The effect of jasmine extract on *Drosophila melanogaster* development

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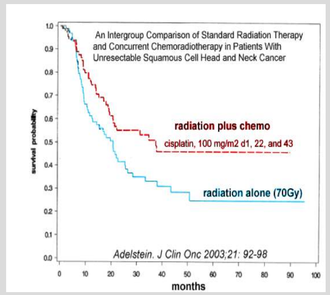
Abstract

Our experiment aimed to target the avoidance of apoptosis amongst head and neck cancers through the use of *Jasminum sambac* on rapidly proliferating *Drosophila melanogaster* larvae. We chose jasmine as the experimental compound because previous research showed evidence of anti-proliferative properties. Pure jasmine essential oil (concentrations from 10% to 0.125%) and jasmine tea (steeping from 15 minutes to 24 hours) were used to treat the larvae. The vials contained dead clumps of black larvae in the jasmine oil, tea, and control vials after the incubation period, meaning that our data was not viable. Due to this inconclusive data, we re-evaluated the experimental design, and used ethanol to extract the compound (concentrations from 10% to 0.125%) from the oil form so that it would mix into the food more completely. These methods and results are attached as a supplement. However, due to the initial low survival rates, further research should be conducted on a more specific component of jasmine in order to develop a more precise test and eliminate extraneous variables.

Introduction

Cancer is the second leading cause of death in the United States, accounting for an estimated 1,670 deaths per day [1]. Currently, research is aimed to reduce this toll; however, studies thus far have been helpful in explaining the origins of cancer and how cancerous cells progress. The onset of cancer can be due to a number of environmental and genetic factors, and its progression is aided by one or many cancerous hallmarks. These hallmarks include evasion of regulated cell death, self-sufficient growth signals, insensitivity to anti-growth signals, the development of a tumor-regulated vascular system (angiogenesis), limitless replicative potential, and of course, tissue metastasis [2]. Current treatments include surgery, radiation, and various chemotherapies. In particular, chemotherapies target one or more of the aforementioned hallmarks, but only 10%-15% of patients treated experience disease remission [2].



Research has suggested that a combination of lower chemotherapy doses and radiation treatment results in higher tumor toxicity than either of these treatments alone. This cytotoxic increase is not largely significant, however, and the search continues for alternative compounds compatible with combination therapy. Homeopathic remedies are growing increasingly popular in the United States, so research into the effects of naturally occurring compounds such as flower extracts and herbs on various cancers has increased. One of these plants is the jasmine flower. Historically, different species of jasmine were used by alchemists and healers in treating ringworm, ulcers, leprosy, and other ailments and diseases [3]. In modern times, it is used in oils and teas to relieve stress and anxiety, and it has been claimed to be a cure for many diseases, cancer included. However, without extensive research, this claim is hardly viable. A study by Pandeti et al. [4], showed that a chemical transformation of night jasmine extract promoted apoptosis and cell cycle arrest in human carcinomas, suggesting that jasmine may be useful in restoring regular cell death (apoptosis). It is because of this research that we have chosen to test the effects of jasmine essential oil, extracted jasmine oil and jasmine tea on cancer. Using third-instar *Drosophila melanogaster* larvae as a model organism for head and neck cancers and common jasmine (*Jasminum sambac)* we hypothesize that jasmine in oil, extracted oil, or tea form, will have a negative effect on larvae cell proliferation in grapes (cell cycle checkpoint) mutated larvae.

Hypothesis

Using third-instar *Drosophila melanogaster* larvae as a model organism for head and neck cancers, we hypothesize that common jasmine (*Jasminum sambac*), in oil, extracted oil, or tea form, will inhibit larvae cell proliferation.

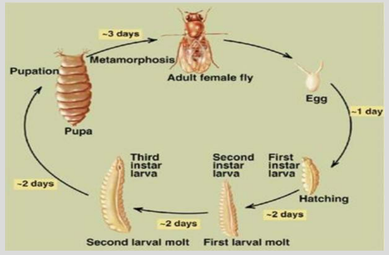
Methods

Experimental Design

Jasmine essential oil and jasmine tea were used to treat irradiated third-instar larvae, according to the following protocols. The vials were then incubated for approximately four days at room temperature (until larvae fully eclosed). We marked GFP- (non-glowing) and dead (not fully eclosed) larvae, then quantified survival and analyzed results.

Model Organism - *Drosophila melanogaster*

Drosophila melanogaster, or fruit fly, was the model organism in this experiment, as its third-instar larvae resemble head and neck tumors. The procedure and techniques used to maintain the population of interest is outlined in Gladstone and Su *Chemical Genetics and Drug Screening in Drosophila Cancer Models* (2011) [5].



Jasmine Essential Oil Protocol

Five vials were labeled according to the following table. Once the vials were labeled, 3 mL of warm food was placed into each vial and either jasmine or coconut oil was added, based on the table. We made sure that the oils were mixed with the food before allowing them to cool. After all vials cooled we placed 50-100 irradiated larvae on the surface of the food with a paintbrush, before plugging the vials and waiting for eclosion.

Vial #	1	2	3	4	5
uL of Jasmine	300 uL	150 uL	75 uL	37.5 uL	3.75 uL
Concentration	10%	5%	2.5%	1.25%	0.125%
Vial (coconut)	1-CO	2-CO	3-CO	4-CO	5-CO
Coconut Oil	300 uL	150 uL	75 uL	37.5 uL	3.75 uL

Jasmine Tea Protocol

We boiled 900 mL of water before placing 3 tea bags of jasmine in the water and allowing it to steep. We removed 1000 uL of tea at every designated steeping time, and then placed 3 uL of each tea concentration (based on what time they were collected) into vials as specified in the table below. 50-100 irradiated third-instar larvae were then placed into the treatment vials using a paintbrush. We then waited the designated incubation period.

Vial #	1 -T	2-T	3-T	4-T	5-T	6-W
uL of Jasmine Tea	3 uL	3 uL	3 uL	3 uL	3 uL	3 uL water (no tea)
Time Collected	15 min	30 min	45 min	1 hour	24 hours	N/A

Radiation Protocol

We obtained third-instar larvae via the sieving protocol outlined by Gladstone and Su [5]. The larvae were then spread in a plastic container and covered with pantyhose to prevent the larvae from escaping while they were irradiated at 4,000 rad.

GFP Protocol

We placed our vials under a blacklight by putting them on their sides on the microscope stage. This allowed us to circle all pupae that were GFP- (did not glow) and recorded the total number of GFP- and GFP+ pupae observed. GFP- pupae were desired because there genotype is not heterozygous for the grapes mutation, making them more susceptible to radiation.

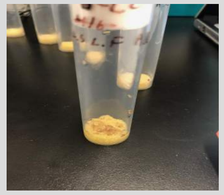
Quantifying Survival Protocol

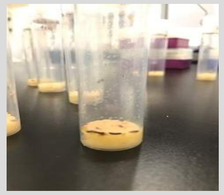
We observed individual pupae in the vials over a light box and determined whether the pupae were full or empty for one genotype (GFP- vs. GFP +) at a time. We then documented how many alive and dead pupae there were for each genotype and then compared this with the GFP protocol results.

Results

The experiment yielded no viable data. Even though pupae did form, there was a limited number that climbed up the side of the vial and could later be marked for GFP and survival. Most of the larvae died in the food, and because of the limited number of pupae that formed on the walls of the vials, a statistical analysis could not be performed, but was good material for future discussion.

Conclusions





Our results showed nearly 0% survival in both jasmine essential oil and tea treatments. This is particularly interesting because the control vial containing water yielded no living organisms, even though the fly-food is water-based [5]. This leads us to believe that an extraneous variable contributed to the low survival rates.In addition, the vials containing jasmine oil and coconut oil were not only similar to one another, but resembled the survival rates seen in the tea treatment group. In this case, the oils did not mix into the food. Lipids do not mix well with water, and in the vials with the largest concentrations of oil, the oil sat on top of the food, possibly drowning the larvae before they had a chance to develop. To combat this, we extracted the essential oil with ethanol in order to remove the target compound from its oil base and create better consistency of the compound in the food. The results of this experiment are in our supplemental materials.

Lastly, the observation of dried, black larvae found in clumps on the top of the food suggests that their development process was also hindered by lack of moisture or the corrosive properties of the oil.

Future Directions

As homeopathic treatments grow in popularity, it is important that research follows these trends in order to determine their validity as treatment options. Though our results were inconclusive, we believe this is still research worth pursuing. Our data suggests that an extraneous variable was responsible for the low survival rate of larvae, not the jasmine treatments themselves. The study that our project was based on had isolated and chemically transformed night jasmine extract to achieve their results. In our case, without equipment or know-how, this was not possible. Having the impure compound resulted in complications such as the larvae drowning in excess oil. In the future it would be beneficial to develop a more effective delivery system of the isolated target compound in jasmine that is aimed at a specific cellular mechanism.

Additionally, the study conducted by Arun et al.[3], used *Jasminum grandiflorum*, which is night jasmine, and we used *Jasminum sambac,* common jasmine, found in essential oils and green-based jasmine teas. It is entirely possible that night jasmine simply contains the anti-cell proliferative properties that common jasmine does not. Further research comparing the two would be beneficial to compare this and analyze the different properties each species possesses

Acknowledgments

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The effect of PDE inhibitors, caffeine and tadalafil, on inducing apoptosis of tumor cells

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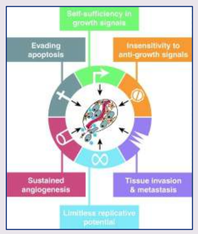
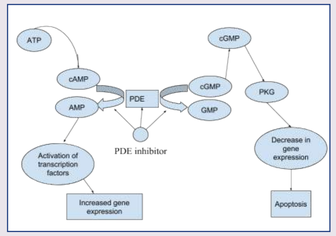
Abstract

Cancer is a very common disease in the United States; virtually everyone knows someone who has been affected by it. The current treatments can be effective, but they have major side effects that can become life threatening, driving a need for research on effective and less harmful chemotherapy drugs. We had chosen our compounds based on their abilities to inhibit phosphodiesterase 5 (PDE5), which is commonly overexpressed in cancer. PDEs are responsible for activating transcription factors, and when inhibited, those transcription factors are not activated and the cell experiences apoptosis. In order to conduct our experiment, we used *Drosophila,* which mimic head and neck tumor cells, due to their rapidly dividing cells and amounts of stem cells. We tested two PDE inhibitors, caffeine and tadalafil, to determine if they were possible effective chemotherapies, in combination with irradiating the *Drosophila*. We believe that tadalafil will be more effective based on the data done by the National Center for Biotechnology Information (NCBI).

Introduction

Cancer is a life altering disease that affects 1.7 million people in the United States alone every year; around 600,000 of these patients die each year. It is extremely difficult to treat because of the different hallmarks of this disease. These include how cancer is growth factor independent, insensitivity to antigrowth signals, avoidance of apoptosis, sustained angiogenesis, developed immortalization, and metastasis. These hallmarks are brought about by differing factors such as drug resistance, cell cycle susceptibility, minimal immune response, tumor heterogeneity, and genetic drift in the tumor after treatment. Common treatments include surgery, radiation, and chemotherapy. One third of patients without metastasis respond to surgery and radiation. Although current treatments can be successful, they often lead to major side effects, which can be deadly. Due to the high mortality and recurrence after remission rate of cancer, there is a push to find compounds that can function effectively as chemotherapy drugs while having less harmful effects to the patient's body and health.





Drosophila were used in the course of this experiment to test the plausible chemotherapeutic drugs we had chosen to research. *Drosophila* mimic head and neck tumor cells as they possess many stem cells and the cells of the third instar larvae divide quickly like that of tumor cells. Additionally, the *Drosophila* used were genetically modified to have a recessive mutation called *grapes* (*grp*), causing them to be more susceptible to radiation; this is paralleled in humans via the Checkpoint Kinase I (Chk1). A mutation in Chk1 is commonly found in patients with head and neck cancers. Combination therapy was used in this experiment in the form of a phosphodiesterase (PDE) inhibitor compound and radiation, because combination therapy typically shows a synergistic effect and allows a lower dose administration of each therapy. The administration of lower doses of chemotherapeutic drugs entails lower occurrences of side effects, which in some cases kill patients faster than their cancer.
In this experiment, compounds caffeine, a nonselective PDE inhibitor and tadalafil, a PDE5 inhibitor, similar to the active ingredient in Viagra, were tested against colchicine, a positive control, and water and DMSO, the negative controls. In many cancers, PDE5 is a tumor biomarker (overexpressed in cancer). We examined the effects of caffeine and tadalafil on head and neck cancers and their possible uses as a combinatorial therapy to reduce toxicities.

Hypothesis and Rationale

Based on articles and information from the NCBI, we hypothesize:

1. Both caffeine and tadalafil will be statistically significant in inducing apoptosis to *Drosophila* (a model for tumor cells), tadalafil will be more effective since it is a PDE5 specific inhibitor, while caffeine will be less effective as it is a nonspecific inhibitor.

2. It is also hypothesized that as dose increases for both drugs, the drug will be more effective.

Often, phosphodiesterases (PDE) are overexpressed in cancer cells. We chose caffeine and tadalafil because of their abilities to inhibit PDEs. PDE inhibition prevents the inactivation of cAMP and cGMP (raising the levels of cAMP and cGMP in the cell), leading to the inactivation of transcription factors, thereby inducing apoptosis.

Methods

Placed adult flies in population cages that are equipped with grape juice/yeast plates that serve as a breeding ground

Collected embryos from grape juice plates, then allowed for growth in food vials

Once third instar larvae were present (~5 days), we used a sieve to separate out the larvae according to size

Irradiated the third instar larvae at 4000 rads for 12 ½ minutes

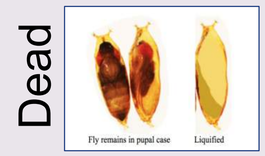
Dissolved caffeine in water and tadalafil in DMSO and performed 1:10 serial dilution

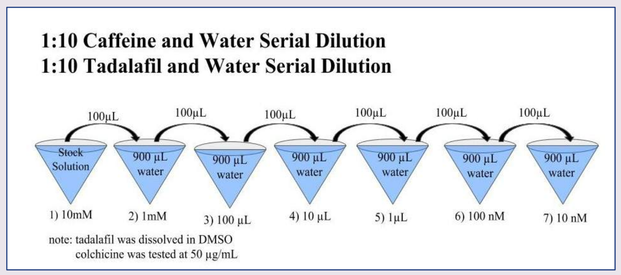
Mixed 3 mL of fly food with 3 µL of the various concentrations, using 42 vials in total

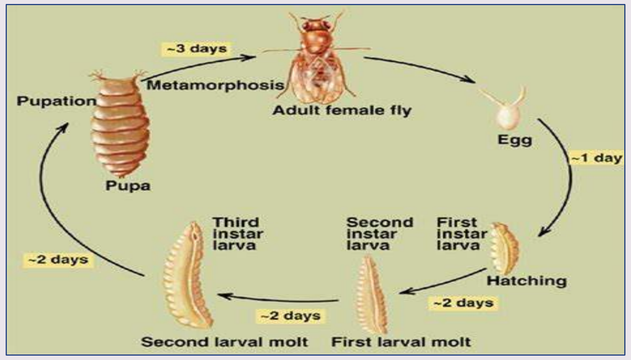
Marked GFP to determine which Drosophila are homozygous recessive for grp1.

If GFP+, they are heterozygous, GFP- they are homozygous recessive for grp1.

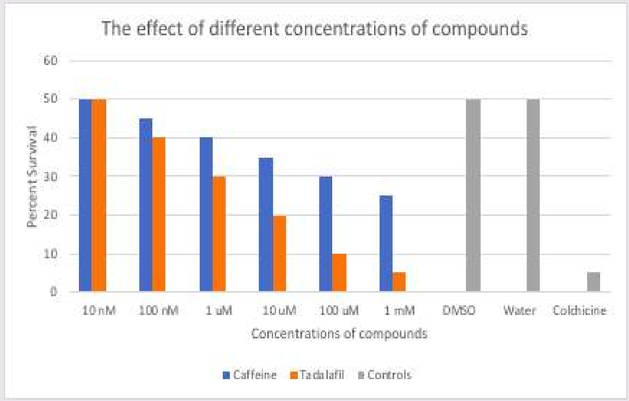
Quantify percent survival by counting alive versus dead pupae after 10 days





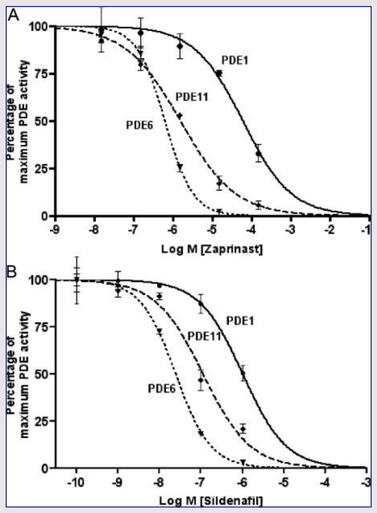


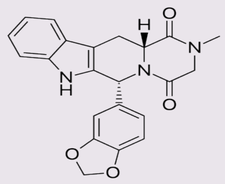
Expected Results



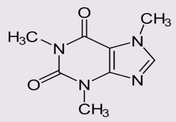
Different concentrations of compounds

These are expected results based off of data taken from other research done by NCBI published journals that conducted experiments on the effects of various concentrations of sildenafil and zaprinast on PDE activity, both selective inhibitors. Tadalafil will have more of an effect on *Drosophila* because of its PDE5 specific inhibition rather than caffeine's nonselective PDE inhibition.





