Modeling the Effects of Small Molecule Therapeutics on Glycolysis and Lactate Flux

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Abstract

Chinese Hamster Ovary (CHO) cells are widely used in the industrial production of commercial therapeutics. One key aspect to the productivity of these cells is their high rate of glucose consumption. The high rate of glucose consumption is paired with a high output of lactate which can lead to negative culture performance. The rate of glucose consumption and lactate production can be modulated by a number of chemicals, some of which are being explored as therapeutic drugs, which affect the activities of the enzymes involved in glucose metabolism. This research aimed to evaluate the effects caused by small molecule therapeutics on CHO cells' metabolism using a mathematic model of glucose metabolism. To model the therapeutics, established kinetic information for these therapeutics was implemented into a metabolic model. Then different concentrations of therapeutics were explored to assess their effects on metabolism. In addition, combinations of therapeutics were examined to study the effects of more drastic changes to metabolism. These therapeutics showed a large impact to the bistability of glucose metabolism as well as the lactate flux. These outcomes were important due to the potential to increase the productivity of CHO cells for industrial use as well as decreasing cell death. Thus, these therapeutics could be used to reduce lactate production in cells allowing for higher productivity.

1. Introduction

The Warburg effect is the phenomenon describing how cancer cells consume glucose at high rates while also outputting lactate at high rates [1]. The Warburg effect is commonly seen in cell culture due to their proliferative nature. Chinese Hamster Ovary (CHO) cells are a cell line commonly used for industrial cell cultures to create products, particularly commercial therapeutic proteins [2]. These CHO cells, even when in environments with excess oxygen, can still display high glucose flux and high lactate flux. The high lactate output can have negative implications on productivity, and cell viability [3]. In batch cultures these cells produce high amounts of lactate which can cause high osmolarity over time due to the necessity to add base to counteract the acidity caused by lactate.

This issue is not new, and different methods to address high lactate output have been used in the past. One method which has shown success was the substitution of glucose for other sugars after the growth phase. The sugars that were promising included galactose, fructose and mannose [4]. These sugars are useful due to their lower binding affinity to transporters, which causes slower uptake. Modified feeding of these cells therefore proves to be a useful method in the controlling of lactate flux [5].

While some genetic engineering in CHO cells has proven to be successful in the past, other changes have been detrimental. An example of this is efforts made to knock out lactate dehydrogenenase (LDH), the enzyme responsible for the conversion of pyruvate to lactate. It should be noted that these efforts have caused cell death indicating that likely knocking down LDH or other enzymes in glucose metabolism would prove to be more effective at lowering flux without causing cell death [6].

When looking at the ways that glucose metabolism has been modified, small molecule therapeutics show up repeatedly in use for treatments. In particular, small molecule therapeutics have been investigated for use in the treatment of many different cancers. Cancer cells, like CHO cells are rapidly dividing, and also tend to be at a high flux state producing lactate. Therefore, small molecule therapeutics were selected to be used in smaller concentrations then when used in cancer treatment, as that was used to induce cell death.

The goal of this research was to model the behaviors of glucose metabolism in the presence of small molecule therapeutics. The therapeutics chosen were inhibitors of LDH, glucose transporter 1 (GLUT1), pyruvate dehydrogenase kinase isoform 2 (PDK2), phosphofructokinase 1 (PFK1), monocarboxylate transporter 1 (MCT1), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB), hexokinase (HK) and an activator of pyruvate kinase muscle isozyme 2 (PKM2). These enzymes were chosen to be inhibited and activated due to their importance to glucose metabolism and as they were frequently targeted in gene expression experiments seeking to lower glucose metabolism.

2. Methods

2.1 Inhibitor and Activator Kinetics

To identify target enzymes for inhibition and activation, multiple past publications were studied. These targets included LDH, GLUT1, PKM2, PDK2, MCT1, PFK1, PFKFB, and PKM2. For majority of the inhibitors, Michaelis-Menten kinetics for competitive inhibition were used [7]. The exception to this was PDK2 inhibitor AZD7545 which could not be directly added into the model due to the fact that PDK2 is not explicitly a part of the model. PDK2 is excluded from the model because it performs a post translation protein modification which is beyond the scope of the model. Instead, the impact of inhibiting PDK2 was approximated by changing the activity of PDHC, which allows an impact on PDK2 without knowing precise concentrations needed for the observed effects. The only activator being considered was TEPP-46, and activator of PKM2. TEPP-46 was added into the model using a multiplier which is currently a part of the model that determines the activity level of PKM2.

