THE CANADIAN SCIENCE FAIR JOURNAL

ARTICLE

The Cranberry Cure: The Efficacy of Cancer Chemotherapy Combinations with Cranberry Flavonoids

David Wu, Dr. Peter Ferguson, Dr. James Koropatnick

Age 17 | London, Ontario

Canada-Wide Science Fair Excellence Award: Senior Bronze Medallist

Cancer is a disease in which mutated cells grow uncontrollably, often spreading around the body and compromising vital organs. Cancer is highly variable; it forms in a variety of tissues from numerous oncogenic mutations[1]. To compensate for this variability, a wide selection of chemotherapeutic agents is needed; this would allow chemotherapy to be optimized depending on the characteristics of the patient and cancer. Flavonoids, a large family of biological metabolites, have shown anticancer potential. Found in many plants, flavonoids may exhibit less toxicity than typical chemotherapy drugs on patients and are proposed to have anti-inflammatory, antioxidant, anti-allergy, and anticancer effects[2]. It would be of great benefit if flavonoids synergized with existing anticancer drugs to kill cancer, potentially reducing chemotherapy toxicity to normal tissues.

However, the mechanisms of action for flavonoids and how they interact with other cancer drugs is unknown. This is of great concern in cancer research, as combination chemotherapy is a cornerstone of modern cancer treatment, offering better specificity and efficacy than single drug treatment[3]. More studies are needed with flavonoids to assess their activity when used in combination with currently used chemotherapy drugs against malignant cells.

This inspired me to begin my project, where I looked at the potential of flavonoids from a more practical perspective by combining flavonoid-rich cranberry extract with four types of anticancer drugs against human head-and-neck cancer cell lines.

PURPOSE

The purpose of this project was to understand how cranberry flavonoids interact with tubulin disruptors and DNA synthesis inhibitors against cancer cells. I also aimed to understand how cancer drug resistance impacts the efficacy of drug combinations with cranberry flavonoids.

HYPOTHESES

Cranberry flavonoids with exhibit additive or synergistic effects with both tubulin disruptors and DNA synthesis inhibitors, more effectively inhibiting the proliferation of cancer cells.

Drug resistance in cancer cells will reduce the efficacy of chemotherapy combinations with cranberry flavonoids.

MATERIALS

Cell culturing

- 96-well tissue culture plates
- Head-and-neck cancer cell lines HN-5a (parental cell line) and HN-5a/V15e (multidrug resistant)
- Phosphate-buffered saline (PBS) and trypsin
- Alpha Modification of Eagle's Medium plus 10% fetal bovine serum (growth medium)

Applying Drugs to Cells

- Growth medium and distilled water
- Cranberry extract fraction 6 (Fr6)
- Tubulin disruptor drug stocks: Vincristine (VCR) and navelbine (NVB)
- DNA synthesis inhibitor drug stocks: Pemetrexed (PTX) and 5-fluorodeoxyuridine (FUdR)
- Plastic troughs and centrifuge tubes
- Pipettes and pipette tips

Data Collection

- Optical miscroscope
- Growth medium
- AlamarBlue reagent
- Spectrophotometer

METHODS

Cell Culturing

1. Cell lines HN-5a and HN-5a/V15e in cell flasks are washed with PBS and detached from flask surface using trypsin.
2. Cells are counted using an automated cell counter and concentration calculations are carried out.
3. Cell lines are subcultured into 96-well tissue culture plates, with 2.0×104 cells and 200 µL of growth medium in each well.

Applying Drugs to Cells

1. One day after subculturing, drug volumes and concentrations are prepared from stock for each of the 4 drugs selected. Concentrations were determined based on preliminary experiments (single drug dose-response), centered around the IC50 values for each drug.
2. Cell plates are treated with combinations of cranberry extract and selected anticancer drugs. Each treatment has six replicates.

Data Collection

1. Four days after drug application, images of cells in each well were taken using an optical microscope at 100 times and 400 times magnification.
2. 100 µL of medium is removed from each well and 100 µL of

Alamar Blue reagent is added (diluted 1:12 with growth medium). Cell plates are placed in the incubator.
3. After three hours of incubation, fluorescence of cells were read with a spectrophotometer to determine cell viability.

RESULTS

Collected data was analyzed from 3 perspectives for a more complete understanding.
1. Trend analysis was done using IC50 bar charts.
2. Point-by-point analysis was done using dose-response curves.
3. Changes in cell morphology and proliferation were observed using photos of cells after treatment.