Tadalafil



Caffeine

Results from Screening a NCBI Compound Library
The data tests 6 different concentrations of two different compounds, both PDE inhibitors. On the y-axis, the percentage of PDE activity is shown, which is compared to percent survival of our *Drosophila*.

Conclusion

From our research, we expect that both tadalafil and caffeine will be effective in killing *Drosophila*. Our results should also display that tadalafil would be more effective than caffeine based on its ability to be a selective PDE5 inhibitor, rather than caffeine's' non selective PDE inhibition. Additionally, we predict a negative linear trend for our results, showing that as concentrations of both drugs increase, going from 10 nM to 1 mM, percent survival of *Drosophila* will decrease. We predict that DMSO and water will have little to no effect on the percent survival. A statistical hit will be determined by looking two standard deviations outside of the mean survival for both negative controls. Supplemental data based on our completed experiment is attached below.

Future Directions

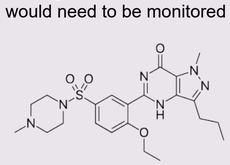
While tadalafil and caffeine have both been tested and are currently being tested, there is still much more that can be investigated. With additional time and resources, here are a few directions future research could go:

1. Test the effects of sildenafil (the active ingredient in Viagra) on the larvae, as it is the most potent PDE5 inhibitor.

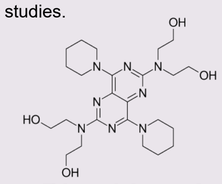
2. Alterations in expression of PDEs and PDE-gene mutations (especially mutations in PDE6, PDE8B, PDE11A and PDE4) have been seen in various diseases and cancers. Therefore, combining a PDE5 inhibitor with another PDE inhibitor listed above could result in an even lower percent survival. There is also currently a need for PDE8 inhibitors, so researching that would be a priority.

3. Test tadalafil and caffeine on the third instar larvae without any radiation to see how effective the drug is alone in order to accurately determine whether radiation and chemotherapy combined have a synergistic effect or an additive effect.

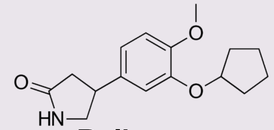
4.Combine a PDE5 inhibitor with an anti-cancer drug. The PDE5 inhibitor could potentially enhance the strength and sensitivity of the anti-cancer drug due to the inhibition of PDE5 and specific ATP-binding cassette (ABC) transporters. The effects of this combination would need to be monitored in future studies.



Sildenafil



Dipyridamole



Rolipram

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Harvey, Pamela. Course Manual for MCDB2171. Course Manual for MCDB2171.

Oleuropein as a chemotherapy

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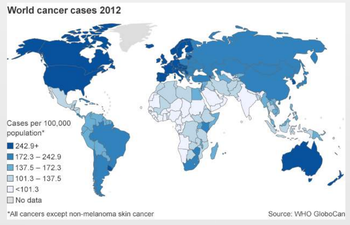
Abstract

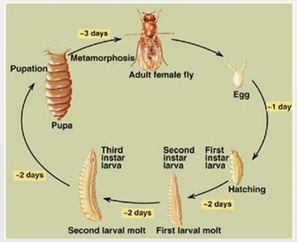
Oleuropein is an olive oil leaf extract commonly found in the Mediterranean area. In past research, scientists have found that Oleuropein has anti-inflammatory and antioxidant properties. Because it has a similar structure to estrogen and can bind to the estrogen receptors, it reduces the presence and progression of hormone related cancers. Oleuropein inhibits anti apoptosis and pro-proliferation protein NF-kB and its main oncogenic target cyclin D1.

During our experimentation, we are testing the effects of Oleuropein on *Drosophila* larvae survival. We added different dilutions to the food vials that we then added the *Drosophila* larvae to, in order to test their survival rate. We tested a fairly strong solution and diluted it down in order to observe the effects that it has on the larvae. In addition to the Oleuropein dilutions, we are using olive oil (in which Oleuropein can be extracted) also in different concentrations. This is in order to compare whether it is Oleuropein or another compound that can be found in olive oil that may or may not assist in finding a possible hit in future cancer drugs.



Introduction

In Mediterranean countries, there is a decreased occurrence of cancer. In recent research, this is believed to be because of the compounds found in olive oil. For our experiment we chose to test Oleuropein, which is one of the main components of olive oil and has been shown to act as an anti-tumor agent. We chose to use *Drosophila melanogaster* (common fruit fly) because *Drosophila* are excellent models. They reproduce and mature quickly, have genetics incredibly similar to humans, and are very inexpensive to keep and maintain.



During Stage 3 of *Drosophila* development, the larvae grow the quickest, very similar to how cancer cells multiply at an accelerated rate. Therefore, we are testing whether Oleuropein will prevent *Drosophila* from reaching adulthood, which could be indicative of Oleuropein's ability to kill cancer cells in humans. When the larvae are at the stage just before pupation, they are growing the quickest and are eating the most. By adding the drug when the larvae eat the most leads to the most consumption of the drug. When we look for survival we'll be observing if the *Drosophila* has eclosed out of their pupa.

The Problem

Cancer is second leading cause of death worldwide. Roughly every 1 in every 6 global deaths is caused by cancer. Cancer costs the world approximately $1.16 trillion every year. While millions of people have cancer, the treatment is deplorable. If you live in a high income country, treatment typically includes radiation as well as chemotherapy. While this treatment does a decent job of "curing" cancer, the side effects are awful for the patients quality of life, which is just as important in medicine as the quantity of life.

Hypothesis & Objective

We are testing Oleuropein with radiation on third-instar *Drosophila* larvae to see if it may be a possible hit to be further tested for a possible future anti-cancer drug.

The objective of this experiment is to determine whether Oleuropein acts as an effective candidate to become a lead compound for cancer research. In order to accomplish this we will be testing Oleuropein, through serial dilutions, on drosophila melanogaster.

Methods

Making the Stock Solution
To the bottle in which it arrived (which contains 10 mg oleuropein), we added 1 mL of DI water to create the stock solution. The concentration is 10 mg/mL Oleuropein.

Making Dilutions
1) From the bottle, using the micropipettes, remove 500 µL of stock solution and add it to an empty 1.5 mL centrifuge tube.
2) Using a new micropipette tip, add 500µL of DI water (the dilution is 5 mg/mL).
3) Use the vortex for 5 seconds to mix the solution so that the entire solution is homologous and at the bottom of the tube.
4) Using another micropipette tip take 500 µL from the 5 mg/mL vial solution and add it to a new centrifuge tube.
5) Continue to create serial dilutions by taking 500 µL from the previous solution and adding 500 µL of DI water. Do this 4 more times for a total of 5 dilutions and the stock, thus creating a serial dilution of a 1:2 ratio.

The following is the concentrations and ratios of the dilutions that will be made:
Stock: 10 mg/mL, 18.5mM
Dilution 1: 5 mg/mL, 9.25mM
Dilution 2: 2.5 mg/mL, 4.63mM
Dilution 3: 1.25 mg/mL, 2.31mM
Dilution 4: 0.625 mg/mL, 1.16mM
Dilution 5: 0.3125 mg/mL, 0.578mM
Negative Control: No Oleuropein, only DI water

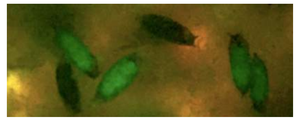
In addition to the Oleuropein dilutions, we will be utilizing olive oil in the food vials
10% olive oil (300 µL of olive oil to 3 mL of food)
8% olive oil (240 µL of olive oil to 3 mL of food)
6% olive oil (180 µL of olive oil to 3 mL of food)
4% olive oil (120 µL of olive oil to 3 mL of food)
2% olive oil (60 µL of olive oil to 3 mL of food)

Irradiation
1) We irradiated the flies when they were third instar larvae. To get the third instar larvae from the bottles we will added water to the food bottles in order to break up the food.
2) We poured the food into a series of three sieves.
3) Then collected the embryos from the middle sieve.
4) We then created a 1 instar larvae thick layer onto a plastic plate, covering it
5) Then we Irradiated the larvae at 4000 Rad, making the flies more susceptible to the drug.
6) After they were irradiated, we added 50-100 third instar larvae to each drug vial.

Making Food Vials
1) Using an infusion needle (syringe) we slowly added 3 mL of food to the food vials.
2) Using a micropipette, we added 3 µL of one of the concentrations of Oleuropein solution directly into the food and stirred using the pipette tip. (Making sure that the food is cool to the touch as hotter temperatures may negatively affect the stability of the drug)
3) Then letting the vial sit to cool and solidify before adding a flug
4) Then we added approximately 50 irradiated larvae to each vial (Making sure the food has solidified before adding the larvae; as they may get trapped and die if the food has not yet solidified)
5) Following these same steps, we added the olive oil with the micropipette using the amounts listed to the left.
6) We created each concentration in triplicate: there was a total of 36 food vials.

Results and Discussion

Unfortunately, due to the unpredictable nature of the *Drosophila's* life cycle as well as the timing of our experiment, we were unable to produce data. Please see additional sheet for results as well as discussion.



Drug vials containing an unknown drug(left)
Example of fly quantifying(top)

Drosophila melanogaster

Future Direction

The results will provide us with a direction for future experimentation. There are several factors to keep in mind. For example, the concentrations of different vials can be adjusted accordingly based on our results to better examine the proper concentration of Oleuropein in future experiments. We should also keep other variables in mind. In our experiment, *Drosophila* larvae are radiated to simulate the radiotherapy that usually accompanies chemotherapeutic drugs. We can conduct more experiments to investigate the effect of radiation on *Drosophila* larvae when treated with Oleuropein.

If our data proves Oleuropein to be a promising lead compound, we will continue our experimentation with Oleuropein. We will perform initial safety tests and optimize the lead compound. Before moving on to testing the compound in humans, it is also necessary to conduct *in vitro* and *in vivo* tests: experiments conducted in lab test tubes and experiments conducted in live cell cultures and animal models. The FDA will examine data from pre-clinical trials, the candidate drug's chemical structure, how it works in the body, and potential side effects. It is critical to ensure that people who participate in the clinical trials will not face unreasonable risks. Only after passing these extensive tests can a compound move on to clinical trials.

Statisticians and others from the Institutional Review Board at the institutions where the trials will take place will closely monitor data from these clinical trials as it becomes available. Finally, after years of clinical trials conducted in multiple stages, the compound may become a FDA-approved drug.

Acknowledgments

We would like to thank Dr. Tin Tin Su, the lead researcher in this lab in which we are using Drosophila for cancer drug screening. We would also like to thank Jesse Kurland, Lindsey Visscher, and Nima Shokrani for being our TAs and guiding us throughout this research. We would additionally like to appreciate Dr. Pamela Harvey for providing, conducting, and overseeing this lab research.

This work could not have been possible without funding from the Molecular, Cellular, and Developmental Biology Department, the Howard Hughes Medical Institute, and the Biological Sciences Initiative.

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Arcyriaflavin A, as a potential chemotherapy

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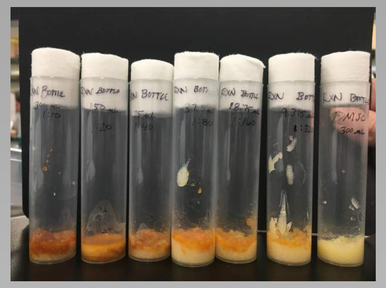
Abstract

Cancer is one of the most prevalent diseases in the world, with approximately 40% of all men and women being diagnosed with cancer at some point in their lives. Even with current cancer treatments, thousands of people die from cancer each year, highlighting the need for continued research for better treatments.

In this experiment, Drosophila larvae were used as model organisms to simulate human cancer cell growth. The larvae were exposed to the compound Arcyriaflavin A, a Cyclin D1 inhibitor. The goal was to determine whether a combination of irradiation and drug exposure effectively inhibited cell growth in the larvae, which was quantified by measuring the larval survival rates to adulthood. Larvae were cultivated and placed into vials containing food and various doses of the drug, and were then irradiated. After about five days, the survival rate was determined.

Results indicated that Arcyriaflavin A was effective at decreasing the larval survival rates compared to the positive and negative controls, and that the survival rate decreased as drug concentration increased. These results indicate that the drug is a potential chemotherapy, and should be analyzed in further research studies.



Introduction

Cancer is one of the most prevalent diseases in the United States, with roughly 1.7 million new cases expected to be diagnosed in 2018 alone. While there are chemotherapy and radiation treatments available for cancer patients, there is currently no cure for this devastating disease. Cancer cells exhibit several unique hallmarks that make them difficult to treat, such as insensitivity to anti-growth signals, evasion of cell death signals, limitless replicative potential, metastasis, and constantly evolving genetic drift. Therefore, continued cancer research and drug development is imperative in the fight against cancer.

Our experiment involves disrupting the cell cycle in the model organism, Drosophila. These flies have been bred with a particular mutation in the *grapes* gene, which normally produces a checkpoint protein that stops the cell cycle when DNA is damaged. This mutation allows for the flies to be more susceptible to irradiation. The presence of the *grapes* mutation is determined by a balancer chromosome containing GFP (green fluorescent protein), which causes the flies to glow green. Flies that contain the *grapes* mutation will not glow, while flies without the mutation will glow. This will help determine which flies are more susceptible to irradiation.

One of the potential targets for cancer cell treatment is Cyclin D1, a regulatory subunit of a protein that promotes the G_1/S-phase transition of the cell cycle. Cyclin D1 activation leads to increased cell growth, and there is strong evidence suggesting that Cyclin D1 overexpression leads to increased cancer growth. Our goal in this experiment is to use a Cyclin D1 inhibitor, Arcyriaflavin A, to stop the cell cycle in Drosophila. Drosophila mimics cell-signaling pathways in humans, particularly the pathways used in cancer cells. Therefore, by inducing cell death in Drosophila with irradiation and drug treatment, we are effectively testing potential chemotherapies in human cancer cells.

Statements of Hypothesis

The use of the cyclin D1 inhibitor, Arcyriaflavin A, on larval Drosophila will cause disruption of the larval cell cycle and result in lower survival rates. Also, the drug will be effective in both grapes mutants and wild-type larvae, with or without irradiation.

Methods

The effectiveness of Arcyriaflavin was tested using wild type Drosophila to determine whether the Cyclin D1 inhibitor will stop the cell cycle in wild type flies. Additional trials tested irradiated flies homozygous for the grapes mutation to examine how this drug operates in a model tumor. A dosing series was used for each trial, as were positive and negative controls.

1. **Cultivate** the homozygous mutant (or wild-type) flies at 25°C, and collect third-instar larvae.
Rationale: By testing the drug on different types of flies, we can determine whether the drug could be effective only with the *grapes* mutation present. We could also determine the effectiveness of the drug without the presence of the grapes mutation in the wild-type flies.

2. **Prepare** food drug vials by using a dosing series. A 1:10 dilution of the stock solution of Arcyriaflavin A was prepared. Then, dilutions of the drug were mixed with the fly food with a 1:2 dilution, with the highest concentration at 75uM and the lowest concentration at 1:1280. 75uM of the positive control, colchicine, and 75uM of the negative control, DMSO, were also created.
Rationale: By using different dilutions, we can determine the effectiveness of different doses of the drug.

3. **Irradiate** the mutant third-instar larvae once they have been placed into the food/drug vials. The wild-type larvae will not be irradiated.
Rationale: By irradiating the mutant larvae and not the wild-type, the effectiveness of the drug in the absence of irradiation can be determined.

4. **Mark GFP** in the vials containing the pupae of the mutant flies after 2-3 days with a UV lamp.
Rationale: The pupae that are negative for GFP have two copies of mutated *grapes*, and are susceptible to irradiation. These pupae are most similar to cancerous cells, so their death or survival will help determine the potential effectiveness of the drug as chemotherapy. Wild-type flies are not marked, due to no *grapes* mutation present.

5. **Count** the number of live versus dead flies in both the wild type and mutant vials by observing the pupae, and compare the results with the controls by calculating the mean standard deviation of the survival rates.
Rationale: By using the control vials as references for the effectiveness of ineffectiveness of a drug, we can determine the effectiveness of Arcyriaflavin A by comparing the number of alive versus dead flies with the controls. If the vials with the drug present contain many more live flies than colchicine, than the drug is most likely not an effective chemotherapy. However, if the live count is comparable to colchicine, then the drug will have potential as chemotherapy.

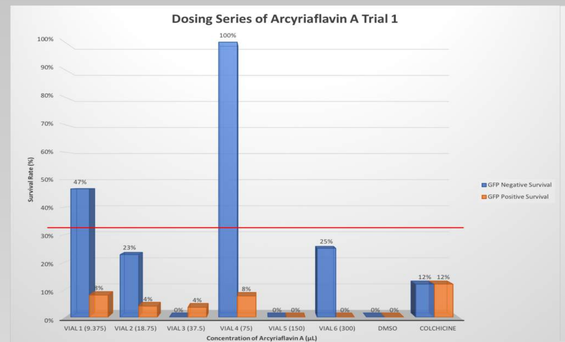
Results

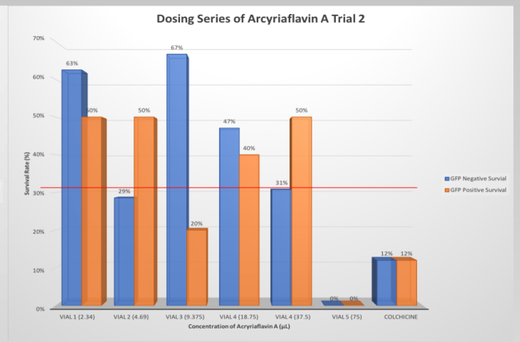
Effect of dose on survival rate of Drosophila exposed to Arcryiaflavin A.

