The Effect of Trastuzumab, Paclitaxel, and XL147 Treatment on Aerobic Glycolysis in Breast Cancer Cells

Re O. McClendon, Joe T. Sharick, Melissa C. Skala

KEYWORDS. Breast cancer, metabolism, glucose, lactate

BRIEF. This study evaluated the impact of select cancer treatments on the metabolic glucose consumption and lactate production of breast cancer cells.

ABSTRACT. During glycolysis, cells consume free glucose to generate energy through substrate level phosphorylation. Lactic acid fermentation may then reduce the products of glycolysis into lactate molecules via electron carriers[1]. This sequence of bioenergetics is often appropriated by cells residing in primarily hypoxic or anoxic conditions; however, many cancer cells adopt this metabolic pathway in order to meet the demands of oncogenic proliferative stress[2].This cancerous tendency towards glucose fermentation is known as the Warburg Effect[3], stemming from the aerobic glycolysis pathway described above. Because of the Warburg effect, glucose and lactate levels may be used as indicators of neoplastic activity. Measuring the glucose and lactate levels in cancer cell media thus serves as an effective tool to examine the metabolic effects of cancer treatments. Using this method of glucose and lactate measurement, the effects of XL147, paclitaxel, and trastuzumab (Herceptin) were examined on the BT474 and the MDA-MB-231 breast cancer cell lines. Preliminary results from this study support the prediction that trastuzumab and paclitaxel treatment significantly ($P<0.05$) reduce the lactate production from the BT474 cells, but not the MDA-MB-231 cells. Current methods of mapping the impact of cancer treatments, such as examining tumor growth and assessing glucose uptake via positron emission tomography (PET) scans, are often slower, more expensive, and potentially less effective. The data produced from this study validates optical imaging assessments of cellular responses to treatment, serving as a calibration tool for computerized cellular metabolic analyses.

INTRODUCTION.

Cancer occurs when cells begin to uncontrollably and abnormally proliferate. It is classified as a group of diseases and is further differentiated by the cells it affects. In a healthy state, cells regulate the cell cycle and reduce the frequency of mutation through a series of checkpoints. Despite this phenomenon, mutations still arise and give way to up-regulated proliferation genes (oncogenes) or down-regulated tumor suppressor genes. Namely, breast cancer occurs when the cellular qualities of uncontrollable or abnormal cell growth (malignant neoplasms) are observed in the breast tissues.

2014 statistics posit that about 12% of women will develop a form of invasive breast cancer over their lifetime leading to an estimated annual death toll of 40,000 [4]. Breast cancer incidence and mortality rates have been on a steady decline since the year 2000[7]; however, treatment methods could be improved. Frequently, chemotherapy drugs such as paclitaxel are used to fight off neoplastic cells. Paclitaxel works as a mitotic inhibitor involved in stabilizing microtubules and interfering with their breakdown [5]. More recently, antibody therapies such as Trastuzumab have been used to treat certain forms of breast cancer. These work by specifically interfering with an important cell signaling receptor, HER-2, in order to block the pathway and prevent proliferative growth [6]. Targeted pathway inhibition is another modern technique for treating breast cancer. An example of this is the XL147 small molecule inhibitor which targets the PI3K pathway (commonly associated with tumorigenesis), and has been shown to contribute to tumor regression [7].

One of the main impediments to treating breast cancer is the current lack of understanding of the effects of particular treatments. Current methods of mapping the impact of cancer treatments, such as examining tumor growth and analyzing via positron emission tomography (PET) scans, are often slow, expensive, and potentially ineffective.

Cell metabolism pathways can be analyzed to generate reliable and timely data regarding the efficacy of cancer treatments. Cells consume glucose to generate energy through substrate level phosphorylation. Products of glycolysis are then reduced into lactate molecules via electron carriers[1]. Non-cancerous cells residing in primarily hypoxic or anoxic conditions commonly use this metabolic pathway; however, the demands of proliferative stress cause many cancer cells to adopt this sequence[2]. Glucose and lactate may be used as indicators of neoplastic activity because of their link to the cancer cell metabolism. Measuring the metabolism of the cells via the glucose and lactate levels in the media can be used to examine the efficacy of certain cancer therapeutics.

