METFORMIN MODULATES GLUCOSE UPTAKE AND TRANSPORT IN CACO-2 CELL MONOLAYERS
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ABSTRACT

Metformin is a longstanding anti-diabetic drug with a poorly understood mechanism of action in the intestine. Using the radiolabeled glucose surrogate \[^{14}\text{C}\text{2-deoxy-D-glucose}\] (2DG) that does not undergo metabolism, this study investigates the effect of metformin on glucose transporters that mediate the intestinal absorption and transport of glucose in Caco-2 cell monolayers, a well-established model of human intestinal epithelia. Real-Time Polymerase Chain Reaction (RT-PCR) confirmed an adequate presence of the intestinal glucose transporters GLUT1, GLUT2, and GLUT3 in Caco-2 cells compared to human small intestinal tissue. \[^{14}\text{C}\text{2DG}\] uptake and transport was assessed in Caco-2 cells (grown in Transwell™ plates) by liquid scintillations spectrometry. Results showed that metformin may increase glucose absorption and glucose apical to basolateral transport, but further studies are needed to confirm these effects at lower concentrations of 2DG. The concentration of 100mM used in this study saturated transporters and maximized its effects on GLUT2 translocation, rendering the effect of metformin inappreciable. Data from this study could be used to develop other hypotheses on the effect of metformin on glucose trafficking and metabolism.

INTRODUCTION

Metformin and diabetes

Metformin is an anti-diabetic drug widely used to treat type 2 diabetes mellitus (T2DM). T2DM is a chronic disease characterized by hyperglycemia and glucose intolerance due to insulin resistance, and is managed with a combination of diet, exercise, and medication. Metformin combats the condition through its activity in the liver, skeletal muscles, and intestine. Studies show that metformin decreases
hepatic glucose production and intestinal glucose transport, and increases intestinal glucose utilization,
all of which contribute to blood glucose-lowering effects of metformin in diabetics\textsuperscript{1-3}. Little is known of
the mechanism of action of metformin in the intestine, which is the focus of this study. Clinically,
metformin is understood to decrease glucose intestinal absorption and increase glucose utilization, the
latter of which is thought to contribute to metformin-induced intestinal lactic acidosis. However,
literature is conflicting whether metformin decreases the intestinal glucose absorption\textsuperscript{2,5}.

\textit{Cell physiology}

Studies show that the highest metformin accumulation occurs in the intestine following oral
administration\textsuperscript{4}, which raises the question whether high metformin intestinal concentrations modulate
cell function. Since intestinal glucose absorption occurs from the apical (AP) and basolateral (BL)
membranes, it warrants investigating whether high metformin concentrations affect intestinal glucose
absorption and glucose transporter (GLUT) translocation to membranes of intestinal cells. Little is known
about how metformin regulates GLUT transporters, although some evidence shows that metformin
promotes their translocation to the AP membrane\textsuperscript{5}. Under normal physiology, GLUT2 translocates to the
AP membrane of intestinal epithelia in response to a meal, while the sodium-glucose transporter (SGLT)
handles glucose transport at low concentrations\textsuperscript{6}. A previous study suggests an increase in GLUT2
translocation to the AP membrane of mouse jejunal tissue following metformin treatment due to the
activation of the cellular energy sensor, adenosine monophosphate (AMP)-activated protein kinase
(Figure 1)\textsuperscript{5}. This study aims to elucidate the effect of metformin on glucose uptake and transport via
GLUT transporter translocation to AP and BL membranes in Caco-2 cell monolayers.
Figure 1. Intestinal Glucose Absorption via Glucose Transporters

Intestinal glucose transporters, primarily GLUT 2, translocate to the apical membrane as a result of the AMPK activation by metformin. The focus of this study is the overall effect of glucose transport due to these transporter changes.

Hypothesis

We hypothesize that metformin decreases systemic glucose by decreasing intestinal absorption (apical to basolateral transport) in the human intestinal epithelium.
Specific Aims

In the cellular model of intestinal epithelium, i.e. Caco-2 cell monolayers, determine the effect of metformin on (1) AP to BL transport of metabolically stable 2DG, (2) AP and BL 2DG uptake, and (3) on GLUT transporter translocation to explain its effect on glucose absorption and uptake.

METHODS

Caco-2 Cell Culture

The Caco-2 cell model, being derived from human colon carcinoma, was chosen for its ability to form polarized cells and tight junctions with AP and BL membranes. Caco-2 cells were cultured at 37°C in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and 1% antibiotic-antimycotic in 5% CO₂ and 90% relative humidity. Cells were seeded at 120,000 cells/cm² on 12-well Transwell™ plates and cultured for 21-28 days prior to transport experiments.

RT-PCR

Total RNA was isolated from Caco-2 cells and reverse transcribed using a Superscript® III First-strand synthesis kit (Invitrogen). Human small intestinal total RNA was purchased from Zyagen. RT-PCR was conducted using Taqman assays (Applied Biosystems) to determine relative expression of GLUT1-3. All mRNA levels were normalized to 18s rRNA.

14C 2DG Uptake

Caco-2 cell monolayers were preincubated for 60 minutes with transport buffer (Hank’s Balanced Salt Solution with 10 mM HEPES) with or without 5 mM metformin. To initiate uptake, a solution of glucose and 2DG (10mM) was added to the donor compartment. After 10 minutes, cells were washed with ice
cold transport buffer three times and then lysed with 0.1 N NaOH/0.1% SDS. 2DG was quantified by liquid scintillation spectrometry.