Initial dosing series of the compound began with 300 □L of Arcryiaflavin A, diluted by half in each successive dose, resulting in dilutions from 1:10 to 1:320. Low survival rates at each dilution show the Arcryiaflavin effectively inhibited cell growth, with decreasing survival rates at increasing concentrations. The data also showed that wild-type larvae were more affected than larvae with the *grapes* mutation. This data was compared directly to the results of our positive control, Colchicine, a known chemotherapy. The red line represents the average survival rate for colchicine, minus two standard deviations, with any lower survival rates representing a “hit”. While the first dataset resulted in 12 overall hits, the volume of drug in the initial dilutions was too high, resulting in fly death due to drowning, rather than reaction to the drug. This is clear as the negative control, DMSO, resulted in an equally low survival rate as the highest concentration of drug. Taking this into account, the first drug trial resulted in 8 overall hits, and demonstrated the need for an additional set of dilutions at lower concentrations. In addition, certain concentrations had an unavoidable lack of data, due to the lack of GFP-negative pupae present in the designated sample, as seen in the 75 □L concentration.

Effect of dilution series two on survival rate of Drosophila exposed to Arcyriaflavin A.

Second dosing series began with 75 □L of Arcryiaflavin A, resulting in dilutions from 1:40 to 1:1280. These lower concentrations still effectively lowered the survival rate of the fly pupae of both wild-types an mutants, with 5 overall hits. The second data set also shows decreasing survival rate due to increasing concentrations of Arcryiaflavin A, with no flies surviving with 75 □L of the drug present.





Conclusion

The results confirmed our hypothesis that Arcyriaflavin A could act as a potential chemotherapy treatment in cancer patients, as the drug killed larvae through Cyclin-D1 inhibition with 13 overall hits. These hits signify that our drug treatment was effective compared to the mean standard deviation of the negative control, DMSO. A few of the hits were even more effective than our positive control, Colchicine, further supporting the potential of Arcyriaflavin A as a chemotherapy. The lower concentrations yielded higher survival rates, which could indicate an inverse relationship between survival rate and concentration of Arcyriaflavin A. A surprising result of the two trials was that the survival rate for wild-type larvae was lower than the survival rate for larvae with the *grapes* mutation. This could be due to the *grapes* gene having an additive effect on the potency of our drug, or the difference could be due to a different mechanism of the drug that causes the death of the larvae.

There were a few limitations to our experiment. The highest concentrations samples contained too much liquid to provide reliable results, including the DMSO control. Also, the absence of a sufficient balance of GFP-negative and positive larvae was limiting, particularly in the 1:40 vial in the first trial.

Future Directions

The data resulting from these trials has presented significant potential for future experiments. Further testing is necessary to reveal specific information regarding the effectiveness of Arcyriaflavin A as a potential chemotherapy, with an emphasis on collecting more comprehensive data. The future direction of this experiment should be as follows:

- Conduct multiple trials similar to trial 2, with similar dosing and irradiation levels. Also conduct more trials comparing the effects of the drug on wild-type larvae.
  - -This will create a much larger pool of data to eliminate the effects of outliers which may skew results. This will also mitigate the effects of minor mistakes in each trial.
- Conduct multiple trials while manipulating the dosage of drug and irradiation.
  - -Based on the results of the repeated trials using the same dosing series as trials 2 and 3, the dosage that yields the lowest survival rate can be determined.
- Repeat the experimental methods in other Cyclin D1 inhibitors
  - -This will validate the mechanism of Cyclin D1 inhibition as a chemotherapeutic pathway, and could reveal additional potential chemotherapy drugs.
- If Arcyriaflavin A continues to demonstrate chemotherapeutic effects during these auxiliary trials, experimentation could be continued in more complex model organisms.
  - -In more complex model organisms, testing can then be focused on the metabolic mechanisms, the side effects, dosage, and long-term effects of the drug.
  - -If the drug continues to be successful in other model organisms, the next step would be to conduct human trials.

Acknowledgements

We would like to thank Dr. Tin Tin Su for the unique opportunity to contribute to cancer research by allowing us to participate in this lab. Special thanks to Pamela Harvey for her constant support and instruction throughout this experiment, and to our teaching assistants Alia Alsaif, Isabella Shelby, Julianna Rohn, and Ben Huxley. Also, this experiment would not be possible without funding from the Howard Hughes Medical Institute and Biological Sciences Initiative (BSI). Lastly, we would like to thank the Molecular, Cellular, and Developmental Biology Department at the University of Colorado boulder for the continued support of this research lab.

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Discovery lab “hits” affect model tumor-cell survival in dosing series

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Abstract

Cancer is the one of the leading causes of premature death in the United States. With cancer’s intrinsic genetic heterogeneity and related drug resistance, discovering novel chemotherapies is a critical element in the ongoing effort to improve patient care. A screen using checkpoint -protein mutant *Drosophila melanogaster* larvae as a model for cancerous cells identified multiple known chemotherapeutics and several hereto unstudied compounds for their potentially synergistic effect on tumor mortality when paired with the conventional modality of ionizing radiation.

This experimental procedure was repeated with two of the novel chemicals identified, NCS 104129 and 681744, in a one-half dilution, eight-fold dosing series. Though it was expected that the series would reveal a dose-effect relationship, no such correlation was discerned. However, even in lower doses, both compounds demonstrated a statistically significant reduction in modeled tumor cell survival relative to the negative control groups.

Limited stock of the two compounds allowed for only a single iteration of each dosing-series. Further, certain doses yielded confounding outliers in survival rate data. Repetition of the experimental procedure would add confidence and clarity to the results obtained. Moreover, that both compounds consistently exhibited significant variance from the negative control suggests additional study may be warranted.

Introduction

Cancer, a group of diseases characterized by the unconstrained growth and spread of abnormal cells secondary to genetic mutation, is the second most prevalent cause of death in the United States. In 2018 alone, it is estimated that 1.7 million new cancer diagnoses will be made. In that same time-period, over 600,000 Americans are expected to die from cancer.

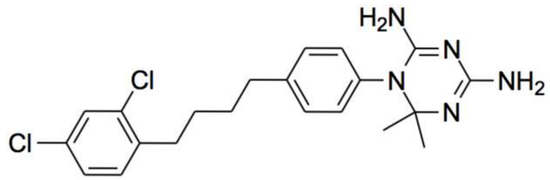
Expected 2018 cancer diagnoses by state.

Considering the prevalence and consequence of cancer, expanding the number of treatment options is a necessary endeavor. Both chemotherapeutic agents and targeted radiation have demonstrated efficacy in eliminating rapidly dividing tumor cells; unfortunately, these modalities have deleterious side effects. Further, cancer cells commonly have intrinsic or acquired resistance to single agents when they are applied alone. As such, combinatorial therapy, where the two approaches are applied concurrently and at relatively lower doses, can minimize side effects and increase treatment efficacy.

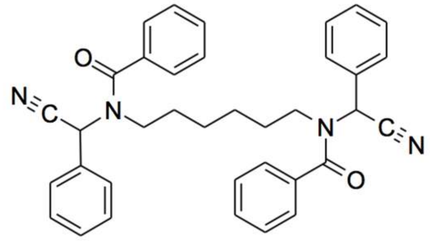
Exploring multi-modality combinations through clinical trials has proven costly and ineffective. By identifying and investigating potential chemotherapeutic agents from the National Cancer Intsitute’ s Mechanistic Set IV that synergize with radiotherapy using *Drosophila Meanogaster* checkpoint mutants as an *in vivo* model, the Discovery Lab presents a novel and economical avenue to expanding and improving the oncologic armamentarium.

Hypothesis

The two compounds, NSC104129 and NSC681744, will exhibit drug like behavior in a dosing series. These compounds were selected based off the results of the screen performed by MCDB 2171 students. In the initial screen, both compounds resulted in a reduced percent survival in *Drosophila* third-instar larvae in line with the positive control, Colchicine. If the compounds behave like drugs, at lower concentrations the percent survival will be higher, while at higher concentrations of compound, percent survival will decrease.



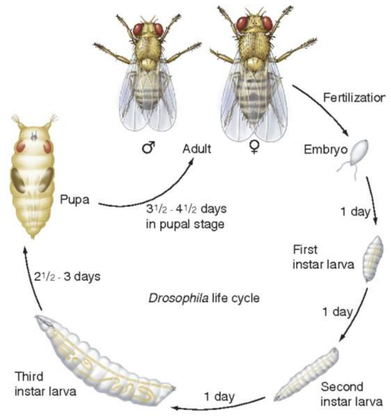
NSC Compound 104129



NSC Compound 681744

Methods

1. **Larvae exposure to radiation and to compound:** *Grp* mutant *Drosophila melanogaster* third instar larvae were exposed to 4000 rad of ionizing radiation. Irradiated larvae were added to vials containing a dosing series of our two chosen compounds, NSC104129 and NSC681744. The series consisted of eight 1:2 dilutions ranging from 5 µM to 0.039 µM of drug.
Rationale: The use of *grp* mutant *Drosophila* ensured the flies were susceptible of radiation, which provided the basis for identifying a drug with synergistic effects.



***Drosophila melanogaster* life cycle.**

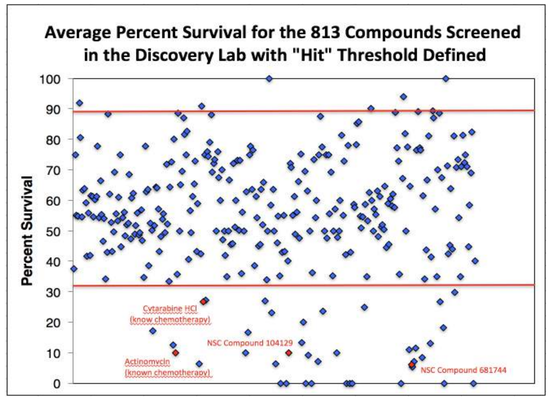
A dosing series of each compound was used to gauge whether the compound behaved as a drug or as a toxic substance that is unsafe at any concentration.
C

2. **Identification of *grp* mutants:** *Grp* mutant pupae were identified using blue light seven days after larvae were added to vials. Non-grp mutants had a green fluorescent protein that appeared green under blue light.
Rationale: Distinguishing between *grp* and *GFP* positive flies ensured the survival percentages calculated were for flies that were radiosensitive, enabling identification of a compound with synergistic effects. *GFP* is maintained in the population on a balancer chromosome to allow for differentiation between radiation susceptible *grp* mutants and heterozygotes that are radiation resistant.

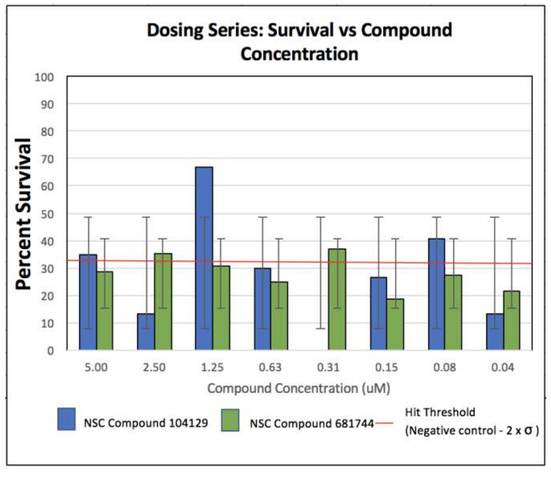
3. **Quantification of survival:** The survival of *Drosophila* larvae was determined by counting the number of pupae that had eclosed three to five days after marking the vials.
Rationale: The larvae in our model represented cancer cells; quantifying those that were are unable to survive exposure to the compounds and irradiation allowed for identification of potential chemotherapies.

4. **Data analysis:** Survival data was analyzed for each drug vial by calculating survival percentages and exploring the dose-response relationship. A negative control of DMSO and a positive control of 50 ug/mL colchicine was used to determine compound efficacy.
Rationale: Identify a potentially effective dose of the compounds tested on larvae.

Results



The compounds tested were among thirty two “hits” that included marketed chemotherapies.



The expected dose-response relationship was not observed in the dosing series trial.

Conclusions

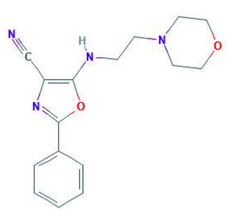
Based on the collected survival data, no clear correlation exists between the concentration of compound administered and larvae survival for either NSC681744 or NSC104129. While the low percent survival seen in the majority of vials validated that the compounds were effective at killing third instar larvae, further tests are needed to determine if the compounds simply have toxic properties or are able to predict survival with any success as a drug in a dosage series.

As the *grp* mutation present in our experimental *Drosophila melanogaster* population is homologous to the checkpoint kinase 1 mutation often present in head and neck cancers in humans, the success of our experimental compounds to prevent survival of the larvae could be indicative of their success in preventing tumor growth in humans suffering from cancer.

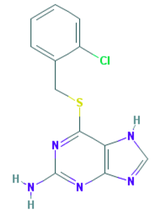
Future Directions

Due to limited availability of library compounds, only one trial of each dosing series was completed in this experiment. Additional trials could further validate our results and provide more reliable evidence of each compound’s behavior in a dosing series.

There were two additional compounds in the National Cancer Institute library that were screened as hits and shared similar functional groups and structural features with the compounds tested in this experiment. Tested compound NSC681744 contains a nitrile group, as does the screened hit morpholine-N-dithiocarbamate. Tested compound NSC104129 contains a dichlorobenzene ring, screened compound NSC36826 contains a chlorobenzene ring. Future testing of these compounds could help identify functional groups with chemotherapeutic effects.



Morpholine-N-dithiocarbamate



NSC36826

Acknowledgments

We would like to thank **Dr. Pamela Harvey** for serving as our course instructor along with our teaching assistants Alia Alsaif, Isabella Shelby, Julianna Rohn and Ben Huxley. We would also like to thank **Dr. Tin Tin Su** for serving as the principal investigator and sponsor for our lab. We also acknowledge funding and supporting organizations: Howard Hughes Medical Institute, CU Boulder Molecular, Cellular, and Developmental Biology department.

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Effects of Blue Scorpion Venom in a Model for Head and Neck Cancers

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Abstract

Using our model organism, *Drosophila* larvae, we were able to mimic head and neck cancer tumor cells. This is due to the *Grapes* mutation we induced in our larvae, homologous to the Checkpoint kinase 1 gene mutation seen in head and neck cancers. In addition, the larvae's rapid growth mimics the cell division rate of cancer cells. Our drug, Vidatox, is derived from 5 peptides found in the Cuban Blue Scorpion (*Rhopalurus junceus*) venom.

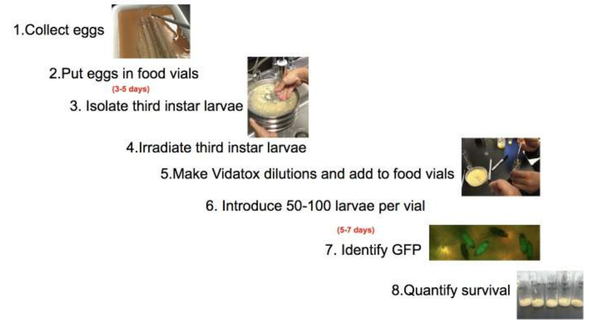
This specific scorpion venom has been used in traditional Cuban medicine. Our drug has been speculated to stop angiogenesis, spark apoptosis, and stimulate the immune system, all to fight tumor cells. We will be testing Vidatox's effectiveness in terms of fly survival in order to determine if this compound has the potential to become a chemotherapeutic drug. We believe that our more concentrated dilution of Vidatox will yield lower survival rates, indicating a potential hit. By comparing our data to DMSO, we will be able to confirm the validity of our results.

Introduction

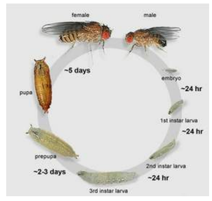
One in three people will be diagnosed with cancer in their lives. Cancer is caused by unusual gene expression. In cancer cells, oncogenes may be activated and tumor suppressor genes inactivated, causing an uncontrolled dividing of mutated cells. Using this idea of rapidly dividing cells, we can test possible cancer drugs on rapidly dividing Drosophila larvae. To mimic radiation treatment of cancerous tumors, we irradiated 3rd instar larvae that have the *grapes* gene mutated. The *grapes* gene is a homologue to the Checkpoint Kinase 1 gene in humans. This Chk1 mutation is most common in head and neck cancers. This mutation allows the cell to pass the first checkpoint in the cell cycle; in normal cells the replication process would stop the cell to repair DNA damage.

The drug we will be testing is called Vidatox. It is currently registered as a homeopathic drug to a 30CH dilution in liquid form. Its dosage for a human is 1-2 drops. It is derived from the venom of the Cuban Blue Scorpion (Rhopalurus junceus). Vidatox uses five of the peptides found in the venom of the Cuban Blue Scorpion. Historically the scorpion venom was used in traditional Cuban medicine as a pain relieving and anti-inflammatory drug, which gives Vidatox promise of also being used for palliative care. Also, the venom has been studied and shown to stimulate the immune system, induce apoptosis, and block angiogenesis. Both avoiding apoptosis and sustained angiogenesis are hallmarks of cancer which indicates that Vidatox will be successful in cancer treatment. Vidatox is however a complementary and alternative medicine (CAM), so we must be skeptical with results, as other studies have shown it could activate tumors more.

