CALIFORNIA LUTHERAN UNIVERSITY - DEPARTMENT OF BIOLOGY

The Anti-Cancer Effects of Berberine on Cancer Cell Lines and Its Role in Apoptosis

Nicholas Arredondo, Chad Barber Ph.D

California Lutheran UNIVERSITY

ABSTRACT

The anti-cancer properties of natural compounds such as curcumin and berberine have been studied on cancer cells *in vitro*. Studies into berberine indicate the ability to reduce proliferation in tumor cells[1] and induce apoptosis in U937 cells[2]. The objective of this study is to treat cancer cell lines with berberine *in vitro* to determine the effects on adherence, proliferation, and viability. In order to observe the effects of berberine in this study, cancer cell lines will be studied with varying amounts of berberine *in vitro*, compared to positive controls. In addition, scratch wound assays will be used to observe the effects of berberine on adherent cell migration. Using flow cytometry, cell viability and apoptosis levels can be monitored in response to berberine treatments. Overall, berberine decreased proliferation and cell density of both cell lines. Migration assays also indicate a decrease in SY cell migration in response to berberine treatment.

BACKGROUND

- Apoptosis is the process of self-induced cell death.
- Proliferation is the rapid replication of cells and plays a big role in tumor progression.
- Berberine has been studied and found to repress tumor progression by reducing cell proliferation.
- A previous study[1] on Berberine Chloride treatment of Liver Cancer cells indicate an effect on the apoptotic caspase-dependent pathways in the carcinoma cells studied. Caspase has a direct role in the apoptosis mechanism within cells.
- Using similar treatment methods, the goal of this study will be to use cytometric and proliferation assays to determine the effects of berberine on apoptosis, proliferation, and viability in different cancer cells.

AIM

- To treat U937 and SY cells *in vitro* to determine the effects of berberine on cell proliferation, migration, density, and apoptosis.

METHODS

Cell Culture

- Cancer cell lines U937 (suspension) and SY (adherent) were treated in this study.
- Cultured cells will be treated with varying treatments from negative control to 150µg/mL

Scratch-Wound Assay

- Used to determine how berberine affects cancer cells' use of EMT to migrate (Figure 1).
- Can provide qualitative data about cell density and proliferation.

Hemocytometry

- Used to count live cells under a microscope and quantify the number of cells in samples.
- Hemocytometry can determine proliferation rate.

Flow Cytometry

- Utilizes lasers to detect various cell characteristics including apoptosis.
- Can quantify both live and apoptotic cells using 7-AAD stain

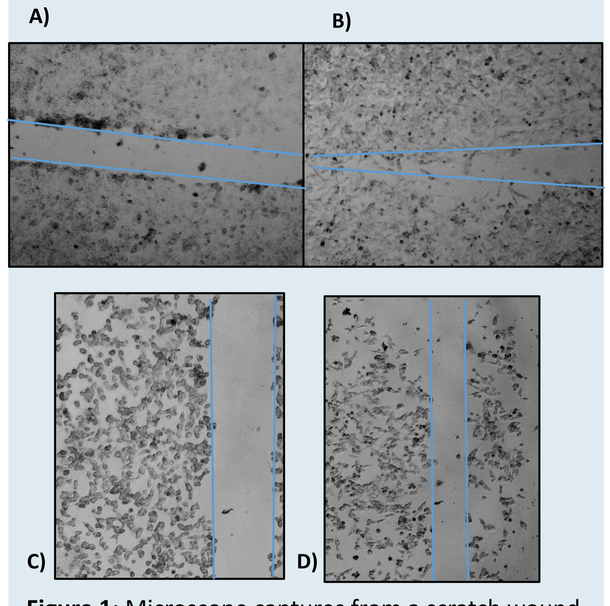

Figure 1: Microscope captures from a scratch wound assay with SY cell cultures. A) Day 0 Control Cells B) Day 2 Control Cells C) Day 0 Berberine Treatment 50 ug/mL D) Day 2 Berberine Treatment 50 ug/mL. Blue lines were added to illustrate difference in percent closure