Enzyme	**Therapeutic**	**K_i**	**Mechanism**	**Citation**
GLUT1	WZB117	6.2 μM	Competitive	[8]
HK	2-Deoxglucose	0.3 mM	Competitive	[9]
PFKFB	YZ9	0.094 μM	Competitive	[10]
LDHA	FX11	8 μM	Competitive	[11]
MCT1	AZD3965	1.6 nM	Competitive	[12]
PDK2	AZD7545	IC50 = 87 μM	Competitive	[13]
PFK-1	Palmitoyl-CoA	1 μM	Competitive	[14]
PKM2	TEPP-46	0.1 nM	Activator	[15]

2.2 Gathering Therapeutic Data

After all therapeutics were incorporated into the model, each one was tested individually over a range of concentrations within which metabolism was sensitive to changes using the MATLAB model and the Minnesota Supercomputing Institute. The concentrations over which the most drastic changes in behavior where focused on and tested in combination for a few inhibitors. Units used when measuring the amount of inhibitor added are in mM unless otherwise stated.

2.3 Testing Conditions

All of the concentrations of inhibitor were tested at steady state using a kinetic metabolic model developed previously [16]. Steady state is used because the conditions that CHO cells are held in are kept close to steady state so it is a good approximation. All inhibitors and activators were tested independently of each other except for in a select few tests seeking the effects of combinations. In the simulations with changing inhibitor concentrations, the amount of lactate is

held constant at 6mM so that bistability can be observed. This concentration was also chosen as it is a reasonable concentration that occurs in CHO cell cultures.

3. Results and Discussion

3.1 Inhibitor and Activator Simulation

For each therapeutic selected concentrations were tested until the apparent maximum effect was achieved. The graphs representing each individual run of the model are shown in Figure 1. In addition, the inhibitors impact over a range of inhibitor concentrations with a constant lactate concentration are also shown.

The first inhibitor shown in Figure 1 is WZB117, a competitive inhibitor of GLUT1. The graph shows the deterioration of the bistability specifically a slant towards the low concentrations of glucose. This behavior could be explained that as glucose levels become lower the inhibitor prevents most glucose from entering the cell therefore extremely lowering glucose metabolism. It appears as though the high flux state has been completely eliminated with this concentration of WZB117.

The next inhibitor displayed is AZD7545, a competitive inhibitor of PDK2. The bistability is heavily affected by the addition of 0.06 mM of AZD7545. Not only is the overall flux of glycolysis lowered but a slant also occurs with the graph slanting downward towards high lactate concentrations.

TEPP-46 is an activator of PKM2 and is shown in Figure 1 at 5 mM. At this concentration the overall flux of glycolysis is increased, and the rate of lactate flux is increased as well. This effect on its own does not accomplish lowering the lactate flux but could be useful if paired with an inhibitor to get to a high flux state which produces less lactate. This combination is expanded upon in the combinations section below.

Palmitoyl-CoA is an inhibitor of PFK-1 and is shown at a concentration of 1mM in Figure 1. Its affects were similar to that of AZD7545 with a lower overall glycolysis flux as well as lower lactate flux.

Past research on the knock down of LDH has shown a lowering in the overall flux of glucose metabolism [17]. The competitive inhibitor FX11 did this, and at a concentration of 50mM it can be seen on Figure 1 that while bistability remains, the former high flux state has been cut nearly in half. In addition, the rate of lactate export is also reduced in a proportional manner. The impact of a range of FX11 concentrations can be seen below in Figure 2. Again, an increase in the concentration of FX11 causes a lowering in glycolysis flux. The concentration of lactate is held constant for the range of inhibitor concentrations at 6 mM.

