Abstract

Human immunodeficiency virus-1 (HIV-1) glycoprotein-120 (gp120) is known to target lipid rafts for entry into the host organism’s cells (S. Fuchs & Schiller, 2011). HIV-1 gp120 has been noticed to also cause elevated levels of ceramide (a precursor molecule for glucosylcerolipids) within cells (Campbell, Crowe, & Mak, 2002). Patients with neurological defects, such as Alzheimer’s disease and those associated with dementia (HAD), are known to have elevated levels of ceramide within their cells (Uchida et al., 2002). I hypothesize that the activity of glucosylceramide synthase (GCS), an enzyme that bonds a glucose molecule to ceramide, converting it to glucosylceramide, might be negatively affected by HIV-1 gp120. To test whether GCS is functioning normally, I am developing an enzyme assay to test enzyme activity in the presence of HIV-1 gp120.

Methods

Tissue Culture

I performed tissue culture with SH-SY5Y neuroblastoma cells to test GCS activity in this cell line. The cells were grown to 80%-90% confluence in a 75cm² tissue culture flask. For use in fluorescent microscopy, cells were grown on 15mm tissue culture treated glass cover slips with HAD (Verma, 2009). Ceramide is a waxy lipid molecule that is the precursor molecule for glucosylcerolipids. Too much ceramide is toxic to the cell, because a high concentration of ceramide can cause apoptosis. Glucosylceramide synthase (GCS) prevents the negative effects of too much ceramide by converting ceramide into the non-toxic glucosylceramide (Uchida et al., 2002).

I worked to establish an enzyme assay to measure GCS activity within a cell in the presence of HIV-1 gp120. Using this enzyme assay, further studies will investigate if gp120 affects GCS activity resulting in the observed elevated levels of ceramide. This enzyme assay uses thin layer chromatography (TLC) to determine whether or not ceramide is converted to glucosylceramide in the presence of HIV-1 gp120.

Figure 1. Flowchart of proposed methods

Objective: Develop an enzyme assay to measure GCS activity within cells treated with HIV-1 gp120.

- Establish protocol to measure glucosylceramide production
- Thin layer chromatography (TLC)
- Establish standards for NBD-C6-ceramide, C16-ceramide & glucosylceramide
- Protein assay
- Cell lysis
- Enzyme assay
- Ceramide extraction
- Incubate cells with HIV-1 gp120
- Incubate cells with NBD-C6-ceramide
- TLC
- Effects of HIV-1 gp120 in vivo

Discussion

With the enzyme assay developed, future experiments will answer whether or not HIV-1 gp120 negatively affects GCS. I theorize that either GCS is not functioning properly due to HIV-1 gp120 or ceramide is produced in such a high concentration that GCS activity can’t keep up with the production.

Development of Thin Layer Chromatography Ceramide Standards

NBD-C6-ceramide, C16-ceramide, and glucosylceramide, (purchased from Cayman Chemicals [C6-ceramide] and Avanti Polar Lipids, respectively) were run on silica gel 60F plates. The lipids were loaded onto a TLC using the solvent system chloroform/acetone/methanol/acetic acid/water (10:4:3:2:1 ratio). Two methods of development were tested: 10% CuSO₄ in 8%H₃PO₄ with charring and iodine vapor. Iodine vapor was chosen as the best method to develop the plates.

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Figure 2. Protein assay standard curve

Figure 3. Results of thin layer chromatography of ceramide standards testing different developing methods. Fig. 3A. Development with 10% CuSO₄ in 8%H₃PO₄ with charring. Fig. 3B. Development with iodine vapor. Lanes a, b, and c represent glucosylceramide, NBD-C6-ceramide, and C16 ceramide standards, respectively. RF values are as follows: Fig. 3A. a-0.65, b-0.86, c-0.88. Fig. 3B. a-0.67, b-0.92, c-0.95.

References


Kory M. F. Joe working on lab preparation.