Statistical significance of numeric data was determined using two-way ANOVA (Analysis of Variance) followed by post-hoc Tukey tests. Data was significantly different when $p < 0.05$. For the IC 50 graphs, asterisks indicate significantly different data. These asterisks have been omitted on the dose-response curves to avoid cluttering. Over 80% of the points were statistically significant.

The IC 50 value is the concentration of drug needed to inhibit 50% of cancer cell growth, a good measure of overall trends in drug effectiveness. By analyzing the IC 50 values of my drug applications, I found that the drug-resistant cell line was highly resistant to the combination of Fr6 and NVB. Fr6 and PTX combined are overall additive, but antagonistic against non-resistant cells in HN-5a. Analysis showed no significant differences in Fr6 combined with VCR or FUdR.

Dose-response curves show how cancer cells respond to varying concentrations of drugs. When analyzing the data point by point, drug combinations with Fr6 are primarily antagonistic. The exception is the combination of Fr6 with tubulin-disrupting drugs (VCR and NVB) against nonresistant cancer cells, which

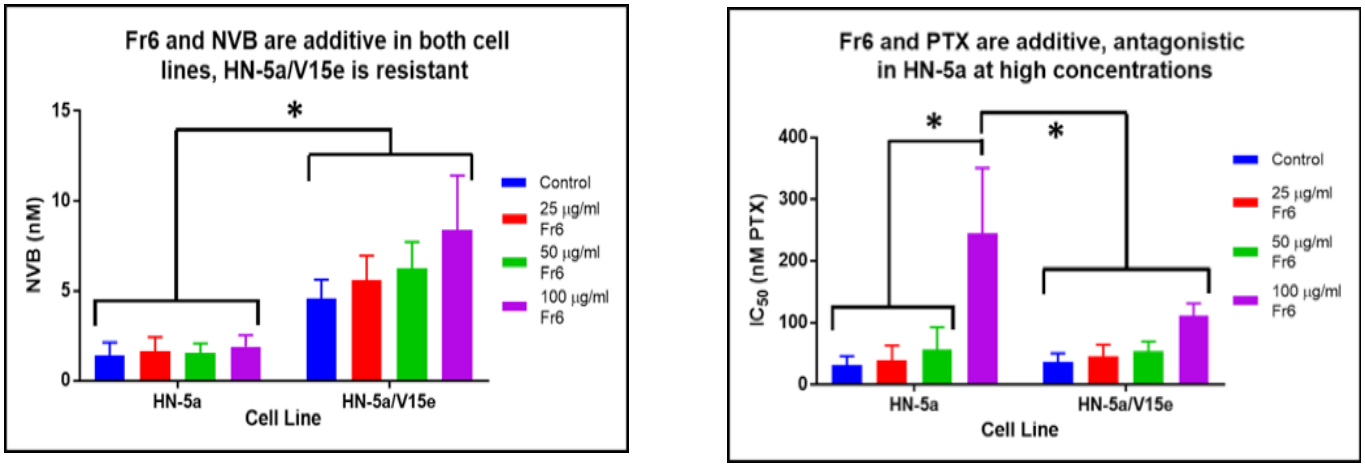

Figure 1. Trend Analysis. (Left): Graph comparing the IC50 values of VCR in combination with Fr6 against normal and drug-resistant cancer cells. (Right): The graph compares the IC 50 values of PTX in combination with Fr6 against normal and drug-resistant cancer cells.
* indicates significant differences. Error bars are ± standard deviation.