Because the BT474 cell line is HER2+, the trastuzumab treatment was expected to significantly reduce the metabolism of these cells; however, due to the lack of HER2 expression in the MDA-MB-231 cell lines, the trastuzumab was expected to have little to no significant effect on them. The XL147 and paclitaxel may affect the cells (statistically) similarly because of their lack of reliance on the presence of the HER2 protein. This may lead to significantly lower levels of lactate production and glucose uptake in the MDA-MD-231 cells, specifically, compared to when treated with trastuzumab.

MATERIALS AND METHODS.

Cell Culturing and Treatment

HER-2 positive (expressive) BT474, and HER-2 negative (non-expressive) MDA-MB-231, cells were cultured in separate rows of a 96 well plate. After 24 hours of incubation at 37°C and 5% CO2, cell plates were taken out and treated with paclitaxel (0.5μM concentration), XL147 (25nM concentration), and Trastuzumab (25μM concentration). Cells were set in 5 wells of each cell type per treatment type. A row of 5 wells for both cells were left untreated to serve as the control. Cells were then re-incubated for an additional 24 hours.

Examining Cellular Metabolism

After 24 hours, the 96 well plate was removed from the incubator and another 96 well plate was prepared. The media from each of the wells were transferred into the new 96 well plate. The media solution was examined for L-lactate and D-glucose following materials and methods suggested by the Eton Bioscience kit (cat no. 800.756.1630) and the (LIFE) Molecular Probes Amplex Red and Glucose Oxidase kit (cat no. 541.465.8300).

Glucose oxidase + D-glucose → H_2O_2 + D-gluconolactone →(added Red Reagent) + H_2O_2 → resorufin (absorbent at 570 nm)

Lactate + NAD^+ + lactate dehydrogenase → pyruvate + NADH → Terazolium salt INT (added) reduced in NADH coupled enzyme reaction → formazan (absorbent at 490 nm)

The assays were conducted using a dilution ratio of 1:50 for the lactate assay and 1:500 for the glucose assay. Plain media was also added and diluted appropriately between each of the test types to provide a comparison. After addition of the tetrazolium salt, the lactate assay was incubated for 30 minutes. Both plates were then evaluated for absorbance data using a plate reader. The plate reader evaluated the relative levels of glucose and lactate in the media based on the absorbance of the resorufin and the formazan in each well respectively, and statistics of the results were taken using a two-tailed T-test.

RESULTS.

Glucose Assay

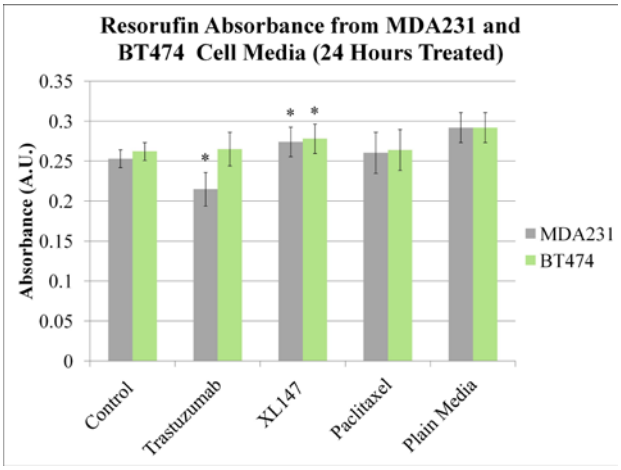

Figure 1. The relative absorbance from the resorufin within the media denotes the presence of glucose remaining after uptake from the breast cancer cells. Lower levels of glucose indicate higher levels of cell viability and metabolic activity and vice versa *=P<0.05 vs control. n=20 reads for each. Statistics were taken using a two-tailed T-test.