Methods



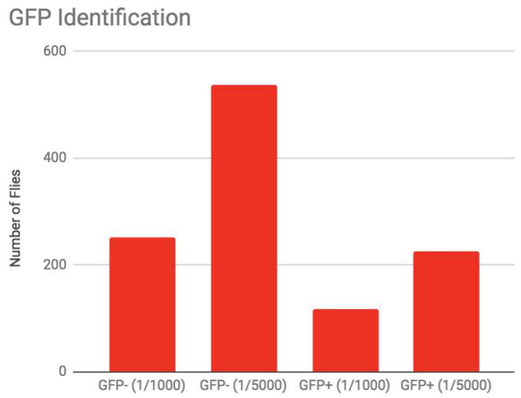
The life cycle of the drosophila begins with the embryonic stage and moves on to larval stage. There are three stages of larvae beginning with the 1st instar larvae growing into 2nd instar and finally 3rd instar larvae. Our experiment focuses on the 3rd instar larvae because their rate of cell division mimics the rate of cancer cell growth and they contain a similar mechanism to the checkpoint kinase found in human cells. The larvae then pupates until it is ready to eclose into an adult fly. We look at which flies have eclosed in order to determine the survival rate of our flies. If there is a whole fly still in the pupa or if the fly has liquified, the flies are quantified as dead. If the pupa is completely empty or partially eclosed the flies are quantified as alive.



Hypothesis

By testing Vidatox on *Drosophila* larvae, we can determine if Vidatox will work synergistically with radiation to treat head and neck cancers. Our model organism is similar to head and neck cancer tumor cells because the larvae cells are are rapidly growing.The Drosophila we are testing already have the grapes mutation, making them more susceptible to DNA damage when they undergo radiation. The *grapes* mutation is similar to the Checkpoint Kinase 1 mutation in humans. In order to identify the grapes mutation, we quantified GFP – and GFP+ to determine which flies were homozygous or heterozygous for the mutation. We wanted to isolate the GFP- as they have the homozygous mutant alleles, thus they are more susceptible to radiation, and better mimic tumor cells. The GFP gene codes for Green Fluorescent Protein and, in flies where it is transcribed, they appear fluorescent green under a black light, allowing selection of GFP positive and negative drosophila. Since the grapes mutation in drosophila is similar to the Checkpoint Kinase 1 mutation which is common in head and neck cancers in humans, drosophila were an ideal model organism for our experiment. Once the flies have ingested Vidatox and pupated, we can quantify for survival based on how many flies have eclosed. We can further compare the percent survival of our flies to our negative control of DMSO in order to get a more accurate idea of the effectiveness of our compound.

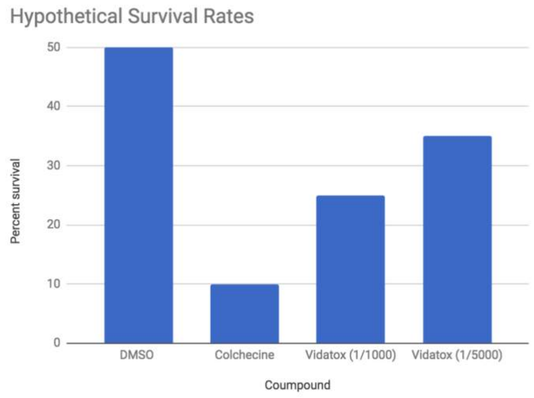
Results



GFP Identification: These results represent the total number of flies that were GFP- or GFP+ for both concentrations of Vidatox (1/1000 and 1/5000)

Analysis: Our results did not follow the expected trend of GFP- to GFP+ ratios. Theoretically, 2/3 of the flies should be GFP+ and only 1/3 of the flies should be GFP-. Our data demonstrates a conflicting pattern as there were significantly more flies that were GFP-. We expect this to affect the survival rates of our flies in both concentrations of Vidatox because our compound could be interacting with the GFP balancer chromosome, explaining the discrepancies in our results. In addition, our number of flies are not representative of a full population since we did not attain the ideal GFP-/GFP+ ratios.

Anticipated Results



Hypothetical survival rates: The graph demonstrates the predicted survival of our compound compared to our positive and negative controls. Because the flies are being irradiated, 50% of the flies exposed to DMSO will be killed. Although, without radiation, DMSO would have no effect on the survival rate of the flies. The Colchecine reduces percent survival to 10%. We expect our 1/1000 dilution of Vidatox to reduce percent survival to 25%, and the 1/5000 dilution to 35%.

Further DIrections

If we could be in the lab for an additional semester and if we had unlimited funding, we would first want to repeat our experiment to see if we could get more standard data in terms of GFP- and GFP+ ratios. Ideally there should be more GFP+ which indicates a genotype that includes a balancer chromosome and one copy of the mutation. However, in our drosophila populations there were more GFP- flies which are homozygous for the mutation, which is not indicative of predicted ratios. There is a possibility that there was an interaction with the *grapes* mutation and our drug, and the ratio of GFP in our drosophila could cause skewed results. Increasing our population size or using later generations of drosophila may yield a more realistic ratio of GFP and produce more accurate results. Additionally, we would want to test different dilutions in order to get an ideal Vidatox dosage. Testing pure scorpion venom extracts is another experiment we would run because it could possibly yield different results since Vidatox is a compromised version of scorpion venom containing only 5 peptides found in venom. Testing the compound on wild-type flies as well as running the experiment without administering radiation would lead to different survival rates and effectiveness, and would be a promising experiment to determine the extent of Vidatox as a treatment or a palliative drug. If our drug was a confirmed hit, outside of two standard deviations from the mean survival rate of DMSO, we would next move onto pre-clinical trials that would involve testing with mice because we would need to test Vidatox in an mammalian organism, since mice would be a model for human biological processes.

Acknowledgments

We would like to thank Dr. Tin Tin Su for being the principal investigator for the laboratory that sponsors our independent experiments. We would also like to thank Pamela Harvey for being lab director and teaching us how to properly conduct research, and for Jesse Kurland's guidance in our research projects. We are grateful for the Molecular, Cellular, And Developmental Biology department and the Biological Science Initiative at University of Colorado Boulder for their continued support of the Discovery Lab. Lastly for the Howard Hughes Medical Institute for funding our research.

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Effects of 6-Shogaol on *Drosophila* Development and its Application to Cancer Treatment

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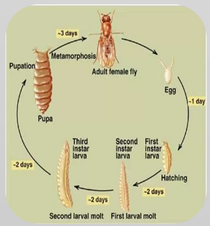
Abstract

[6]-Shogaol, an active constituent in ginger, has shown potential in reducing the size of tumors by targeting rapidly dividing cancer cells, and extended research on the compound could provide insight into the fight against cancer. In our experiment, we used third-instar *Drosophila melanogaster* larvae as a model for cancerous tumors, and tested the effect of [6]-Shogaol on development and survival as adult flies. Although we are their still awaiting results, our findings thus far suggest that nearly all the *Drosophila* either died in the pupal stage, or are developing unusually slowly. However, since this result was also true for the negative control containing ethanol, we cannot credit it to just the [6]-Shogaol. Ethanol may have played a role in significantly slowing development of the *Drosophila* , and the effects of [6]-Shogaol must be studied using a different negative control in order to make any conclusions as to its effectiveness.

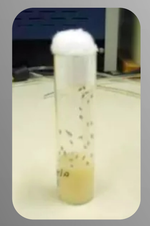
Methods

The procedure of raising and preparing the larvae for drug administration is as follows: First we maintained adult flies in population cages, where they mated and laid eggs. These eggs were then collected and put into food bottles until they developed into larvae. The larvae were put through three stacked sieves, and sorted by size into first-instar, second-instar, and third-instar larvae. The third-instar larvae are collected from the 600-850 µm sieve and irradiated at 4000 rads. This is meant to mimic the administration of radiation before chemotherapy.



After this, they were added to the prepared drug vials for testing. These drug vials were filled with fly food that contained a series of dilutions that we had made from our compound. This series of dilutions included concentrations of the following:

- 1.000 mg/mL [6]-Shogaol in ethanol
- 0.500 mg/mL [6]-Shogaol in ethanol
- 0.250 mg/mL [6]-Shogaol in ethanol
- 0.125 mg/mL [6]-Shogaol in ethanol
- Pure ethanol



Originally, our compound came in a 1mg/200µL of ethanol, so to make a stock solution of 1mg/mL that we could use to make the rest of our dilutions, we added 800µL more of ethanol. With this stock solution of [6]-Shogaol, we created three dilutions of incrementally decreasing concentration in 1:2 ratios. Because our compound was originally a powder that had been dissolved in ethanol, we used ethanol alone as our negative control, and prepared the dilutions using ethanol. In each drug vial we added 3 mL of fly food, 30µL of each dilution, and 100 µL of water.

We incubated the vials for 10 days at 25 degrees Celsius. After 10 days we quantified the fly's survival by shining the vials under a light to show GFP positive and negative flies. We then quantified survival of GFP negative flies by counting how many have successfully eclosed and how many have failed to eclose.

The Problem

Cancer encompasses more than 100 specific diseases, and therefore has a broad range of symptoms, but commonly includes tumors, unexplained fevers, night sweats, and unintentional weight loss. Cancer is a common disease in which abnormal cells divide and destroy healthy tissues. In 2016, approximately 1,685,000 people were diagnosed in the United States, and nearly 600,000 people died of the disease. 39.6% of people will be diagnosed with some form of cancer in their lifetime. Typically, cancer is treated with radiation and chemotherapy; however, drug resistance is becoming an issue and some chemotherapies are becoming too toxic, which is why it is vital that research for new, safer drugs becomes a priority.

Hypothesis

Third-instar larvae treated with the dilution series of [6]-Shogaol will have a reduced eclosure rate of the flies as the doses of the compound increases.

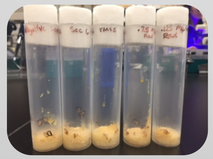
Objectives

The goal of this experiment is to understand the effects of the compound 6-shogaol on the development of *Drosophila melanogaster* from third-instar larvae into adult flies. If successful, the 6-shogaol will either stop larvae development into adulthood or kill the larvae altogether. We will use our results and consider their potential application in treating cancer - more specifically, in shrinking or stopping the growth of cancerous tumors.

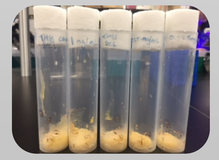
Results

Due to a limited amount of time for the trial, we are still awaiting results. However, *Drosophila* usually take between 3 to 5 days to develop into the third instar larvae- even after that time frame our larvae have yet to eclose. Because this slow development is observed in both the vials containing the drug and vials containing the negative control, we cannot make any conclusions regarding the effectiveness of the [6] -Shogaol compound. This result indicates that ethanol may have played a role in inhibiting *Drosophila* development. Therefore, further studies of [6]-Shogaol would require a different negative control in order to clearly decipher its effects.

Trial 1



Trial 2



Introduction

The nature of cancer has made it very difficult to treat as cancer has many origins, whether from genetic disorders or inherited dispositions, the outcome is often the same. One of the most common causes of cancer is a random mutation whether in the cell cycle or DNA that goes unchecked that allows the cancer cells to divide and spread rapidly.

Third instar larvae of the *Drosophila melanogaster* are used as models of cancerous tumors because of their abundance of rapidly dividing stem cells that mimic the fast growth of cancer cells. They also contain grp genes, which creates a protein that is very similar Chk1 found in humans. Chk1 mutations are often found in head and neck cancers, so the results are specifically for these types of cancer.

The pharmacokinetics of [6]-Shogaol, a compound commonly found in ginger, have been studied and shown to be a potent inhibitor breast cancer as it targets the NF-κB activation cascade, to prevent the spread of cancer by inducing apoptosis. Recent evidence also suggest its ability to inhibit growth in ovarian cancer cells. This is due largely to the phenolic alkanone present in [6]-shogaol, which damages the microtubules and induces mitotic arrest and thus inhibits the production of cancer cells.

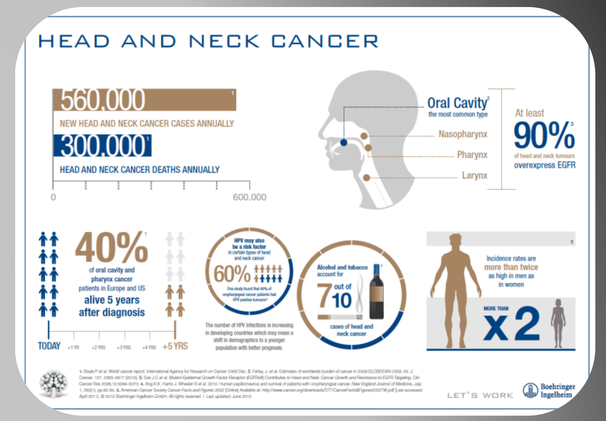
Discussion

While we were not successful in deciphering whether the [6]-Shogaol has a significant effect on *Drosophila* development due to the strength of our negative control, we can still make inferences regarding its application to cancer treatment. From our results, our most likely conclusion is that either the ethanol or the [6]-Shogaol- or the combination of the two- significantly slowed *Drosophila* development without killing them. Because our negative control showed no signs of development past the pupal stage, we can say with more certainty that ethanol alone could have made a big impact on slowing development.

Although our desired outcome in testing a compound is no survival, our actual result has some interesting implications. Today, treatments for cancer are not ideal, not only because of inevitable drug resistance, but also because of their many side effects caused by toxicity. Doses must be carefully assigned by maximizing effectiveness while minimizing effects of toxicity. If ethanol did indeed slow *Drosophila* development without killing the *Drosophila* , it may offer insight into minimizing drug toxicity. This concept could be applied to the growth and development of cancer cells, and the possibility of containing tumors and stopping or slowing metastasis.

Acknowledgments

We would like to thank Dr. Tin Tin Su, the principle investigator of our sponsor laboratory, for providing her provision and expertise, and allowing us to be a part of this incredible research process. We extend our thanks to Dr. Pamela Harvey, who has guided us through our Drug Discovery education and has been a huge promoter in our success as undergraduate researchers . We would like to thank the Department of Molecular, Cellular, and Developmental biology , the Howard Hughes Medical Institute for their funding for undergraduate research education, and the Biological Sciences Initiative (BSI) at CU Boulder for supporting our research. Finally, we would like to thank our Teaching Assistants (TAs), Lindsey Visscher, Jesse Kurland, and Nima Shokrani for guiding and supporting us through the duration of our experiment.



Future Directions

In order to see the effects of the 6-shogaol in combination with other compounds found in ginger, we could test vials with ginger to see how or if the survival rate is affected by the effects of 6-shogaol in combination with gingerol and other compounds commonly found in ginger. This also opens up an exploration in diet; incorporating ginger in the diet of cancer patients could have a different effect than if used strictly as a drug, both as far as concentration absorbed and in how it is broken down in the body's digestive tract. Additionally, we could've chosen to create vials without first radiating the flies, thus observing the effects of combination therapy on the model tumors. Finally, for future experiments we could conduct more research to find the effective dosage of 6-shogaol for humans and then scale it down for flies so we can create a more effective dosing series.

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The Effects of Allicin on *Drosophila melanogaster*

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Abstract

Cancer is one of the leading causes of morbidity and mortality worldwide and it is ranked the second leading cause of death globally in 2015.What makes cancer so problematic is not its large number of cases, but rather its treatment strategies. Since malignant tumors have the ability to build resistance to chemotherapies that they are exposed to, it is essential to pursue and continue to find more chemotherapies that will reduce cancer resistance, but to also prevent cancer cell proliferation. A potential compound that was found that may prevent cancer cell proliferation is Allicin, a compound that is derived from garlic. Based upon previously published experiments, Allicin has been found to have both preventative and cancer suppressant properties. Allicin has been reported to induce cell apoptosis and therefore being able to prevent cancer cell proliferation. Further evidence has indicated that Allicin has an inhibitory effect on many kinds of tumor cells. We aim to test these the results out and see their potential uses using our model based upon third instar larvae because they can replicate at a similar rate as malignant tumors.

Introduction

Allicin is a possible effective compound to prevent cancer as well promote cancer cell apoptosis, which could be a potential approach with anti-cancer treatments, specifically in thyroid cancer. Allicin is extracted from garlic. Thyroid cancer is ranked as the most prevalent endocrine malignancy. Furthermore, thyroid cancer frequently exhibits resistance to standard chemotherapy regimens. Autophagy is a physiological process in the body that deals with destruction of cells in the body. It is supposed to maintain homeostasis and normal functioning of destroyed cell organelles for new cell formation. Allicin induces autophagy-dependent cell death even despite if the cancer cells have developed apoptosis resistance. Allicin was found through an in vitro experiment that Allicin inhibits cancer cell proliferation and induced cell apoptosis. However, tests of Allicin on thyroid cancer remains elusive that it improves multidrug resistance. It is possible that Allicin could serve as an adjunctive therapy.

Unfortunately, it is difficult to determined how effective and the dosage amount of Allicin that could be absorbed into the human body. Allicin cannot be detected in humans up to 24 hours after ingestion because they are found to be rapidly metabolized. It is found to be rapidly metabolized because Allicin breaks down releasing a number of volatile compounds that results and detected in human breath after consumption. It is also detected in urine within about four hours of ingestion, which suggests that it is absorbed into the blood and rapidly excreted.
For the initial drug screening, we used a dose of compound that can commonly be achieved in human serum, 10 mg/mL. The reasoning for this is because there was no information regarding the serum concentrations for Allicin. According to Oregon State University, Allicin is rapidly metabolized to the extent that there is little information about the absorption values. The negative control is Dimethyl Sulfoxide (DMSO) because it does not inhibit the growth of cancer cells. The positive control is Colchicine because it was proven to inhibit cancer cell proliferation. Included in the initial screening, irradiated larvae was used because Allicin is being tested as an effective chemotherapy or not.

