GluT1 mediated Quercetin uptake

Katy Hamilton, Brendan Looyenga, Larry Louters, Calvin College

Calvin COLLEGE 1876

Introduction

GluT1 is transmembrane glucose transporter protein responsible for glucose uptake in many cells but particularly in:

- Erythrocytes
- Nervous System Cells

Understanding glucose uptake and the role of GluT1 adds insight into diseases that exhibit compromised glucose transport:

- Cancer and Diabetes

Quercetin is an antioxidant flavonoid compound found in dark fruits and vegetables.

- Data indicate that Quercetin is a competitive inhibitor of GluT1 and suggests that it is transported into cells by GluT1.

Objectives

- To characterize the transport of Quercetin via GluT1
- To determine if Quercetin uptake via GluT1 is measurable

Methods

Glucose Uptakes:

- L929 cells were grown in a 24-well plate & exposed to Quercetin and/or inhibitors as indicated.
- Uptake was measured using radioactive 2DG, an analog of glucose.
- Results were analyzed using a scintillation spectrophotometer

Flow Cytometry:

- Due to the natural florescence of Quercetin, experiments performed with the compound could be analyzed with this technique
- This technique allows for single cell analysis generating very accurate results

Results

Figure A: Dose Dependent Response

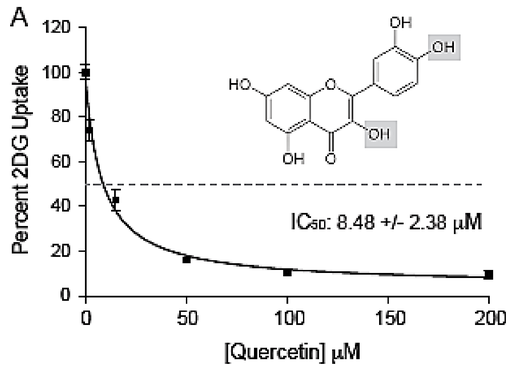

Figure A: Cells were exposed to varying concentrations of Quercetin during 2DG uptake. Results indicate that half maximal inhibition (IC_{50}) was achieved at 8.48 µM along and maximum inhibition at 100 µM.

Figure B: Quercetin Transport Over Time

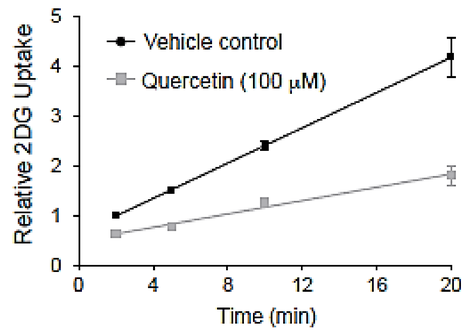

Figure B: 2DG uptake is linear in both control and 100uM Quercetin treated cells for 20 minutes. The data indicate that Quercetin inhibition is immediate and no additional modes of inhibition occur over time.

Figure C: FLOW can be used to measure Quercetin uptake

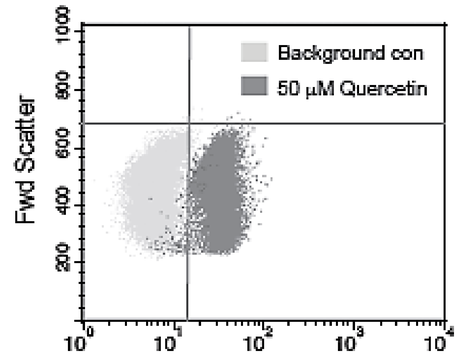

Figure C: These data indicate that the florescence of Quercetin can be detected by FLOW Cytometry. This technique will be used to measure the binding and/or uptake of Quercetin to GluT-1.

Figure D: FLOW can be used to measure Quercetin binding to cells

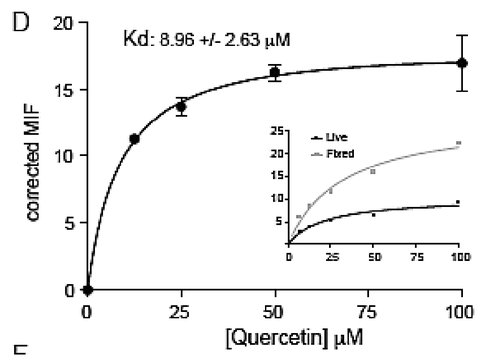

Figure D: Quercetin fluorescence (MIF) was measured at increasing concentrations of Quercetin. The K_D of Quercetin Uptake (half maximal) was 8.96 µM, closely matching its IC50 for inhibition of 2DG uptake. As expected, there is enhanced binding in fixed cells where internal GluT1 proteins are accessible.

Figure E: Quercetin Transport Over Time

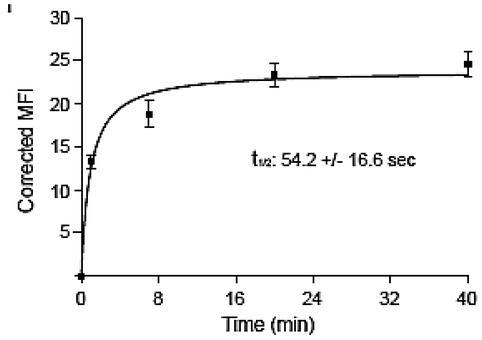

Figure E: The Quercetin florescense (100 µM) as a function of time was measured by FLOW cytometry. Total florescence is acquired quickly, with half maximal response of 54 seconds. This suggests that Quercetin may not be transported, but simply be binding to GluT1.

Figure F: Quercetin competitive Inhibition with Various Inhibitors

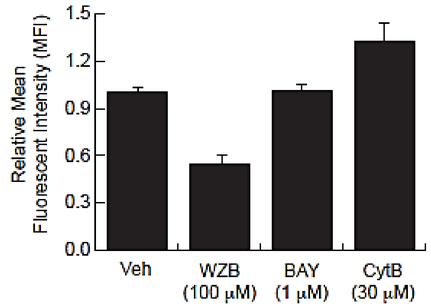

Figure F: The graph shows that results of an FLOW experiment that evaluated Quercetin's interaction with known inhibitors of GluT-1. Based on the results of the experiment, WZB117 appears to compete for the same binding site as Quercetin. Cytochalasin B seems stabilizes GluT-1 into a conformation that allows for more binding of GluT-1. BAY-876 binds to another site far enough away that it does not influence the Quercetin binding location. The Cytochalasin B results support the hypothesis that Quercetin is only binding to GluT-1 and not being actively transported.

Conclusions

Quercetin and GluT-1

The data suggest that Quercetin is not being transported on a significant scale by GluT1

- Data supports a hypothesis that Quercetin binds to GluT1 and may be internalized only as individual transporters are recycled from the cell surface.
- Data supports the theory that Cytochalasin B causes a conformational change to GluT1 that promotes Quercetin binding.

Future Work:

Continuing to characterize the GluT1 protein through work with inhibitors.

Acknowledgements

- Drs. Louters, Looyenga and Arnoys
- Calvin College Department of Chemistry and Biochemistry
- National Institute of Health