Lactate Assay

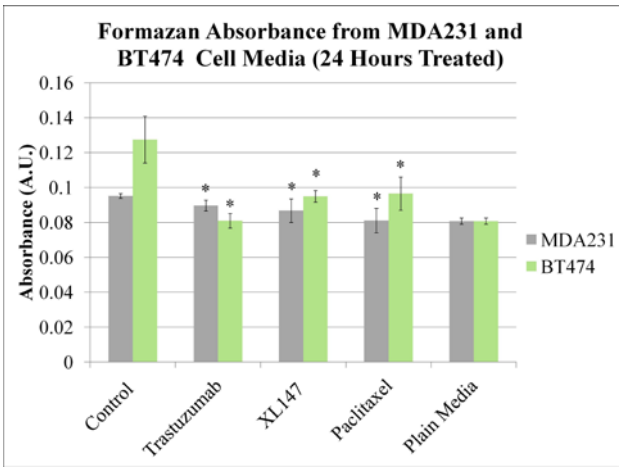

Figure 2. The relative absorbance from the formazan within the media denotes the presence of lactate produced by the breast cancer cell metabolism within the media. Higher levels of lactate indicate higher levels of cell viability and metabolic activity and vice versa *=P<0.05 vs control. n=20 reads for each. Statistics were taken using a two-tailed T-test.

DISCUSSION.

Paclitaxel did not yield a significant change in glucose uptake in 24 hours when compared to the untreated cells for both cell lines. This is perhaps because the drug requires more time to have an effect on glucose uptake.

A significant decrease in glucose uptake was observed after 24 hours of XL147 treatment, supporting the prediction that interfering with cell metabolism by inhibiting the PI3K pathway decreases glucose uptake.

Trastuzumab treatment for 24 hours did not cause a significant change in glucose uptake in BT474 cells, which does not support our prediction that this treatment would cause a decrease in glucose uptake due to the HER2+ status of these cells. The same treatment in MDA-MB-231 cells resulted in an increase in glucose uptake, which contradicts our prediction that trastuzumab would have no effect on these cells based on their lack of HER2 receptors. Future tests may analyze the effects of trastuzumab on other HER2- cells to see if the glucose and lactate results show a similar pattern to the MDA-MB-231 cells in this experiment.

All treated cell types produced less lactate than untreated cell types, but the BT474 cells when treated with trastuzumab showed the lowest average lactate production. Results suggest that these breast cancer drugs affect metabolism by inhibiting the ability to produce lactate. Results also show that the metabolic impacts of each drug (besides trastuzumab) on the cells are relatively similar in terms of glucose uptake and lactate production. This mostly supports our prediction, except that we did not expect trastuzumab to have an effect on lactate production in MDA-MB-231 cells which lack HER2 receptors. All treated groups still showed a small amount of background lactate production.

Glucose and lactate analysis is a time efficient method of determining the level of efficacy of a cancer treatment on cellular metabolism and may be used to precede, supplement, or replace current popular methods. Implications of these methods may be beneficial in clinical trials and future work utilizing these analyses may serve to alter the perceived impact of widely used or experimental cancer treatments.

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REFERENCES.

1. "Glycolysis, Krebs Cycle, and Other Energy-Releasing Pathways." *Glycolysis*. N.p., n.d. Web. 15 June 2014.
2. Vander Heiden MG1, Cantley LC, Thompson CB. "Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation." *National Center for Biotechnology Information*. U.S. National Library of Medicine, Apr.-May 2009. Web. 17 June 2014.
3. "Aerobic Glycolysis and the Warburg Effect." *Sigma-Aldrich*. N.p., n.d. Web. 17 June 2014.
4. "U.S. Breast Cancer Statistics." Breastcancer.org. N.p., n.d. Web. 17 Nov. 2014.
5. "Taxol (paclitaxel) Drug Information: Description, User Reviews, Drug Side Effects, Interactions - Prescribing Information at RxList." *RxList*. N.p., n.d. Web. 17 June 2014.
6. "Herceptin (trastuzumab) Drug Information: Description, User Reviews, Drug Side Effects, Interactions - Prescribing Information at RxList." *RxList*. N.p., n.d. Web. 17 June 2014.
7. "Definition of Class 1 PI3K Family Inhibitor XL147 - National Cancer Institute Drug Dictionary." *National Cancer Institute*. N.p., n.d. Web. 17 June 2014.

Re. O. McClendon is a student at Hume Fogg Academic Magnet High School in Nashville, Tennessee. He participated in the Research Experience for High School Students (REHSS) program at Vanderbilt University.