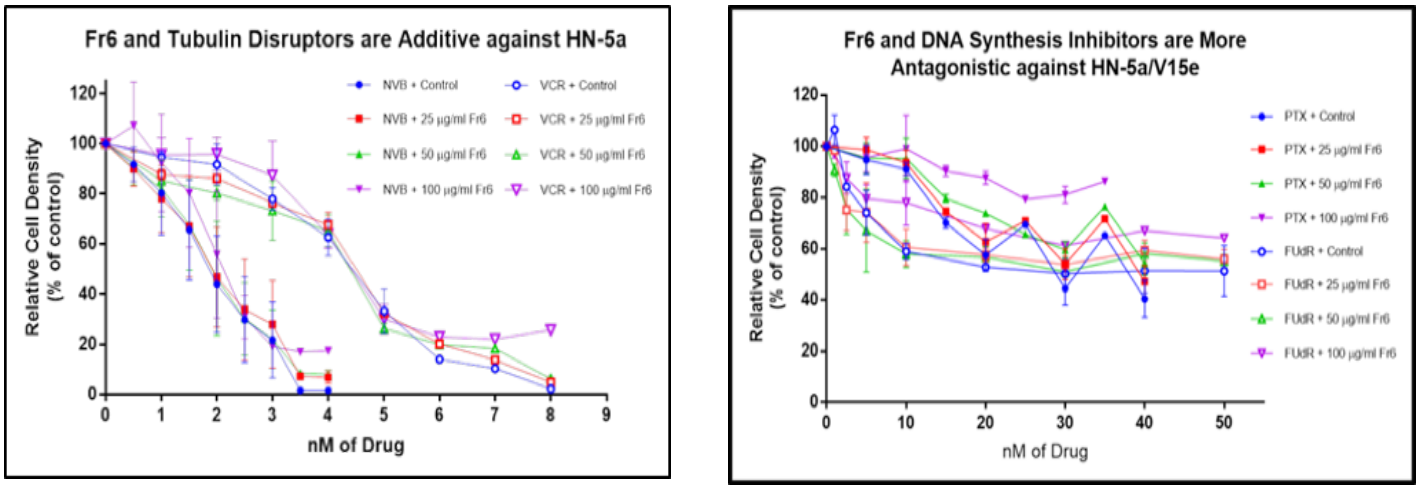

Figure 2. Point by Point Analysis. (Left): The graph is a dose-response curve for Fr6 plus DNA synthesis inhibitors against nonresistant cancer cells. Each data point is the average of 6 replicates ± standard deviation. (Right): The graph is a dose-response curve for Fr6 plus DNA synthesis inhibitors against drug-resistant cancer cells. Each data point is the average of 6 replicates ± standard deviation.

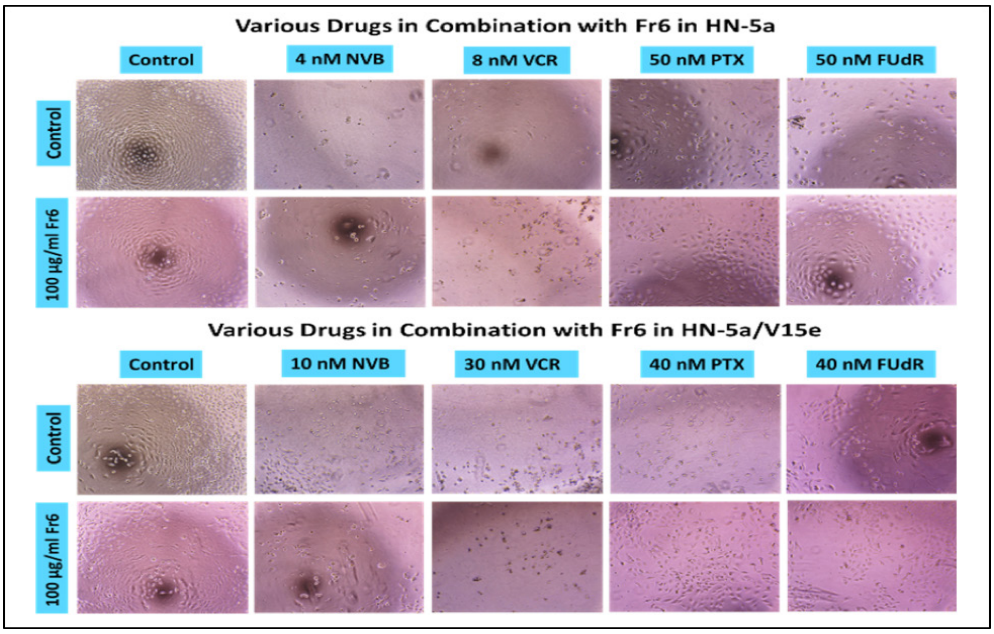

Figure 3: Cell morphology. Photos of both cancer cell lines are shown after 4 days of drug application with an optical microscope at 100X magnification with phase contrast."

demonstrates an additive effect.

The qualitative analysis of cancer cell photos after applying the drug combinations aligned with the conclusions drawn from the trend and point-by-point analyses. Inhibition of cancer cell growth can be seen through the density of cells on the plate in each photo; drug-resistant cells display much higher density. Morphological changes can also be seen. Cells treated with tubulin disruptors (NVB and VCR) have irregular shapes due to damaged cytoskeletons. Cells treated with DNA synthesis inhibitors (PTX and FUdR) are slightly larger, a common effect of DNA damaging drugs.