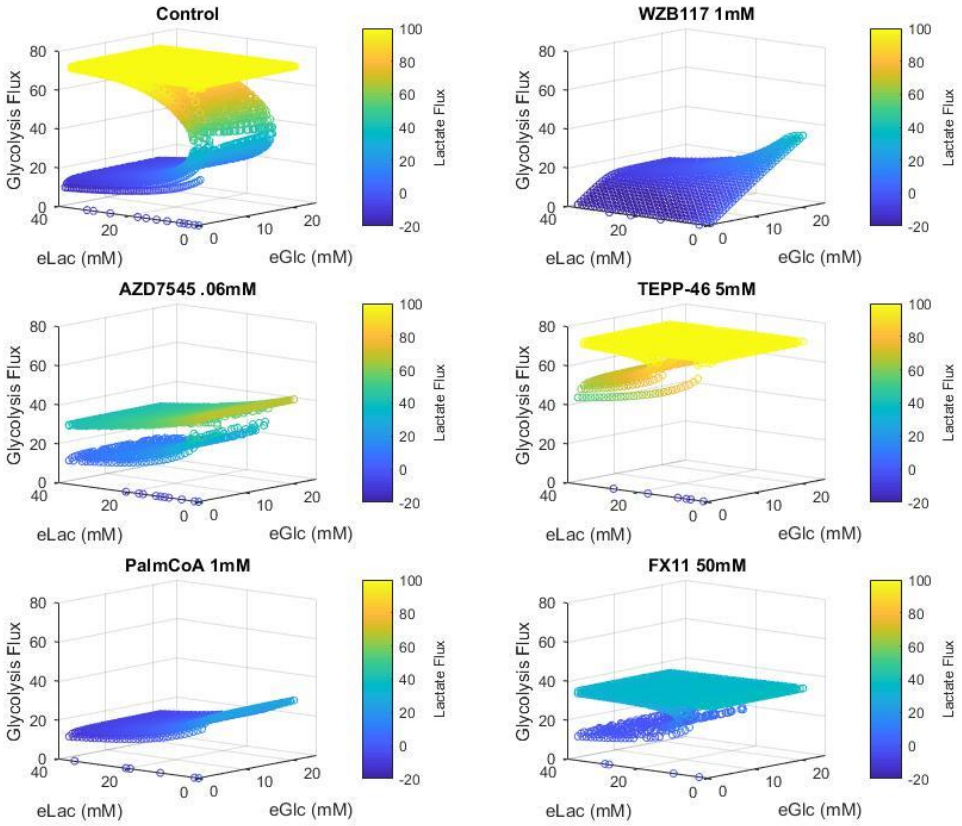

Figure 1 Displays inhibitors and the activator at different concentrations. Included is a negative control with no inhibitors or activators. The plots shown are plotted with extracellular lactate vs extracellular glucose vs glycolysis flux.

3.2 Effect of Inhibitors on Glucose Flux

In addition to testing at a constant inhibitor value with varying lactate and glucose, we also tested across a range of inhibitor values while holding lactate constant. This was done for a few of the inhibitors across their most sensitive concentrations. The therapeutics shown this way are FX11, YZ9, and TEPP-46.

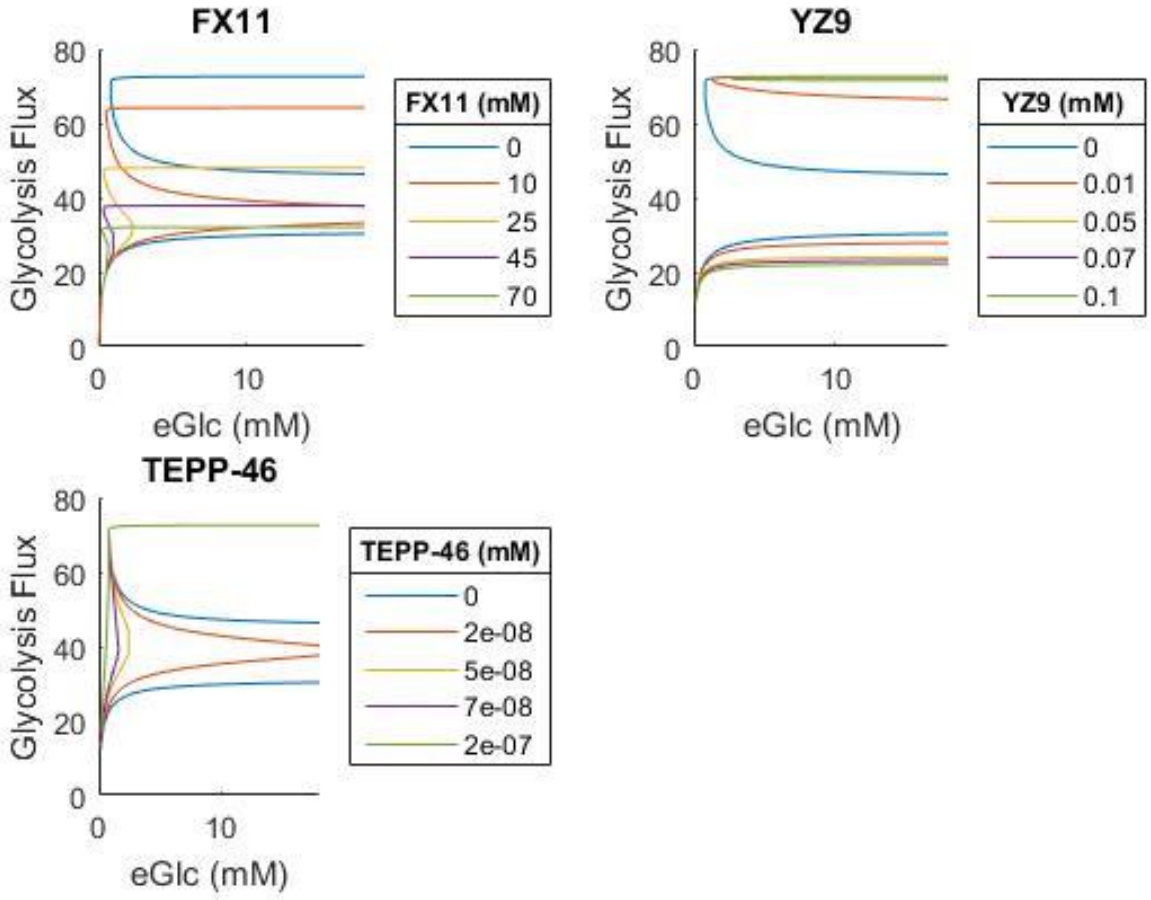

Figure 2 shows the graphs of different inhibitor concentrations for LDH inhibitor FX11, PFKFB inhibitor YZ9 and PKM2 inhibitor TEPP-46