Grapes (grp) is the gene that encodes Checkpoint Kinase 1. Checkpoint Kinase 1 prevents entry into mitosis if it senses that the cells has a problem with DNA. This is a similar process in humans. We are using grp^1/grp^1 larvae to act as the mutations found in human tumors that disrupt cell cycle checkpoints. We mark GFP of the larvae to determine which larvae has the mutated grape gene. The larvae with GFP are susceptible to radiation. Based on the markings of GFP, we quantify survival by counting the number of closed and empty pupal cases

Hypothesis

Currently, the drugs that are available for treatment of cancer are quickly becoming unusable due to the the resistive properties of cancer. We need to be able to find more and more possible treatments to cancers. These cancers are increasingly difficult to treat due to them being multidrug resistant.

Specific Hypothesis: We hypothesized that Allicin, a compound in garlic, would be an effective chemotherapy compound for the treatment of tumors that were commonly found in the head or neck. Due to Allicins ability to induce apoptosis, we determined that it would be a good compound to test in cancer treatment.

Purpose

1. Test compounds for their ability to limit growth and survival of third instar Drosophila larvae representing tumors typically in the head and neck.
2. Hypothesize the effectiveness of promising compounds based on their ability to inhibit survival of third instar larvae.
3. Conduct multiple trials and experiments that allow us to determine whether a “hit” may be an effective chemotherapy.

Methods

1. Raise and Maintain Adult Drosophila

Collect eggs from population cages and allow them to grow in culture bottles full of fly food. This allows the flies to develop and undergo rapid cell division. We incubated transferred flies for 5 days. This step is expressly controlled with regards to the environment in which we allow the eggs to hatch and develop.

2. **Collect Third Instar Larvae from the Culture Bottles**

We then collected the third instar larvae from the egg basket by running water over the sieve so that the smaller and less dense fly larvae that are in the early stages of development wash out. This leaves us with only third instar larvae. We use the third instar larvae because they are an effective representation of a head and neck tumor due to rapid cell proliferation.

3. **Irradiate Third Instar Larvae**

After collecting third instar larvae, we irradiate them using 4000 rad gamma radiation for approximately 12 minutes. Providing a coupled treatment such as radiation allows us to administer our compound at lower doses. We know that stem cells are more radiosensitive than somatic cells. The ionizing radiation breaks the DNA double helix to damage the DNA of the rapidly dividing cells.

4. **Drug Administration and Experimental Setup**

After collecting and irradiating the third instar larvae we placed 3 mL of fly food mixed with either a concentration of 10 mg/mL or 5 mg/mL of Allicin mixed into the food. We then placed roughly 50-150 irradiated third instar larvae respectively into the culture vials with the food and the drug.

5. **Mark GFP (Green Fluorescent Protein) in Flies**

Two days after administering the drug, using modular stereomicroscopy we marked the flies for the expression of green fluorescent protein. This allows us to determine the fly genotypes. GFP-negative flies are homozygous for the mutation in the grapes gene and GFP-positive flies are heterozygous for the mutation. Heterozygotes are more susceptible to the radiation than the GFP-negative flies. All of the wild type flies end up dying. This is a crucial step to effectively quantifying survival.

6. **Quantifying Survival**

The final step is to quantify survival by counting the number of empty (alive) and closed (dead) pupal cases. We quantify survival of GFP-positive and GFP-negative flies separately in order to get more accurate and controlled results. Using these results, we can determine the effectiveness of the drug.

Results

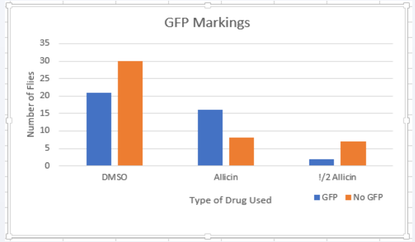

Figure A: This graph indicates the number of flies that had GFP. Those marked that had GFP express grp^1/grp^1, which means that they were susceptible to radiation. The concentration of Allicin was 10mg/mL. The volume of DMSO and the Allicin were the same to account for any toxic effects of the compound or nutrient depletion. Half of the dosage for Allicin was also tested. The blue bars are designated to show the flies that had GFP and the orange bars are designated to show flies that did not have GFP.

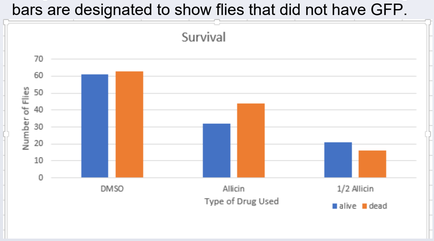

Figure B: This graph indicates the number of flies that survived or died after going through radiation and chemotherapy. In comparison between the negative control (DMSO) and Allicin, the data shows that Allicin was working as a chemotherapy because there were less flies surviving, which is what is wanted since that indicates that the model cancer cells are dying. In comparison between the full dosage of Allicin and the half concentration of Allicin, the data indicates that the chemotherapy would have worked more effectively with half the concentration of Allicin. This may not be accurate because only one trial was ran for having half the concentration of Allicin. The blue bars show the living flies and the orange bars show the dead flies.

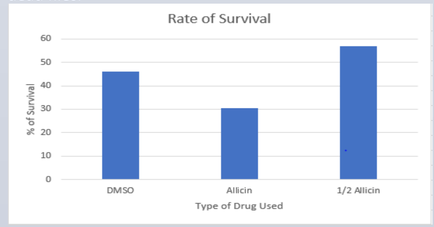

Figure C: This graph indicates that percent of survival that is was calculated from Figure B. In comparison between the negative control (DMSO) and Allicin, the data shows that Allicin was working as a chemotherapy because there was a lower percent of flies surviving, which is what is wanted since that indicates that the model cancer cells are dying. Comparing the half concentration of Allicin, this indicates that half the concentration of Allicin may not be toxic enough to kill the flies. The bars show the percent of living flies.

Conclusions

Since the value for Allicin is less than two standard deviations from the mean survival of the negative controls, it is identified as a “hit.” Our results suggest that testing Allicin on tumor cells in vitro would be an even more effective way to determine the overall therapeutic effect. One limitation to our experiment was the lack of literature on metabolic rates and dosing for Allicin. As a result of this we chose to use a dose that is commonly found in human serum of 10 mg/mL as well as 5 mg/mL. Because we observed a significantly lower survival rate in flies given the full dose of 10 mg/mL than the half dose of 5 mg/mL, a full dosing series would be a logical next step to determine a more therapeutic dose. Overall, Allicin significantly inhibited tumor representing larvae to survive to adulthood.

% Survival Hit Values (DMSO)	
Mean of Allicin	30.38%
Mean of Controls	46.15%
2 SD Above Mean	83.05%
2 SD Below Mean	36.90%

Future Directions

1. We would like to perform a dosing series for Allicin.
2. We could try different methods of integrating Allicin into the food such as using a diluted solution or adding in the Allicin while we are creating the food.
3. If results are effective, *in vivo* studies may be effective
4. Combining Allicin with Rapamycin, which has been shown to be effective in treating cancers in other studies.
5. We can try working with non-irradiated larvae to see how the effects differ from irradiated larvae.
6. We would also like to try adding 50mg/mL of Allicin to see its effects, and whether or not it kills more flies.

Acknowledgments

We would like to thank Dr. Tin Tin Su and Dr. Pamela Harvey for overseeing our entire operation. At this time we would also like to thank the funding sources for this lab including the Department of Molecular, Cellular, and Developmental Biology, Howard Hughes Medical Institute, and the Biological Sciences Initiative. Other contributors to our success in Drug Discovery include our wonderful TA’s Alia Alsaif, Katie Franks, Erin Kneeskern, Jack Schutz, and anybody else without whom our work would not have been possible.

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Effects of Thalicarpine as a Combinational Therapy with Radiation

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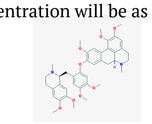
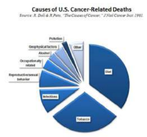
Abstract

Our project's goal was to identify a hit from our class's drug screen to further test its effectiveness in lower doses. We tested the drug in several concentrations and compared it to our negative control in our model organism, Drosophila Melanogaster. Drosophila was a good model organism for our purposes in identifying a hit due to its similar biological pathways relative to a human. Furthermore, Drosophila has a genetically modified *grapes* gene that allows cells to continue through mitosis without correcting DNA damage, similar to cancerous cells. In our independent project, we decided to test the compound Thalicarpine on the Drosophila with the genetically modified *grapes* gene. Thalicarpine binds to and inhibits p-glycoprotein, the multidrug resistance efflux pump. In our experiment, we wanted to create a dosing series to determine if the compound will be effective at lower doses. We also had a negative control of dimethyl sulfoxide (DMSO); a solvent for our compound. Our expected results were that our dose of 5 micromolar will be effective; however, we did not believe the 2.5 micromolar conentration will be as effective as the other doses.



Introduction

Cancer is the second leading cause of death in the United States. According to the American Cancer Society, in 2018, there will be an estimated 1,735,350 new cancer cases diagnosed and 609,640 cancer deaths in the United States. As well, the number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024. Cancer can affect men and women, healthy or sick, and young or old. The disease can be caused by genetic mutations, poor lifestyle choices, or chance. Cancer does not have a set of symptoms, as it can affect any part of the body. It can be extremely hard to diagnose early. For example, pancreatic cancer is usually diagnosed at a late stage because it does not show symptoms until the cancer has spread throughout the body. Because cancer is hard to catch early, it is difficult to treat.



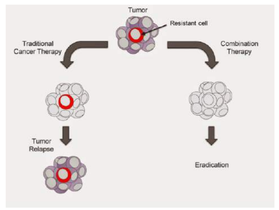
Cancer is a genetic disease that occurs when DNA is damaged or mutated, leading to to abnormal patterns of expression or replication. Thus, the effects of the normal genes that control cell growth, survival, and spread are enhanced and those that suppress these effects are repressed. Driven by two classes of genes, oncogenes and tumor suppressor genes, there are key changes that occur in cancer cells that drive their malignant behavior. Also known as the "Hallmarks of Cancer", some of these changes include: evading apoptosis, growth factor independence, insensitivity to antigrowth signals, and invasion and metastasis. These factors are essential in the identification of possible drug functions that can inhibit cancer cell growth.

As of right now, only three main treatments are administered to combat this illness including: radiation, surgery, and chemotherapy. 50% of all cancer patients undergo chemotherapy treatment, but it only has a "success" rate of 10-15%. The main problem with commonly used chemotherapies is that they are highly toxic to normal somatic cells, which lead to various side effects. Side effects can be short term, or develop over time and affect patients for the rest of their lives. Short term side effects consist of hair loss, fatigue, nausea, mouth and throat sores. Long terms side effects include cognitive problems, various heart problems, and in women, early menopause. Finding a smaller effective dose is essential in order to minimize the side effects of chemotherapy to the normal somatic cells.

In our independent project, we decided to test the compound Thalicarpine. Thalicarpine binds to and inhibits p-glycoprotein, the multidrug resistance efflux pump. Thalicarpine also induces single-strand breaks in DNA and arrests cancer cells at the G2/M and G1 phase of the cell cycle. In our initial drug screen, flies with the grapes mutation had a 26.7% survival rate, and flies without the grapes mutation had an 11% survival rate; thus, we identified it as a "hit." The 10 micromolar concentration of the compound resulted in a low survival rate. We want a compound with a high therapeutic index; thus, we can administer lower doses with less side effects. Because chemotherapy is toxic to the surrounding normal cells as well as the tumor cells, our goal is to find a compound we can use at the lowest effective dose so we can minimize side effects from the drug.

Hypothesis

We hypothesize that our compound will still be a hit at a lower dose. When coupled with radiation, our compound will be effective as a chemotherapy even with the lower dosages. We expect that 10 microomolar and 5 micromolar concentrations of Thalicarpine coupled with radiation will be "hits" but, the 2.5 micromolar concentration will not be.



Methods

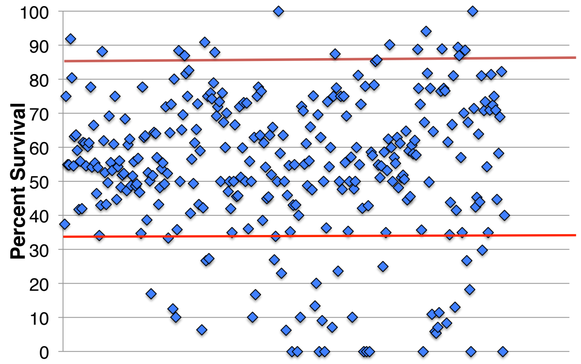
First, we chose a chemotherapy that was identified as a "hit" from Diversity Set IV in a previous drug screen in the Drug and Discovery Lab. We maintained an adult fly population, harvested embryos, and collected 600-800µm larvae. We chose to irradiate the larvae because chemotherapy is commonly administered as an adjunct therapy to radiation therapy. We bred the flies to obtain a *grps* mutation which inactivates a checkpoint protein, causing the radiation to be more effective because mitosis won't be arrested, allowing the DNA damage to persist.

After irradiating the larvae, we transferred the larvae into the vials with the drug and food mixture. We used 11 drug and food vials overall. For each control and dilution we used two trials, 3 µL being the only exception, in order to increase the accuracy of our results. Our positive control was 50 µL/mL colchicine. Colchicine is a proven effective chemotherapy that is known to inhibit mitosis. We also used a negative control: DMSO (Dimethyl sulfoxide). This is a solvent for our chemotherapy which should not harm the larvae. We used controls because it serves as a baseline to determine how effective our compound is. We were given 9 µl of our compound, and we will test 3 µl in our first vial to verify that the compound was indeed a "hit". After the initial test, we will use two vials for each of the different compound amounts. In vials 2 and 3, we will use 1.5 µl of the compound. In vials 4 and 5, we will use 0.75 µl. In vials 6 and 7, we will use 3 µl of DMSO (our negative control) in each vial. Vials 8 and 9 will have 1.5 µl of DMSO, and vials 10 and 11 will have 0.75 µl of DMSO. All vials will have either the drug compound or the DMSO mixed with 3 ml of food. We will conduct this experiment double blinded in order to avoid bias in our results.

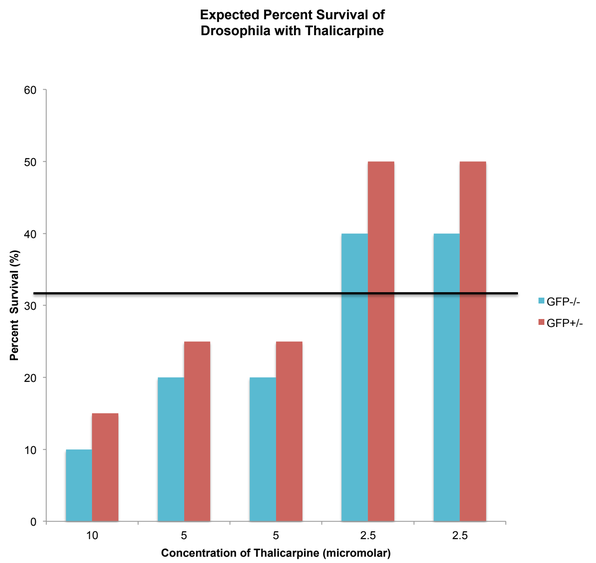
After the larvae incubate and are able to crawl up the walls of the vials, we determine if the flies have to *grapes* mutation. The flies without the *grapes* mutation will glow green under UV light because they have a phenotypic marker known as GFP (green fluorescent protein). The flies with the *grapes* mutation will not glow; we want to quantify the survival of these flies because they accurately model tumor cells. After 10 days of incubation, we account for survival of the flies. After quantifying the survival of all the GFP negative flies for each dilution, we determined the lowest possible effective dose for our given compound.

Results

Below are the results from all the compounds we tested in our initial drug screen. The hits are below the red line. Thalicarpine is one of these hits.



Shown below is the expected percent survival for our varying dosages. There should be slight differences between each concentration due to chance. As the concentration of Thalicarpine decreases, the percent survival should increase. The line on the graph represents the percent Survival that determines if the compound is a hit. It needs to be under 32.9% to be a "hit." This is based of the class data from the first part of our experiment.



Conclusion

In our independent project, we decided to test the effectiveness of Thalicarpine at lower doses. Thalicarpine was chosen because it was a hit from our main lab's original drug screen. As a part of our independent project, we re-tested Thalicarpine to prove that the possible lead compound had been correctly identified. Thalicarpine was tested on our model organism, Drosophila, with the genetically modified *grapes* gene, which allows cells to continue through mitosis without correcting DNA damage, similar to cancerous cells.

To find the compound's lowest effective dose, we had different concentrations of Thalicarpine mixed with 3mL of food, yielding the following concentrations: 10.0 uM, 5.0 uM, 2.5 uM. According to our expected results, 10.0 uM and 5.0 uM proved to be effective, acting in accordance with our hypothesis. Based on the expected data, 5.0 uM of Thalicarpine proved to be the lowest effective dose against our model organism, Drosophila.

However, our independent project was limited in the sense of drug concentration and time. During this lab, our group was only supplied with 9µL of Thalicarpine, thus limiting the range of dosages we could test. Given more of the compound, it would've been possible to test more dosages, thereby getting a more precise measurement of the lowest effective dose. In tangent, given more time we would've been able to obtain real results, either confirming or denying our expected results.