RESULTS

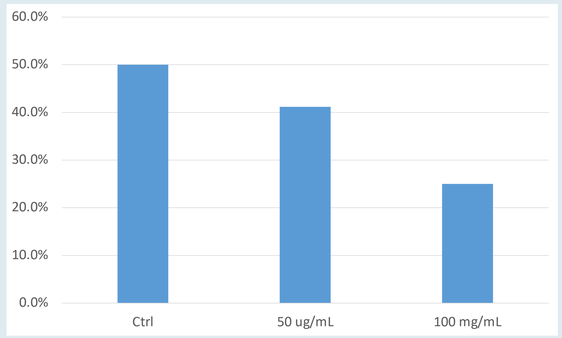

Figure 2: A histogram displaying percent closure after two days in scratch wound assay of SY

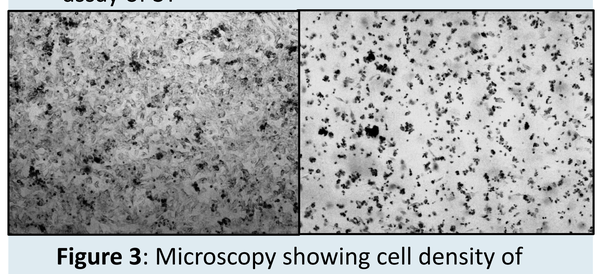

Figure 3: Microscopy showing cell density of the control sample and 100 ug/mL dosage

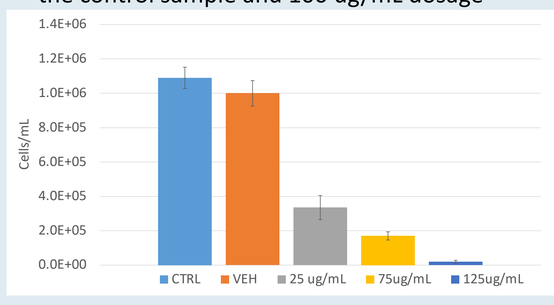

Figure 4: Cell proliferation Assay via Hemocytometry on U937 treatment after 72 hours.

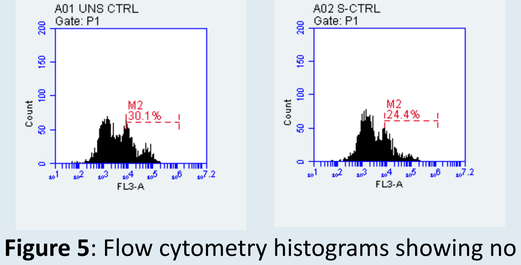

Figure 5: Flow cytometry histograms showing no detectable difference in apoptotic cell count in 7-AAD stained samples.

DISCUSSION

- The scratch wound assay results show a decrease in percent closure as dosage increased. This indicates a decrease in migration of SY cells due to berberine treatment. **(Figure 1,2)**
- Microscopy of SY cell treatments with berberine showed a decrease in cell density as displayed in **Figure 3**.
- Cell proliferation data from U937 treatments with berberine also indicated a decrease in proliferation as dosage increased, as displayed in **Figure 4**.
- Flow cytometry assays showed no detectable differences in 7-AAD stained samples thus far **(Figure 5)**, but berberine decreased proliferation and density in both U937 and SY cell samples.
- Future studies can analyze protein activation via western blots of control cells and treated cells.
- It is hopeful that western blotting will reveal caspase-3 activation, and BCL-2 downregulation. This would reveal a potential mechanism for these anti-cancer effects.

ACKNOWLEDGEMENTS

- Dr. Barber
- ALLIES in STEM
  - Dr. Vargas
- OURCS
  - Dr. Knox
- Writing Center

REFERENCE

[1]Jantova, S., Cipak, L., & Letasiova, S. (2007). Berberine induces apoptosis through a mitochondrial/caspase pathway in human promonocytic u937 cells. *Toxicology in Vitro, 21*(1), 25-31.

[2]Yip, N. K., & Ho, W. S. (2013). Berberine induces apoptosis via the mitochondrial pathway in liver cancer cells. *Oncology Reports*, *30*(3), 1107–12.