In Figure 2 shows four concentrations of FX11 and its impact on glycolysis flux. As the lactate inhibitor concentration is increased the overall flux goes down lowering the high flux state. In addition, the unstable steady state appears to get steeper and this follows as the high flux state is approaching the low flux state. This demonstrates the behavior of FX11 showing how it lowers the flux of glycolysis.

The behavior of PFKFB inhibitor is challenging to observe in 3 dimensions as its changes maintain the overall shape but seem to lengthen the unstable steady state. Figure 2 shows this behavior across a range of concentrations.

The activation of PKM2 in Figure 2 shows an increase in the overall flux of the reaction with the low flux state moving up and the unstable steady state becoming narrower. While PKM2 activation may not be desirable on its own for lowering lactate flux, it is possible that this behavior could be used in tandem with another therapeutic to gain a more desirable outcome.

3.3 Combinations and Future Paths

After modeling all the selected inhibitors and activator on their own, a few combinations of therapeutics were tested. In these combinations, we focused on not only lowering lactate flux but also trying to maintain a reasonably high glycolysis flux. This would be ideal because then the cells would still be productive and would create less lactate. To achieve this balance of affects, we looked to TEPP-46 the PKM2 activator, and FX11 the LDH inhibitor. After combining them at selected concentrations an outcome is shown in Figure 3.

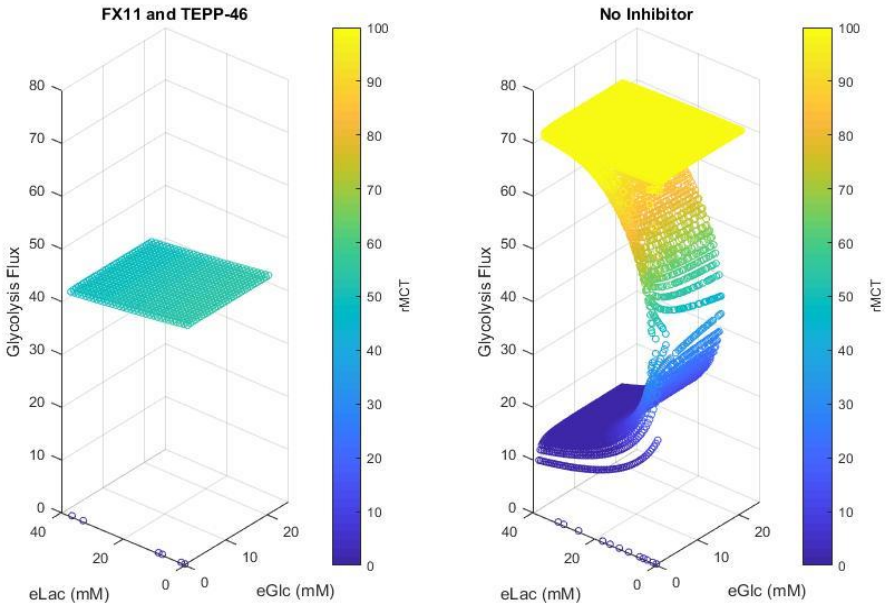

Figure 3 Displays the combination of 30 mM of FX11 and 10 mM of TEPP-46 along with a graph with no inhibitors or activators for comparison.

In Figure 3, there is a single steady state that occurs at around half the rate of the previous high glycolysis flux. This state also produces less lactate, a desirable trait as previously explained. The combinations of said therapeutics would be interesting to examine further possibly with an optimization to more precisely tune the concentrations for desirable behavior could be carried out on the new model, however this is beyond the scope of this research.

Overall this research was able to incorporate inhibitors and activators into a glucose metabolism model as to show their effects over different concentrations. This model can be used for future use to find optimized concentrations of inhibitors and activators to produce the desired impact on CHO cells. Even in this study in which the concentrations selected were not optimized, potent and promising effects were observed on the lowering of lactate flux and glycolysis flux by inhibitors and activators. These therapeutics can be used in cell culture and in the study of cancer metabolism in the future and this model provides a toolbox to probe the potential effects.

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