2DG Transport

Caco-2 cell monolayers were preincubated for 60 minutes with transport buffer with or without 10 mM metformin. To initiate transport, a solution of glucose and 2DG (100mM) was added to the AP compartment. At the designated timepoints (15, 30, 60, 90 minutes), 10µM samples were collected from the BL compartment, and 2DG was quantified by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

RT-PCR results from Caco-2 cells and a human intestine tissue sample found adequate presence of transporters GLUT 1, 2, and 3 in the Caco-2 cell model. This indicates that Caco-2 cell monolayers are an appropriate model to study glucose transport in the human intestine (Figure 2).
Figure 2. RT-PCR results indicate the presence of GLUT 2 and confirm that Caco-2 cell monolayers are a fair model for this study.

Results of the uptake experiments show non-statistically significant trends towards decreased AP uptake of 2DG and its increased BL uptake (Figure 3). While this result was unexpected, it may be indicative of the 10mM AP glucose concentration being below the threshold to recruit GLUT2; therefore observed transport was primarily mediated by SGLT. The decrease in 2DG transport is explained by the deactivation of SGLT by metformin through its known ability to deactivate energy-dependent processes through AMPK activation\(^5\). At glucose concentrations higher than the chosen 10mM, uptake may in fact have been increased and reflects the resultant GLUT2 recruitment. Thus, these results if statistically significant, would be consistent with the current understanding of the activity of metformin.
Figure 3. Metformin (10mM) treatment was associated with a trend towards decreased AP uptake of 2DG and its increased BL uptake. Trends favoring decreased AP glucose uptake may be reflective of the inhibitory effect of metformin on energy-dependent transport of 2DG concentrations, but not high enough to recruit GLUT 2 transporters. N=3

Interestingly, results of the transport experiments suggest non-statistically significant trends towards increased AP to BL glucose transport with the addition of metformin (Figure 4). While this contradicts the hypothesis of decreased glucose absorption, this mechanism, if statistically significant, may in fact aid intestinal glucose absorption for metabolism.
Figure 4. A trend towards enhanced 2DG transport was observed with metformin (10mM) treatment using 100 mM Glucose/2DG solution in the AP compartment, and varying concentrations of Glucose/2DG solutions in the BL compartments.

N=3

There could be a few possible reasons for the lack of statistical significance in trends of decreased AP and BL uptake of 2DG and increased AP to BL transport of 2DG. Firstly, transporters may have been saturated at the high glucose concentration (100mM) used in these experiments. To investigate this, concentration-dependent transport of 2DG was measured and transport flux results were found to decrease with higher glucose concentrations, confirming that transporter saturation occurs between concentrations of 25mM and 50mM (Figure 5).
Figure 5. Concentration-dependent transport of 2DG suggests transporter saturation. Transport flux ($P_{\text{app}}$) decreases with higher glucose concentrations, suggesting transporters reached $V_{\text{max}}$ between 12.5mM and 25mM glucose. $N=1$

Secondly, a high glucose concentration may be masking the effect of metformin on GLUT2 translocation, as glucose itself is known to activate GLUT2. It is likely that this activation leaves little room for metformin to elicit its effect. Therefore, the lack of statistical significance may be due to the high 2DG concentration that both saturated transporters and usurped the metformin’s translocation effect on GLUT2.
Finally, it is important to note that the 2DG glucose surrogate used in this study is a metabolically stable form of glucose, and therefore limits the study from measuring effects of metformin on glucose metabolism. In light of this perspective, the results may have shown an increase in 2DG uptake into intestinal tissue, observed from both increased basolateral uptake and increased apical to basolateral overall transport, as a possible mechanism to increase glucose utilization. Because of the nature of the 2DG glucose surrogate, it was not possible to measure this mechanism by the technique used in this study. The increase in glucose uptake may have been a mechanism for increased utilization, which was unfeasible due to the nonmetabolizable property of 2DG and therefore resulted in the increased transport through the basolateral membrane.

The results of this study may be used to develop other hypotheses on the effect of metformin on glucose trafficking and metabolism. Further studies are needed to confirm the effects of metformin on glucose absorption in the intestine, using lower glucose concentrations that could allow more accurate measurements.

**CONCLUSIONS**

Caco-2 cells have a fair representation of GLUT1, GLUT2, and GLUT3 compared to human intestinal tissue. Metformin may increase glucose absorption and glucose AP to BL transport, but further studies are needed to confirm these effects at lower concentrations of 2DG. The chosen concentration of 100mM of 2DG both saturated transporters and overwhelmed the activity of GLUT2 translocation. The inability to measure glucose metabolism by using the unmetabolizable form of glucose, namely 2DG, is a limitation of this study. Future directions may include a model that incorporates glucose metabolism into intestinal absorption and transport. Other techniques such as confocal microscopy and western
blotting could be utilized to elucidate the mechanistic details of transporter trafficking. Although metformin has been in use for several decades, more information on its mechanism of action in the intestine can be clinically significant in choosing optimal drug combinations, predicting drug interactions, and predicting and understanding the possibility of metformin-induced lactic acidosis.

REFERENCES