DISCUSSION

Flavonoid Mechanisms of Action

It was observed that the effectiveness of drug combinations with cranberry flavonoids was dependent on the type of cancer drug used with it and cancer drug resistance.

Fr6 antagonizes in combination with DNA synthesis inhibitors (PTX and FUdR). This suggests that cranberry flavonoids exhibit a DNA-protective effect. Both PTX and FUdR interrupt the synthesis of precursor nucleotides, preventing DNA from replicating properly. Flavonoids may be helping to enhance the expression of DNA repair enzymes, counteracting this effect.

However, Fr6 is favourably additive in combination with tubulin disruptors (VCR and NVB), causing the treatment to be more effective. This suggests that cranberry flavonoids act parallel to tubulin disruptors without interacting with them. However, without further experimentation, we can not determine through exactly what pathways the flavonoids act.

Drug resistance in cancer cells seems to significantly reduce the effectiveness of the combination treatments. This suggests that flavonoids are a substrate of the drug efflux pumps, or perhaps enhance drug resistance pathways.

FUTURE STEPS

Future research will focus on the isolated flavonoid components within the Fr6 extract.

Protein and DNA assays can be carried out to determine how cranberry flavonoids interact with biochemical processed within the cell, and through what pathways the flavonoids exhibit their anticancer effect. This information would be important in determining optimal drug combinations to use with flavonoids.

Flavonoids will be tested in combination with other types of cancer drugs with different mechanisms of action. For example, nucleotide analogue drugs could be considered. Combinations will also be tested against other cancer cell lines such as lung cancer and tested against cancers with different mechanisms of drug resistance.

Eventually, flavonoid drug combinations may be tested in vivo in mammals to assess their toxicity and how the body metabolizes them.

CONCLUSIONS

New information has been found about cranberry flavonoid interactions with currently used cancer drugs, and how drug resistance effects their efficacy.

Flavonoids antagonize with DNA synthesis inhibitors but have a favourable additive effect with tubulin disruptors, a fact that could be investigated for future cancer therapies. Cancer drug resistance reduces the efficacy of these treatments.

Flavonoids may act parallel to tubulin disruptors while protecting DNA and potentially enhance drug resistance pathways. Future studies will be looking to more accurately understand and quantify these interactions.

In conclusion, flavonoids show promising potential as anticancer agents in combination with tubulin disruptors against non-drug resistant tumours.

ACKNOWLEDGEMENTS

For their indispensable guidance and advice, I would like to thank Dr. Koropatnick and Dr. Ferguson. As well, I thank the Koropatnick research group for providing the technical resources needed for my project. This project would not have been possible without their help

REFERENCES

[1] Nguyen, A., Yoshida, M., Goodarzi, H., & Tavazoie, S. (2016, May 3). Highly variable cancer subpopulations that exhibit enhanced transcriptome variability and metastatic fitness. *Nature Communications, 7*(11246). http://doi.org/10.1038/ncomms11246

[2] Panche, A., Diwan, A., & Chandra, S. (2016, December 29). Flavonoids: an over-

view. *Journal of Nutritional Science, 5*(47). Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5465813/

[3] Bayat-Mokhtari, R., Hoaouni, T., Baluch, N., Morgatskaya, E., Kumar, S., Das, B., & Yeger, H. (2017, June 6). Combination therapy in combating cancer. *Oncotarget, 8*(23): 38022-38043. http://doi.org/ 10.18632/oncotarget.16723

ADDITIONAL BIBLIOGRAPHY

Ferguson, P., Kurowska, E., Freeman, D., Chambers, A., Koropatnick, J. (2004). A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. *Journal of Nutrition, 134*: 1529–1535.

Higdon, J., Drake, V., Delage, B., Crozier, A. (2016, February). *Flavonoids*. Retrieved from: http://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/flavonoids

---

DAVID WU

I am a Grade 12 student at London Central S.S. in London, ON. Though I was born in Taipei, Taiwan, I've lived in Canada ever since I was one. I have a wide range of interests and participate in a variety of extracurricular activities. I lead my school's chess club and Science Olympics team, and I am part of my school's DECA team, senior concert band, and senior jazz band as well. My project studied the potential of cranberry flavonoids in cancer chemotherapy from a more practical perspective than current literature offers. I combined flavonoid-rich cranberry extract with various anticancer drugs and treated head-and-neck cancer cells, looking at the efficacy of these combinations. I was able to find great success with my project, winning a Gold Medal at my regional science fair and a Bronze Medal at the Canada-Wide Science Fair. I look forward to continuing with medical research for the rest of high school and university!