Future Directions

This experiment suggests possible future research including:

1. Quantify survival of the third instar larvae and determine if Thalacarpine is a "hit" at a lower dose.
2. Obtain more of the Thalicarpine (compound 93-8 from Diversity Set IV) drug in order to test a wider range of possible effective dosages. This will lead to a more refined graph that will hopefully reveal the lowest therapeutic dose in our model organism *Drosophila.*
3.Test Thalicarpine *in vitro* and *in vivo* on an isolated tumor cell that would be commonly found in the head and neck regions of an affected patient .
4.Test Thalicarpine on a model organism with cancer, such as a mouse. These tests will provide information on the pharmacokinetics of the drug.
5.Modify Thalicarpine to reduce its effects on other chemical pathways, while also increasing its efficacy on the desired chemical pathway. This will result in an optimized lead compound that can spearhead further preclinical studies.

Acknowledgments

We would like to thank **Dr. Pamela Harvey** for serving as our research instructor and **Dr. Tin Tin Su** for serving as the principle investigator and sponsor for our lab. We also acknowledge funding and **supporting organizations**: Howard Hughes Medical Institute, CU Boulder Molecular, Cellular, and Developmental Biology department, and Biological Sciences Initiative (BSI) at CU Boulder.

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Vidatox coupled with radiation treatment demonstrates anticancer properties in *Drosophila*

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Abstract

Cancer is a disease notoriously known for being relentlessly hard to overcome and all too common in our society. In summary, cancer results in rapid tumor growth due to a malfunction in our cells causing them to replicate at an uncontrolled rate. Two of the most effective treatments of cancer used today are radiation and cytotoxic chemotherapies. Their goal is to kill malfunctioning cells. Using a specific species of fly as our test subject, we have investigated new and possibly more effective ways to kill these cells.

Third Instar *Drosophila melanogaster* were used in this experiment. In this stage of development, the larvae's cells are rapidly replicating which mimics tumors cells. The flies are also an excellent model for cancer research because they are cheap, extremely susceptible to radiation, their cells are very similar to those of humans, and *Drosophila* are simple organisms with a short life span. These are necessary components to have in the model chosen for this experiment because of the time restraints placed on the study, and that is easily comparable to other chemotherapy research done in other labs.

We used a dosing series of 1:10, 1:100, and 1:1,000 this was pipetted to each vial to create a total of 7.5 mL of solution, and fly food. i.e 6.75 mL of food and 1,000 µl of Vidatox, 7.5 mL of food and 100 µl of Vidatox, and 7.5 mL of food and 10µl of Vidatox. This dosing series was performed in triplicate. We also used colchicine as our positive control and deionized water as our negative control. Our findings suggest that when using Vidatox as a form of chemotherapy, radiation paired with the drug is more effective than either form of treatment alone. This is consistent with common cancer treatment options.

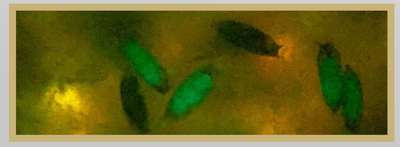
Introduction

The type of scorpion that the venom is extracted from a unique blue scorpion Junceus Rhopalurus, that can only be obtained in Cuba. The venom is seen as a natural trial chemotherapy. The formula used is a diluted solution called Vidatox.

The venom is diluted to 3 µg of chlorotoxin in 75ml of water. It was registered by Labiofam, a Cuban company, in March 2011. Before the release, there had been about 15 years of research and trials done on the drug. Vidatox can be found on easily accessible sites like Amazon which is where we obtained the compound. The experiments that we based our research off of was done by Escozul. They suggest that the best way to use Vidatox is by placing two drops of the solution directly onto the tongue. It is produced from five proteins found in the venom which have the the effects of anti-inflammatory and anti-carcinogenic on cancer cells. We tested our drug on both irradiated and non irradiated *Drosophila melanogaster* and were interested in *Drosophila* with a mutation in the *Grapes* gene (Checkpoint Kinase 1 in humans). This mutation is common in human head and neck cancer. If the *Drosophila* are heterozygous for the *Grapes* gene they contain Glowing Fluorescent Protein (GFP) and will glow green under a blacklight. Homozygous mutants will not glow and are more susceptible to radiation than wild type *Drosophila.*

Hypothesis

It is predicted that the combination of Vidatox and radiation will have a greater effect (less survival of the larvae) than just applying the drug alone to the food.This will hopefully be proven through a dosing series of vidatox. As a form of comparison, there will be two groups of larvae. One will be coupled with the use of radiation, and one will not.

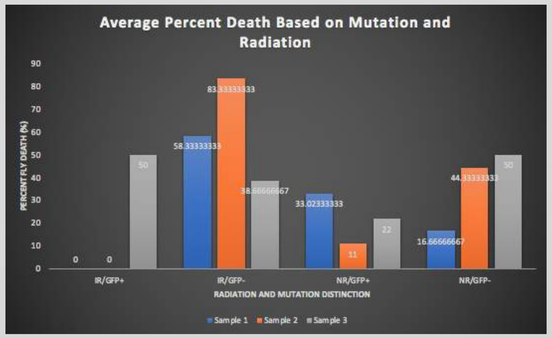


Materials and Methods

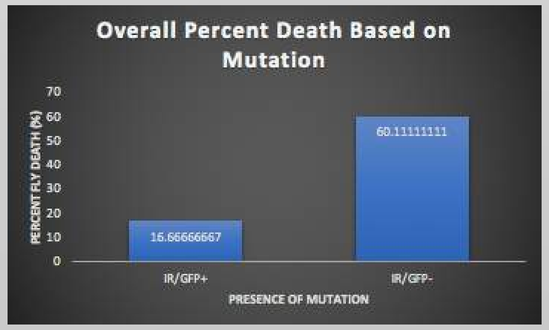
1. Obtain 28 drug vials and flugs. 18 of these will contain either a 1:10, 1:100, 1:1,000 dose of Vidatox solution. 4 will contain dilutions of 50 µg/mL colchicine for the positive control, and 4 will have water for the negative control.
2. To prepare the 18 vials of Vidatox and food mixture, six will be prepared at 1:10, six at 1:100, and six at 1:1,000. For the 1:10 dose, place 6.75 mL of fly food into each vial using a syringe. For the 1:100 dose and the 1:1,000 dose, place 7.5 mL of fly food measured with the syringe.
3. Once each vial contains food, the first six should have 750 µL of Vidatox pipetted into the vials intended to be the 1:10 concentration (vials containing 6.75 mL of food). The next six (containing 7.5 mL of food) will have 75 µL pipetted into each to prepare the 1:100 concentration. The last six (containing 7.5mL of food) will have 7.5 µL pipetted into it to prepare the 1:1,000 concentration.
4. Prepare 4 drug vials of diluted 50 µg/mL colchicine as the positive control. 2 of these will be a 1:100 dose of colchicine (one for the irradiated larvae and one for the non irradiated larvae), and 2 of these will be a 1:1,000 dose (one for the irradiated larvae and one for the non irradiated larvae). Place 7.5 mL of fly food into each drug vial using the syringe. Then pipet 75 µL of colchicine into the first 2 drug vials and mix with pipet tip to make a 1:100 dose. Then pipet 7.5 µL of colchicine into the other 2 drug vials and mix with the pipet tip to make a 1:1,000 dose. If there is excess food mixture on the side of the vial, push it back down with the remainder of the food with a clean paintbrush. Do the same procedure to prepare 4 drug vials of water as the negative control.
5. Take 3 vials of each dose of Vidatox and 1 vial of each of the 50 µg/mL colchicine and water doses and add approximately 50 irradiated *Drosophila melanogaster* third instar larvae (roughly the size of a dime) into each vial (a total of 13 vials will be used here; 9 Vidatox vials and 4 control vials). Take the other 3 vials of each dose of Vidatox and other 4 vials of the positive and negative controls and add the same amount of non irradiated *Drosophila* to them (again there will be 13 vials used). Flug the vials after adding the larvae.
6. Incubate for 7-10 days and then quantify the survival of each dose and of the control vials.

Results

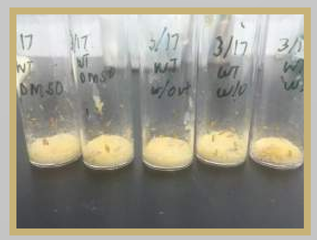
The results we received from our experiments were rather scattered and inconclusive at first. The goal was to understand the effectiveness of the Vidatox while paired and unpaired with radiation. However, there seemed to be no trend between these variables.



After some formatting of the data arrangement we were able to see that the defining factor in our data was the presence of the GFP marker or lack thereof. Our data shows a relatively high percent death in the flies that were GFP-/GFP-. This means that those flies lacking the GFP marker, being the flies with the grapes mutation, were more susceptible to Vidatox paired with radiation.



It should also be noted that many more larvae were present on the walls of the vials containing the Non-Irradiated *Drosophila*. This could be a sign that the radiation paired with the Vidatox was more effective in killing the flies before they were able to form their pupal casing.



Conclusion

This study demonstrates that the combination of Vidatox and radiation increases the success rate over the use of Vidatox alone. The hypothesis stated has been proven to be true. Combination therapy has always been seen to work better than using the chemotherapy by itself. Using the larvae as the tumor model, the most effective combination of Vidatox and radiation was the 1% Vidatox dilution with 4000 RAD. The results are conclusive because the difference between the non-irradiated and irradiated data were enough to show that radiation has a greater effect with Vidatox. Therefore, the results prove that combination therapy consistently works. The study performed used the same concentrations and configurations as the published work, but the use of radiation in our experiment was used in addition to Vidatox where as the published experiment used Vidatox alone. Combination therapy was used for this study to make it more relevant to cancer trials used in the real world on humans.

Future Directions

With more time and funding we would continue to pursue research on the active molecule in our compound, chlorotoxin.
Further steps in our research would entail:

1. Investigating higher concentrations of the chlorotoxin; the dilution we used for our experiments was relatively weak.
2. Preparing more samples (vials). We were not able to prepare as many samples as we would have liked due to time constraints
3. Look into the cytotoxic effect of chlorotoxin on more complex organisms, other than *Drosophila melanogaster*

Acknowledgments

We would like to first thank Dr. Tin Tin Su for being the presenting the incentive for this lab and acting as the sponsor. Next it is important to recognize Dr. Pamela Harvey for coordinating this entire lab and providing many students with their very first lab experience. In addition, we thank our three Teaching Assistants, Jess Colmenero, Jack McLeod, and Ryan Fleischer, these three were available to help us the whole way. Lastly we would like to acknowledge the Biological Sciences Initiative, The Howard Hughes Medical Institute and the Molecular Cellular Developmental Biology Department for providing the means and funds to perform research in this lab and grow our lab experience.

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The Impact of The Compound EGCG, The Major Component In Green Tea, On Drosophila Larvae Survival.

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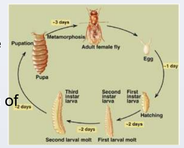
Abstract

Scientists throughout the years have been working tirelessly conducting studies on the best way to cure cancer. The acknowledgment that standard cancer treatments are problematic has only allowed for us to discover more mysteries of the disease. In our study, we examined the effect of EGCG on tumor development. More specifically, we observed the impact different concentrations of ECGC can have on the development of Drosophila Melanogaster. Our reason for using the Drosophila as our model organism is because Drosophila contain many genes that are conserved in humans. The Drosophila's transition between 3rd instar larvae to adulthood is similar to tumor progression in many forms of head and neck cancers. It was because of this we were able to use the percent survival of larvae to statically analyze whether or not our drug compound was a “hit”, as well as to test the effectiveness of adjunct therapy. In fact, we found that there was a correlation between the concentration of EGCG with tumor development, and with further testing, EGCG may become a “hit”. We also examined the possible enhanced effect radiation sensitivity can have on the treatment of cancer when combined with drug therapy. We saw how Drosophila who were genetically more sensitive to radiation had a lower percent survival rather than Drosophila that didn’t. Thus, depicting the impact of radiation sensitivity has on the general effectiveness of radiation treatment of cancer.



Introduction

Cancer is a disease in which abnormal patterns of gene expression lead to mutations in the DNA sequence that result in uncontrollable cell growth and damage to normal body tissue. In humans, cancer forms when an abnormal and unregulated cell develops into a group of mutated cells, called a tumor. These tumor cells can attack and kill nearby healthy cells, spreading and infecting more of the body with the disease.

Almost 2 million new cases of cancer are diagnosed every year, with over half a million people estimated to die from cancer. Many of these deaths can be prevented by administering successful treatment. However, approximately 50% of cancer patients will undergo chemotherapy, but only about 10-15% are cured. An adjunct therapy is given in combination with another strategy, an example of this being treating cancer with both chemotherapy and radiation in an effort to maximize the death of cancer cells and minimize the death of healthy cells. Essentially, chemotherapy drugs in combination with radiation can be more powerful than either treatment alone.

Overcoming drug resistance, in which the cellular target mutates so that it no longer binds to the drug, is the main goal of current chemotherapy research. While chemotherapy drugs may initially present as effective, cancer cells are capable of overcoming their cytotoxic impacts by mechanisms of reduced drug uptake, increased energy-dependent drug efflux, and altered responses to drugs because of changes in apoptosis. With the increase in drug-resistant cancer cells, it is necessary that research continues to explore new chemotherapies that can overcome drug resistance and minimize damage to surrounding healthy tissues.

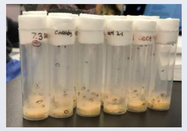
Green tea is a very popular drink and has been advertised as having many health benefits. Green tea contains a high concentration of a powerful antioxidant compound, Epigallocatechin Gallate (EGCG). According to previous studies, it has been suggested that EGCG and other tea catechins have anticancer properties. In a study done by researcher Hirota Fujiki in 1999, it was discovered that EGCG is able to suppress tumor development and formation in mice by inhibiting the release of tumor necrosis factor-alpha, a tumor promotion pathway (Hoffman). Many other studies found similar results that also suggest EGCG has various cancer-fighting properties. Essentially, these studies suggested that EGCG has the ability to induce apoptosis and regulate cancer cell growth—showing that EGCG may be a promising chemotherapy treatment.

Hypothesis

For our experiment, we hypothesize that we will be able to identify the most effective dose of the compound EGCG, found in green tea extract, which can then be used as a possible chemotherapy in order to treat cancer. Further, we hypothesize that the vials containing a higher concentration of EGCG will have a lower percent survival and perform similarly to the positive control Colchicine when compared to the lower doses of EGCG.

Methods

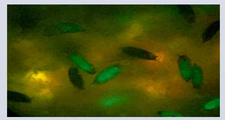
1. Dilution
- Start with 100 mg of the green tea powder and 1 mL of Dimethyl sulfoxide (DMSO)
- Begin series dilution with 100 µL of the previous solution and 900 µL of DMSO (a 1:10 dilution). Repeat the process in order to obtain 5 total dilutions.
- The concentration of solution in the food is as follows: 100 ug/mL, 10 µg/mL, 1 µg/mL, 0.1 µ g/mL, 0.01 µg/mL.



2. Assembling Drug Vials
- Add approximately 3 mL of fly food per vial
- Mix 3 uL of each solution into the fly food
- Vials 6.1-6.3 contain the positive control Colchicine
- Vials 7.1-7.3 contain the negative control DMSO
- Add approximately 100 irradiated third instar Drosophila larvae per vial
- Repeat this process to assemble three vials for each concentration and three vials for both the positive and negative controls.

3. Marking GFP

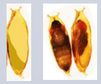
5 days after vial assembly, look at the pupae under the blue light of the microscope and mark the pupae that do not glow green (GFP-) .
- GFP- pupae do not glow green and are homozygous for a checkpoint protein mutation, which is ideal for the experiment because they are the most susceptible for radiation.
- GFP+ pupae glow green and are heterozygous for a checkpoint protein mutation, which makes the pupae less susceptible for radiation.



4. Quantifying Survival

One week after marking GFP- pupae, observe the individual circled pupa in the vials over a light box in order to quantify the survival of drosophila in response to the green tea supplement.
- The flies that have survived are those that have either fully or partially eclosed, and will appear light in color.
- The flies that are dead are those that have died in the pupal case, and will appear dark in color.

Pupae on the right represent flies that are identified as dead



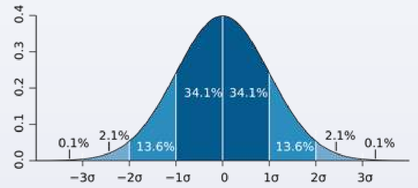
Pupae on the left represent flies that are identified as alive

5. Compare Tumor Survival to Positive and Negative Controls

Larvae treated with Colchicine, the positive control, should have a very low survival rate. Larvae treated with DMSO, the negative control, should have a higher survival rate. Vials treated with concentrations of the green tea powder that resemble the survival rate of Colchicine will be considered “hits” because we know that Colchicine is an effective treatment.

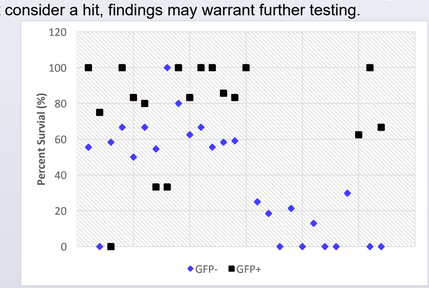
Results

To determine if there were “hits” in our drug set, we compared the average percent survival of the drug set to the +/- 2 standard deviations of the negative control. Anything outside of this range will be considered a statistical hit. About 68% of values fall within one standard deviation of the mean, and about 95% of values will lie within two standard deviations. In our study, drugs with survival over 87.2% and beneath 32.8% are considered hits, but because these qualities are precise, the data has to fall within in this rage.

