Mechanism of Curcumin inhibition of Glucose Transport in Adipocytes

By: Armond Minor, Patsy Heisler

Faculty Sponsors: Dr. Allan Green

ABSTRACT

In this project we are investigating the mechanism by which the compound Curcumin inhibits the transport of glucose across the cell membrane. Curcumin is a compound found in Turmeric, one of the spices used to flavor curry in Northeastern cooking. Curcumin is the major yellow colored component of Turmeric, and has been used in traditional Indian medicine to improve digestion and circulation. Studies have suggested that it may have potential as a treatment for, or in prevention of Type 2 Diabetes. Curcumin has been shown to inhibit the uptake of glucose across the cell membrane via the GLUT4 transporter, an insulin controlled transporter. The mechanism by which curcumin has these effects is not well understood. In this study we investigate what type of inhibitor curcumin is for the GLUT4 transporter. We use adipose tissue from Sprague-Dawley rats to investigate the inhibitory effects of curcumin by varying the concentration of substrate (glucose) and measuring the uptake of glucose by the cells after incubation with curcumin.

INTRODUCTION

In previous studies, it has been shown that curcumin is effective in lowering blood glucose level in rats (Weisberg et al. 2008) and also to have a positive affect on blood glucose and insulin sensitivity in mice (Mahade et al. 2005). Curcumin has been shown to inhibit the uptake of glucose across the cell membrane via the GLUT4 transporter, which is an insulin controlled transporter. How curcumin has these effects is not well understood. One of the specific areas that has not been thoroughly investigated is what type of inhibitor curcumin is for the GLUT4 transporter.

For many years, the incidence of Type 2 Diabetes has been increasing, and there have been large numbers of studies done to find ways to both prevent and treat this condition. The condition is characterized by an insulin insensitivity developed due to diets high in refined carbohydrates. One result of this insensitivity is that the GLUT4 transporters, which are found on vesicles inside cells until they migrate to the cell surface due to the action of the hormone insulin, do not remove glucose from the blood effectively, leading to elevated concentrations of glucose circulating in the blood. The GLUT4 transporter is the main protein responsible for the uptake of glucose in cells so that it can be used by those cells to provide energy through oxidation or for storage for later use (Nelson & Cox 2005).

Since earlier studies suggested that there may be some positive effects of curcumin on treating type 2 diabetes, there have been recent investigations of the effects of curcumin on the uptake of glucose by cells and its interaction with insulin signaling. An experiment, recently conducted by Dr. Allan Green, has shown that curcumin’s action is likely not through the insulin signaling pathway because if this were the case there would be a time delay in its effects that was not observed (Green, A., et al. 2013). Since it doesn’t affect the movement of the GLUT4 transporters from the interior of the cells to the surface membrane it has been proposed that its action is through direct inhibition of the GLUT4 transporter. As inhibitors work in various ways further investigation as to the mechanism of this inhibition are needed to characterize the action of curcumin on the transport of glucose across cell membranes. In order to determine the mechanism by which curcumin inhibits glucose transport across the GLUT4 transporter we will design an experiment to find out what type of inhibitor curcumin is. Inhibitors fall into four categories: competitive, noncompetitive, uncompetitive, and mixed. Using adipose tissue from rats we investigate the inhibitory effects of curcumin by varying the concentration of substrate (glucose) and measuring the uptake of glucose by the cells after incubation with curcumin. Determination of inhibitor type is done through analyzing the data on the uptake of glucose by the cells and applying the principles of Michaelis-Menten kinetics to the data. This is done by plotting the results using a double reciprocal graph of substrate concentration vs. velocity (Nelson & Cox 2005). In this way we are able to determine the type of inhibitor curcumin acts as in its inhibition of glucose transport across cell membrane in the cells.

METHODS

Adipocytes (fat cells) are isolated from the epidymal fat pads of male Sprague-Dawley rats. The cells are isolated by digestion (137mM NaCl, 5mM KCl, 4.2mM NaHCO₃, 1.3mM CaCl₂, 0.5mM KH₂PO₄, 0.5mM MgCl₂, 0.5mM MgSO₄, 20mM Heps (pH 7.4), and 4% bovine serum albumin (BSA)) at 37°C for 45 minutes using 4mg of Collagenase. We then isolate the cells with 3mm nylon mesh, followed by 3 washings, after which 18 samples are prepared in test tubes by resuspending the cells in 0.8 ml of a buffer solution prepared as above but with only 1% bovine serum albumin (BSA).

To the samples we added 50 µl insulin at a concentration of 500 ng/ml and glucose at concentrations of 0, 0.1, 0.2, 0.5, 1.0, 2.5, 5.0, 7.0, and 10.0 mM. This was done for 9 samples containing curcumin (solvent DMSO) at a concentration of 20µM. As a control a series of identical samples was prepared with differing only in that they contained none of the curcumin (DMSO was used in place of the curcumin). The samples are then incubated at 37°C for 1.5 hours. Following this we separate the fat cells from the solution and incubate the samples for 10 minutes at 70°C in order to stop further metabolic activity. The samples are then frozen for use in an assay on the following week.

FURTHER STUDIES/DISCUSSION

In order to quantify the glucose uptake of the cells we will measure the lactic acid production via the reduction of NAD, using Lactate Dehydrogenase to catalyze the reaction. As each of these reactions is 1:1, by quantifying the amount of NADH produced we will determine the amount of glucose taken up by the cells. We will preform an assay on the samples by adding Lactate Dehydrogenase and NAD to 100µl of each sample in 0.8ml in buffer solution prepared as above but with only 1% bovine serum albumin (BSA).

In order to determine the mechanism by which curcumin acts as to inhibit the uptake of Glucose by the adipose cells.

LITERATURE CITED

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