:500 for the glucose assay. The relative absorbance of the lactate assay and the relative fluorescence of the glucose assay were then analyzed using a plate reader.

RESULTS.

Lactate Assay.

Results for the lactate assay were consistent between both the SCC25 and SCC61 cell lines. The plain DMEM/F12 media exhibited the lowest absorbance, signifying the lowest lactate concentration. Media from cells treated with cisplatin and BGT226 exhibited comparable absorbencies significantly greater than that of the media alone, denoting a higher lactate concentration. The cetuximab treated cells also showed decreased absorbance in the SCC61 cell line. The cetuximab treated media exhibited absorbance similar to that of the untreated media for the SCC25 cells.

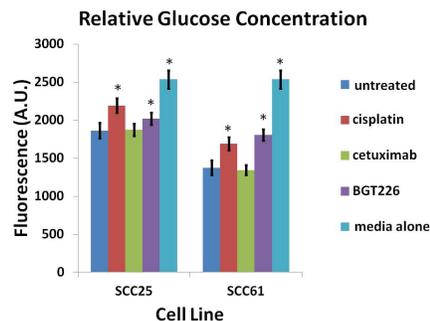
Figure 2. Relative lactate secretion measured by relative absorbance (490nm) of cell media from SCC25 and SCC61 cell lines after treatment with cisplatin, cetuximab, and BGT226, compared to untreated cells and media alone. Greater absorbance corresponds with higher lactate concentration in the media (* $p < 0.05$ compared with untreated cell media). Values represent mean from four samples of each.



Glucose Assay.

Results for the glucose assay were consistent between both the SCC25 and SCC61 cell lines as well. The plain DMEM/F12 media had the greatest fluorescence, and therefore the highest glucose concentration. Treatment with both BGT226 and cisplatin resulted in comparable fluorescence levels below that of the media alone. The media treated with cetuximab exhibited the lowest fluorescence, almost identical to that of the untreated media.

Figure 3. Relative glucose consumption measured by relative fluorescence (545nm) of cell media from SCC25 and SCC61 cell lines after treatment with cisplatin, cetuximab, and BGT226, compared to untreated cells and media alone. Greater fluorescence corresponds with higher glucose concentration in the media (* $p < 0.05$ compared with untreated cell media). Values represent mean from four samples of each.



DISCUSSION.

As expected, media from control cells had increased lactate concentration and decreased glucose concentration as compared with media alone (Figures 2 and 3). Treatment of both SCC25 and SCC61 cell lines with either cisplatin or BGT226 resulted in an overall decrease in glycolytic activity compared with untreated cells, as cells treated with these two drugs consumed less glucose and produced less lactate. Both did consume some glucose and produce some lactate, compared with the media alone. Cisplatin, a standard of care chemotherapy drug, is designed to target and kill rapidly dividing cells. By killing cells, fewer cells remain alive in the media to consume glucose or produce lactate, as demonstrated by our results. BGT226 is designed to inhibit cell growth by blocking the PI3K/mTOR signaling pathways, inhibiting cells' survival and proliferation [4]. This would slow any cellular growth, resulting in higher measured glucose levels and lower lactate concentrations in media. The cetuximab had no significant impact on either glucose or lactate levels. This result is surprising, given that cetuximab is commonly used for cancer treatment. In clinical usage, however, cetuximab is most commonly used in conjunction with radiation therapy and chemotherapy, which may account for its low impact in this setting [5]. Furthermore, *in vivo*, cetuximab works with a body's immune system by triggering the affected cell's destruction by the body [6]. This would also contribute to its apparent inefficacy in affecting the glucose and lactate levels in cell culture.

CONCLUSION.

Altered glycolytic rates can provide insight into the efficacy of cancer treatments. As shown here, the EGFR inhibitor cetuximab had no effect on glycolytic activity in either of the two cell lines, while the standard chemotherapy agent cisplatin and the experimental PI3K/mTOR inhibitor BGT226 decreased glycolysis in two head and neck cancer cell lines after 24 hours of treatment. This research shows that cellular studies of glycolytic activity in treated squamous cell cancer lines can reliably reflect treatment efficacy in early drug development.

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