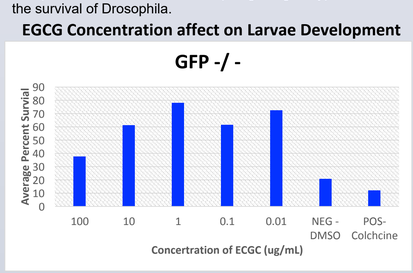


Source: Wikipedia

Our GFP- flies produced a mean percent survival of 37.6% when in the highest tested concentration of green tea powder (100 mg). While it isn't consider a hit, findings may warrant further testing.

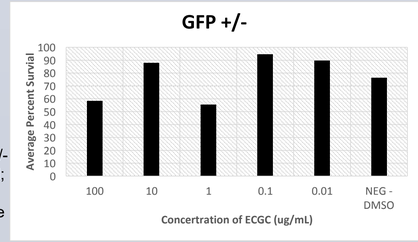


Compound concentration and Grapes gene genotype effect on the survival of Drosophila.



The effect of compound concentration on mean survival of Drosophila with GFP- phenotype .

*Positive control Colchicine for grp+/- pupae are reported; there was no statistical difference between the genotypes.



The effect of compound concentration on mean survival of Drosophila with GFP+ phenotype .

Conclusions

In our study, we hypothesized that we will be able to identify the most effective dose of the compound EGCG in green tea extract. We saw that there was a dramatic increase of mean percent survival between the vials containing concentrations of 100 µg/mL and 10 µg/mL for flies with the *grape* mutation (GFP-). We proposed that because vial 1 had a mean percent survival that was close to being a hit, we should use a closer set of concentrations. We also hypothesized that there was an inverse relationship between the concentration of EGCG with larvae development. We found vials containing a higher concentration of EGCG had a lower percent survival than vials with a lower concentration of EGCG. EGCG is an antioxidant, a molecule that inhibits free radical damage to cells, so to see that our GFP- flies were able to produce more promising data than GFP + flies proves how mutations in *Drosophila’s Grapes* (grp) gene, homolog of the gene that encodes Checkpoint Kinase 1 (Chk1) in humans, causes cells to become more susceptible to ionizing radiation. Overall, some limitations to our study are that the *Drosophila* larvae only represent tumors in head and neck cancers, so we aren’t sure how the treatment would work in other forms of cancer. Additionally, the larvae just serve as models of a tumor, so we are not sure how the drug would interact with actual cancer cells and other tissues in a human body.

Future Directions

Hits from the Discovery Lab will be transferred to Dr. Tin Tin Su’s lab. Recommended experiments to be performed by the lab include:
1. Testing matcha powder in order to determine whether a higher concentration of the EGCG compound will further inhibit growth of Drosophila larvae, which represent tumors.
2. Administer EGCG compound into mice infected with various forms of cancer in order to examine the drug’s effectiveness in more complex organisms and on various forms of cancer.
3. Increasing the variance of the green tea concentrations in order to lessen the difference between each concentration and provide data at higher concentrations.

Acknowledgments

We would first like to thank Dr. Pamela Harvey and Jessica Westfall for their general support in MCDB 2171 as well as for their continuous guidance in the completion of our independent study. We also want to thank Dr. Tin Tin Su for sharing her experiments and materials with our lab and for contributing her knowledge to our work. We also acknowledge the Biological Sciences Initiative (BSI) and the Howard Hughes Medical Institute for funding the research performed in the Discovery Lab. We are also thankful to the Molecular, Cellular, and Developmental Biology Department at University of Colorado Boulder for their continued support of the Discovery Lab.

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Using Thymolphthalein as a Potential Chemotherapy for Head and Neck Cancers

D12

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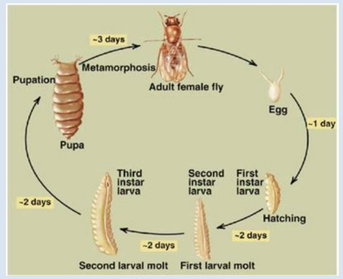
Abstract

Cancer is one of the leading causes of death around the world. Some of the most common anti-cancer therapies include radiation, surgery, and chemotherapy treatments. These treatments have been shown to help fight cancer though are not enough to cure the diseases. New and improved cancer treatments are necessary, including in the form of chemotherapies. The use of multimodal therapy, the use of two or more agents in combination, exhibits a potential for a more effective way to treat cancer. In this experiment, 813 different compounds were screened in live Drosophila as a model for head and neck tumors to test their effectiveness as possible chemotherapies. The results of this screen indicate that several compounds identified as hits are already in use as chemotherapies or should be re-tested and validated as potential chemotherapies. One of the hits identified in the preliminary screen was Thymolphthalein, which in this experiment was administered to irradiated Drosophila in different concentrations. The survival rate of the adult flies indicated the success of the drug in killing the tumor model.

Introduction

Cancer, a leading cause of death around the world, affects over 1.5 million people every year. Although this number has decreased very slowly over the last decade, the need for treatments is just as significant. Cancer treatments are present in the form of many different care methods including but not limited to surgery, radiotherapy, and chemotherapy. Combination therapies have proven to treat cancer more effectively than individual cancer care methods while also reducing toxicity. Researchers use various *in vivo* models to discover new cancer treatments. The Drosophila species is a good model for head and neck tumors in order to find molecular compounds which synergize with radiation and treat cancer. This screening method for potential cancer treatment drugs uses the *grp* mutant Drosophila third instar larvae to model tumors because this strain of Drosophila represents Checkpoint Kinase 1 (Chk1) mutations in human cells and simulates rapidly dividing cells similar to tumor cells. The Drosophila used have a balancer chromosome that allows for the identification of the genotype of the larvae. Two copies of a *grp* mutated gene has the greatest sensitivity to radiation, as found by previous experiments.



Small molecule libraries have been screened for chemotherapy "hits", molecules which when administered in conjunction with radiation, killed significantly more *grp* Drosophila than radiation alone. In Spring 2018, 813 compounds were tested for potential "hits" using Drosophila, several of which were found to be potential chemotherapies or are already in use as chemotherapies in the market. The potential "hits" require further validation and further investigation before moving to clinical trials. Thymolphthalein, a small molecule identified as a "hit" in Diversity Set IV from the National Cancer Institute, proved to have a 0.0% survival rate of Drosophila when administered in conjunction with radiation. Due to this favorable result, thymolphthalein has the potential to be a successful chemotherapy. Validation and investigation of the molecule must continue using the Drosophila screening model.

Hypothesis

Thymolphthalein was identified as a radiation sensitizer hit from the Diversity Set IV in Spring 2018 testing of the compound.

We hypothesized that since Thymolphthalein was a hit in the preliminary screening, the compound can be used as a chemotherapy drug to treat head and neck cancers in combination with radiation with an appropriate dosage.

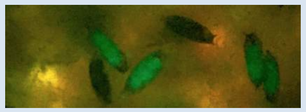
Methods

Prepare

In preparation for this experiment, third instar larvae were collected after being sorted and sieved. Six food and drug vials were set up with equal amounts of food. The vials included four with varying concentrations of Thymolphthalein, one negative control without radiation, and one negative control with radiation. Two vials contained a concentration of 10 uM and two vials contained a concentration of 5 uM.

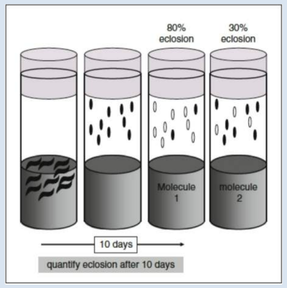
Expose & Incubate

The third instar larvae were exposed to 4000 rad of ionizing radiation. The irradiated larvae were distributed equally into the six food and Thymolphthalein containing vials. The food and Thymolphthalein vials were stored at 25°C and incubated for approximately seven days, until the third instar larvae turn to pupae. When the larvae formed pupae on the sides of the vial, the vials were marked for GFP-/GFP+. The food and drug vials were stored again for approximately three days, until the pupae enclosed into adult flies.



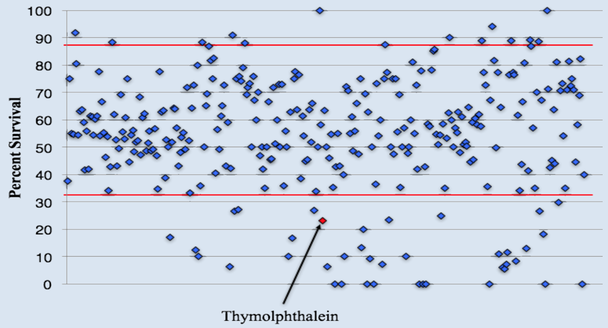
Quantify

Survival of the adult flies was quantified in each vial by counting empty and closed pupae. Empty or half empty pupae shells represent alive flies and full pupae shells represent dead flies.



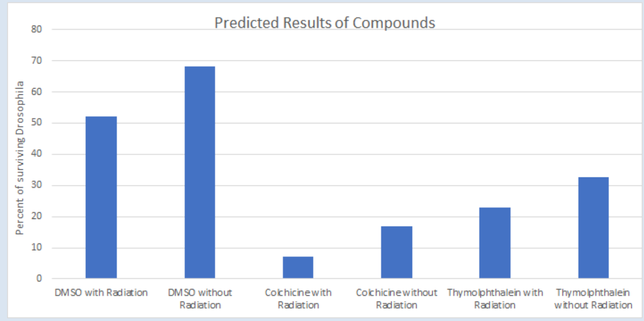
Results

Of the 813 compounds tested in the preliminary drug screening, several compounds were identified as hits for potential chemotherapies. The graph below represents the compounds tested and identified as hits, including Thymolphthalein. This data suggests that all of the potential hits can be re-tested and verified for their effectiveness in killing Drosophila larvae modeling a tumor.



Among the potential hits identified, three FDA-approved chemotherapies were identified (Actinomycin, Cytarabine HCl, and Hedamycin) which all prevent DNA synthesis. These findings validate the drug screen in identifying potential chemotherapies and suggest that Thymolphthalein could inhibit DNA synthesis.

Due to the lack of timely data, the results of the compounds were predicted. Past data of Thymolphthalein and Colchicine from the Spring 2018 initial drug screen was used to predict the survival results of these compounds. The confirmed data on this graph includes DMSO without radiation. Drosophila was used to help predict the results of DMSO with radiation.



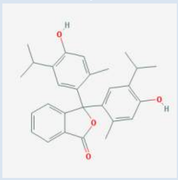
Conclusions

The preliminary drug screen successfully identified potential head and neck cancer chemotherapies which like Thymolphthalein, can be re-tested with the goal of validating the hit. Several hits were identified out of the 813 compounds screened which all have the potential to be successful chemotherapies.

Future Directions

Future experiments may include:

- Testing compounds similar in chemical structure to Thymolphthalein with the same method to find other potential chemotherapies.



- Testing more variations of Thymolphthalein concentrations in conjunction with radiation.
- Testing other identified hits from the preliminary compound screen for validation and exploration of effective doses with similar experiments.

Acknowledgements

We would like to thank Dr. Pamela Harvey for contributing her expertise to our work. Also, we would like to thank Dr. Tin Tin Su for allowing us to participate in this experiment and our TAs for guiding us through this experiment. We acknowledge the Howard Hughes Medical Institute, the Biological Sciences Initiative (BSI), and the Department of Molecular, Cellular and Developmental Biology of the University of Colorado Boulder for their continuous support of the Discovery Lab.

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Effect of Cannabidiol on *Drosophila melanogaster* Survival

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Abstract

The goal of this experiment was to test the effect of CBD, in a dosing series, on both irradiated and non-irradiated 3rd instar *Drosophila melanogaster* larvae, used as a model for cancer cell growth. We used grape mutant *Drosophila* as our model due to the mutation's close resemblance to the *Chk-1* mutation in human's with head and neck cancer. The need for other chemotherapies is becoming more important as cancer is evolving and becoming more resistant to most modern chemotherapies available today. Although we ran out of time during this experiment, we predicted that the CBD will yield positive results as it has been tested in different human cancers and all showed positive results in slowing down the growth of cancer cells.

Introduction

The rates of cancer development are changing, and cancers are becoming more resistant at an alarming rate. Today, approximately 50% of people will get some form of cancer. And, although there are multiple different ways to attack cancer, most modern day practices cannot keep up with the growing resistance rate. Modern chemotherapy practices only work 10 to 15% of the time in completely eradicating the cancer.

To address these issues and expand our repertoire of treatments, we tested the effects of Cannabidiol (CBD) on cancer growth using a drosophila model. Cannabidiol (CBD) is a non-psychoactive drug commonly found in the plant Cannabis or hemp and is usually paired with THC. According to Medical News Today, CBD, when in the body, is shown to encourage the body to use its naturally occurring CB1 and CB2 receptors and improve basic motor skills in the brain as well as the immune system. We have found a review and 2 articles that claim 1) that cannabidiol has inhibitory effects on systemic malignant tumors, 2) that cannabidiol, a non-psychoactive cannabinoid, has antitumor effects on human glioma cell lines, and 3) that cannabidiol has shown to be effective both in vitro and in vivo on neuroblastomas.

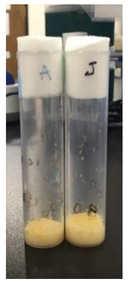
A therapeutic effect of CBD was observed in concentrations of 0.01 - 0.05 μM, where 0.036 μM was the ideal concentration of CBD in plasma. *Drosophila* 3rd instar larvae act similar to cancer cells, with rapid cell division and fast development, so if we want a *plasma* concentration of 0.036 μM, feeding the larvae that same concentration would likely yield that same concentration (0.036 μM) in the larvae, due to minimal processing by the organism. According to the US National Library of Medicine, out of the two compounds tested, CBD and THC, CBD yielded more positive results and was found to be more active in slowing the growth of the tumor. CBD has shown promising results as a cancer cell growth inhibitor.

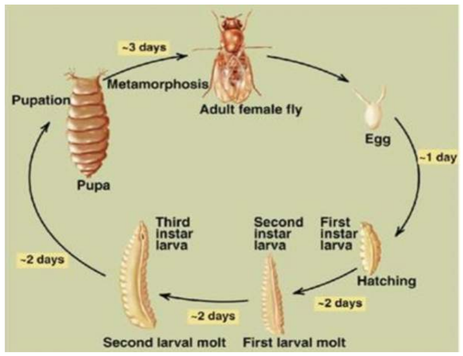
Hypothesis

We hypothesize that CBD will be an effective chemotherapy agent as it should reduce or stop the growth of cancer cells, or in the case of our model, 3rd instar larvae of *Drosophila melanogaster*. We also hypothesize that the therapeutic effect of CBD will differ in *Drosophila* larvae that have been irradiated first and larvae that have not, due to the oxidative effects of CBD.

Methods and Materials

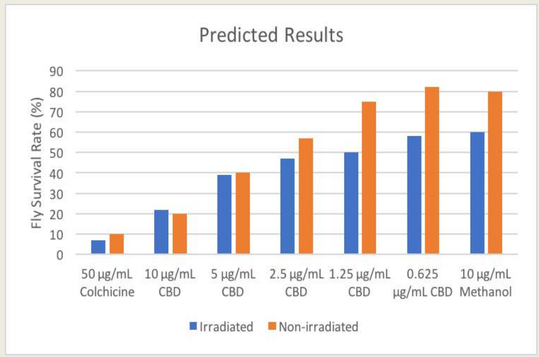
1. Collect eggs of the flies from the population cages and place those eggs in bottles containing fly food.
2. Next, after a few days of growth and development, collect the larvae and sort through all the larvae with a sieve to get the 3rd instar larvae, which are 600μm in size.
3. Irradiate half the 3rd instar larvae collected with 5.33 Rads/sec for 12.5 minutes. Exposure to 4000 Rads should cause double stranded breaks in larvae DNA.
4. While the flies are being irradiated, we prepared 24 vials with 3mL of food.
5. Given that the molar mass of CBD is 314.469 g/mol and 0.036 μM is the ideal therapeutic plasma concentration of CBD, we determined 11.32 μg/mL as our ideal concentration of CBD. Using our initial stock solution of 10 mg of CBD in 1 mL of methanol, we tested the dosing series by altering the volume of CBD added to the food vials as follows:
   a. 3 μL stock in 3 mL food = 10 μg/mL
   b. 1.5 μL stock in 3 mL food = 5 μg/mL
   c. 0.75 μL stock in 3 mL food = 2.5 μg/mL
   d. 0.375 μL stock in 3 mL food = 1.25 μg/mL
   e. 0.1875 μL stock in 3 mL food = 0.625 μg/mL
6. Fill 4 vials with each concentration and fill 4 vials with 3 μL of 100% methanol, the negative control, two of each to be tested with 50-100 irradiated larvae and two to be tested with 50-100 normal larvae. Also test the positive control, 50 μg/mL colchicine, with each larvae.
7. Have one groupmate blind the vials by randomly labeling them A-X and record their code and identity on a separate spreadsheet. This group member will not take part in marking for GFP nor quantification of survival to prevent unnecessary bias.
8. Allow vials to incubate before marking for GFP negative pupae (homozygous for Grp mutation)
9. Quantify fly survival and decode results.





Predicted Results

We anticipate that as the concentration of CBD increases, the rate of fly survival would decrease. As the flies are a model for cancerous cells, a greater incidence of fly death would indicate more success as a chemotherapy. We were unable to predict whether the rate of cell death in the irradiated larvae would be greater than or less than the rate of cell death in the non-irradiated flies as we do not know if CBD has enough of an oxidative effect to protect the flies from radiation damage or not.



Future Directions

In the future, given more time and resources, we would want to re-conduct our experiment in a dosing series with higher concentrations, due to errors that were made in our calculations that left us with lower concentrations of CBD than anticipated. Furthermore, we would want to explore the synergistic effects of CBD by testing CBD with known chemotherapy drugs and treatments and comparing the results to that of the chemotherapy treatments alone. Also, due to human harm of methanol, we would want to try a dosing series using pure powdered CBD in water. It's also important to look at the effects of other 113 types of cannabinoids found so far. They should be tested individually and in different combinations. Testing the synergistic effect of just two cannabinoids at a time in all combinations will be over 6,000 future trials. Testing the synergistic effects in all combinations would result in over 2.6×10^{66} combinations. There are even more tests that should be included if varying proportions are considered. In addition we could test this experiment in vitro in human cancer cells to see how CBD and cancer would interact directly, in addition to testing CBD in various forms of cancer.

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The Effect of Initial Hit, Morpholine-N-dithiocarbamate, on *Drosophila* Survival

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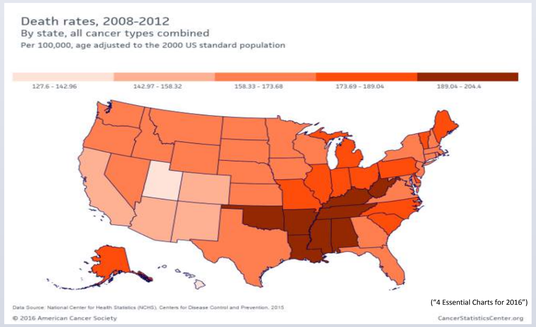
Abstract

Cancer has many potential causes, especially with the increased amount of radiation and toxins we are exposed to on a daily basis. Since an estimated 38.4% of all people will eventually be diagnosed with this destructive disease, finding treatments fast is imperative. Drug research is usually very expensive, but due to Tin Tin Su's lab working together with CU Boulder, we can do a wide variety of preliminary testing to ensure there is a large net to find potential chemotherapies. This lab used *Drosphila* third instar larvae as models for cancer tumors. All lab sections screened 992 potential chemotherapy compounds, and identified Morpholine-N-dithiocarbamate. Our personal results failed to identify Morpholine-N-dithiocarbamate as a hit. These results indicate that this compound might be more effective as a combination therapy. Possible future tests might include repeating this experiment, due to potential errors with our controls. Also, experiments could be done to analyze similarly structured compounds. We concluded that further testing of the drug would be needed to determine its actual effectiveness, especially while using it as a combination therapy.

Introduction

Cancer is a disease caused by the rapid, uncontrolled division of cells in the body. Due to environmental and genetic factors, cancer rates have grown as society has become more and more exposed to toxins that can cause damage to our DNA. On average, 38.4% of all people will be diagnosed with cancer at some point in their lifetime. Because of cancer's incurable nature and severity, research in finding a cure for this disease has the utmost importance in the modern world.



In collaboration with Tin Tin Su's lab at CU Boulder, students perform preliminary tests on compounds from the National Cancer Institute to identify potentially effective drugs, which are later further researched in the Su lab if the drug is seen to be a potential success. By using students to test preliminary drugs, researchers can save both time and money while giving students experience in a lab setting. In this lab, we use the model organism Drosophila melanogaster to research the effects of provided drugs on their survival rates.

Labs use the model organism *Drosophila* because many of the genes present in fruit flies serve similar functions in humans. Drosophila also contain some of the same signaling pathways that humans do, even if they don't express the disease, making it helpful to study the causes of a disease which may lead to new, effective treatments. Third instar larvae of Drosophila reproduce their cells at a high rate, similar to that of a reproducing cancer cell making them an excellent model for cancer cells. Genetically modified flies exhibit a gene called GFP, which is a phenotypic marker that allows us to identify flies that only contain one copy of a mutated grapes gene. Labs breed the flies to get a homozygous recessive fly with the grp gene (the grapes gene). This homozygous recessive gene causes a loss of function result in the protein that normally functions to fix damaged DNA.

This gene has a similar function to the chk1 gene in the human cell cycle. This allows the larvae to become susceptible to radiation. With the goal of reducing side effects of cancer treatment, as well as increase effectiveness, we are attempting to utilize both chemotherapy and radiation. By making the flies susceptible to radiation, we are able to utilize and study this combinatory effect.

The drug we received from diversity set IV was Morpholine-N-dithiocarbamate, which has already been identified as a potential hit by both tests done by the class. Access to two tests of the drug gives us reliable data which we can further apply to our experiment. Since these tests were performed on irradiated larvae, we will be examining the effects on larvae that are not irradiated. This will be done to identify the effectiveness of both combination therapy and only chemotherapy.

Hypothesis

The compound, Morpholine-N-dithiocarbamate, will decrease percent survival of *Drosophila melanogaster* when introduced to the flies in the third instar larvae phase of development. Since third instar larvae behave similarly to cancer cells, low percent survival will indicate that Morpholine-N-dithiocarbamate has the potential to be used as a chemotherapy. Preliminary results from the drug screen identified Morpholine-N-dithiocarbamate as a hit, and the percent survival from the drug screen was 6.34920635%. Elaborating on those results, we hypothesize that non-irradiated larvae will be similarly affected by this compound.

Methods

Prepare and maintain a *Drosophila melanogaster* population cage (reference Population Cage Protocol for detailed instruction).

Isolate fly eggs from grape juice plates and add the eggs to food bottles.

Prepare ten vials by carefully adding 3 mL of prepared food (reference the Fly Food Protocol for detailed instruction) with a syringe. Immediately following the addition of the food, add 100 µL of DI water to each vial to prevent the food from drying out.

Add 3 µL of compound Morpholine-N-dithiocarbamate to one of the prepared vials. The concentration of the stock is 10 mM. It is being tested at a concentration of 10 µM.

Repeat the previous step by adding the compound in concentrations of 10 µM, 8.33 µM, 6.66 µM, 5 µM, and 3.33 µM to four more vials .

Add 3.0 µL, 2.5 µL, 2.0 µL, 1.5 µL, and 1.0µL of DMSO (negative control) to the remaining five vials, separately.

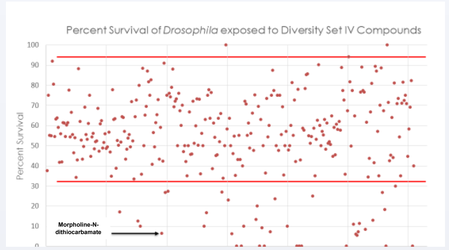
Obtain a prepared vial containing 3 µL of 50 mg/mL colchicine (positive control).
-This vial was prepared by Pam in the lab and provided to us for our experiment.

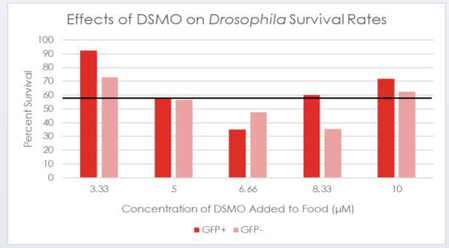
Collect third instar larvae and add 50-100 larvae to each vial.

Mark and quantify GFP+ and GFP- on each vial using a fluorescent light microscope after five days.

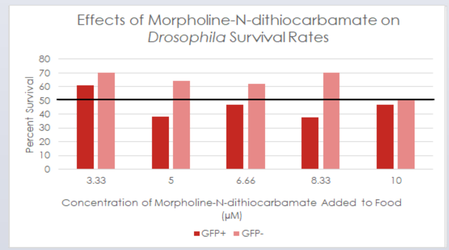
Quantify survival of flies after 4-5 more days by counting the successful and unsuccessful eclosion of the pupae.

Results

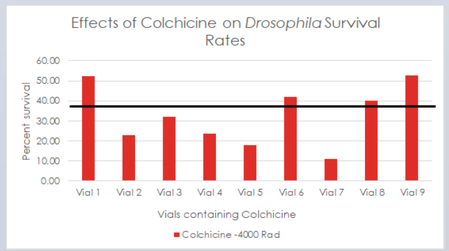




DSMO is used as a negative control in this experiment, meaning it should not have a noticeable effect on survival rates. The value of 2 standard deviations away from the mean was 94.18 and 24.02, which we use to identify if the compounds were hits.



An increase in concentration of Morpholine-N-dithiocarbamate had little impact on survival rates of *Drosophila*, which was unexpected. The mean of this compound was not outside of 2 standard deviation values of DMSO, so it was not identified as a hit.



Colchicine acted as the positive control in this experiment. Colchicine didn't fall outside of 2 standard deviations of DMSO, so was not identified as a hit, which was unexpected. Only grp- flies were recorded. There was no statistical difference between flies with the GFP mutation and those without.

Conclusions

Utilizing third instar larvae as models for head and neck cancer, we administered a range of doses of the compound, Morpholine-N-dithiocarbamate, to Drosophila. It was hypothesized that similar results to those collected during the drug screen would obtained, indicating that the compound is a hit. Upon completion of the experiment, it was determined that Morpholine-N-dithiocarbamate has no significant effect on larvae that has not already been irradiated. Compared to data produced during the drug screen of Morpholime-N-dithiocarbamate, larvae that were initially irradiated had a significantly lower percent survival than those treated without radiation. This suggests that the compound may be more effective when used in combination therapy. The use of both radiation and chemotherapy reveals a synergistic effect where the percent survival was lower in tests that involved both radiation and drug, as opposed to one or the other. The results of our control variables were unexpected. DMSO should have yielded higher percent survival, and the colchicine should have been identified as a hit when looking at the DMSO standard deviations. Since this did not happen, there might have been error in the DMSO test vials.

Future Directions

1. Because Morpholine-N-dithiocarbamate was previously tested during the drug screening as part of Diversity Set IV, we were only able to obtain 10 µl of the compound to use in this experiment. Therefore, the next step would be to validate our results by replicating and performing the experiment again.
2. Moving forward, further studies should be conducted with radiation. Based on our results and the results from the class drug screening, Morpholine-N-dithiocarbamate was more effective in killing the *Drosophila* when it was combined with radiation. The effectiveness of the combination indicates a synergistic effect that should be focused on in future studies. Further experiments can be done by irradiating larvae first, utilizing the drug screening procedure. These studies should include a dosage series in order to identify the therapeutic index for the compound.
3. Finally, since this compound was initially determined to be a hit, relevant future studies might involve tests with compounds with similar structure. For example, Morpholinium4-morpholinecarbodithioate has the same formula as Morpholine-N-dithiocarbamate,. Its structure is slightly different than Morpholine-N-dithiocarbamate, indicating that it might have a different function. However, because of its similarity, conducting research on it might yield relevant results.

Acknowledgments

We would like to extend our thanks to **Dr. Pamela Harvey**, the instructor of this course, for her unwavering support of our research and her extensive educational contributions to our work. We would also like to thank the principle investigator for this lab, **Dr. Tin Tin Su**, as well as the University of Colorado Boulder department of Molecular, Cellular and Developmental Biology for their support and resources. Finally, we would like to acknowledge the Howard Hughes Medical Institute for their support.

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Modeling the Effect of Diet During Cancer Treatment:

The Effect of Ellagic Acid and Curcumin on Chemotherapy and Radiation in *Drosophila Melanogaster*

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Abstract

Diet is the leading environmental cause of cancer and plays a pivotal role in keeping cancer patients stable while undergoing treatment. By tailoring the diet of a patient to compliment his/her cancer treatment, doctors can maximize the efficacy of radiation and chemotherapy. To explore the impact of diet on cancer treatment this research tested two compounds found in everyday foods, ellagic acid and curcumin. We found that while our data initially suggested a synergistic effect as well as a buffering effect in ellagic acid on oxidative damage, after performing a statistical analysis, neither result was statistically significant. However, previous studies that were performed under more realistic conditions (more time, more data, larger sample size) have found statistically significant results in favor of a synergistic effect between ellagic acid and curcumin, as well as evidence of antioxidants buffering oxidative damage of radiation.

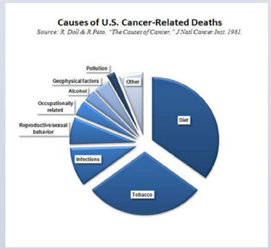
Introduction

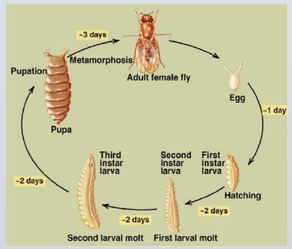
Cancer is one of the leading causes of death worldwide, yet our main form of treatment for cancer, chemotherapy, is only effective in curing 10-15% of cancer patients. The low cure rate of chemotherapy is due to its cytotoxic nature. Cancer cells are normal cells behaving abnormally. Despite targeting cancer cells, chemotherapies also damage normal, healthy cells. Therefore, it is important to develop new treatments that maximize damage to cancer cells while minimizing damage to healthy cells.

Our research tested two compounds: ellagic acid, found in foods such as strawberries, walnuts, and raspberries, (Kumar et al., 2016) and curcumin, an ingredient in turmeric (Kumar et al., 2016). These compounds are thought to play a role in inhibiting tumor cell proliferation by targeting p53. Previous research has tested the effects of ellagic acid and curcumin acting synergistically to induce DNA damage in tumor cells, p53 accumulation and cell apoptosis which are important mechanisms in blocking cell growth. In addition, ellagic acid has been shown to inhibit metastasis of ovarian cancer and is a promising compound against hepatocellular carcinoma (Zaazaa, Lokman, Shalby, Ahmed, & El-Toumy, 2018).

In our research, these compounds were tested individually and in combination to determine their effects in *Drosophila Melanogaster*. Drosophila exhibit many of the same biological processes that humans have. They have simplified systems, are easy to manipulate and their genome contains 75% of disease-causing human genes. Third instar larvae of Drosophila contain rapidly dividing cells, which makes these larvae an ideal model for tumor growth. Part of our experiment includes testing how ellagic acid and curcumin effect radiation. Third instar larvae with homozygous mutation in the grapes gene are more susceptible to radiation, and they are identified by green fluorescent protein. We use the grapes gene because it is homologous to the human checkpoint kinase 1 which is most often mutated in head and neck cancers.

Due to the low effectiveness of current chemotherapies, there is a pressing need to explore more options for cancer treatment. Years of extensive research haven't resulted in a treatment option that optimizes the patient's health while effectively targeting the cancer, therefore the need is greater than ever to find more treatment options.





Hypothesis

Ellagic acid, an antioxidant, will inhibit the effects of radiation by preventing oxidative damage on both cancer cells and normal tissue. Without radiation, curcumin will act synergistically with ellagic acid to induce p53 damage and decrease survival of *Drosophila Melanogaster.*

Methods

- Prepared 31 vials with 3mL of food
- Added specified mass of curcumin (TCI lot # VROND-JL) and ellagic acid (TCI lot # JSFOM-AH) to assigned vials, mixed thoroughly

Dosing Series #1:

Use Irradiated Larvae (4000 Rad)	Mass of Curcumin	Mass of Ellagic Acid	Volume of food
Vial 1, Curcumin	0.01 g	0 g	3 mL
Vial 2, Curcumin and Ellagic Acid	0.01 g	0.01 g	3 mL
Vial 3, Ellagic Acid	0 g	0.01 g	3 mL

Dosing Series #2:

Use Irradiated Larvae (4000 Rad)	Mass of Curcumin	Mass of Ellagic Acid	Volume of food
Vial 1, Curcumin	0.005 g	0 g	3 mL
Vial 2, Curcumin and Ellagic Acid	0.005 g	0.005 g	3 mL
Vial 3, Ellagic Acid	0 g	0.005 g	3 mL

Dosing Series #3

Use Irradiated Larvae (4000 Rad)	Mass of Curcumin	Mass of Ellagic Acid	Volume of food
Vial 1, Curcumin	0.0025 g	0 g	3 mL
Vial 2, Curcumin and Ellagic Acid	0.0025 g	0.0025 g	3 mL
Vial 3, Ellagic Acid	0 g	0.0025 g	3 mL

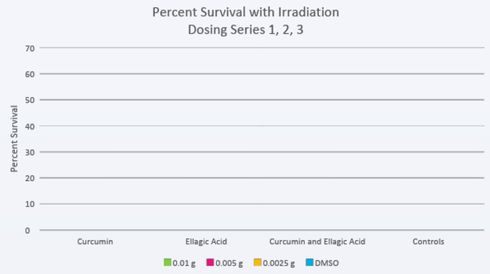
Controls:

Vial 1, 2, 3	3 uL Sorafenib Compound	3 mL food
Vial 4, 5	3 uL DMSO	3 mL food

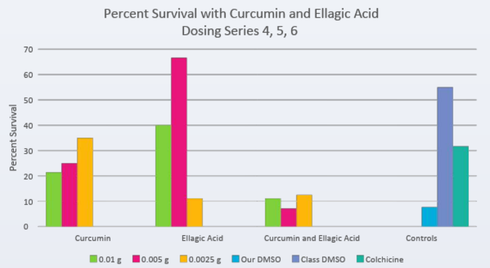
See Attached page for Dosing Series 4-9

- Placed 50-100 third instar larvae into each vial, see table for specified irradiated or not irradiated larvae
- Placed flugs in vials and set aside until larvae climbed up the walls
- Quantified GFP for Dosing Series 1, 2, 3 by circling the larvae that displayed GFP, indicating they were homozygous recessive for the grapes mutation
- Quantified survival by counting the amount of dead and alive pupae for all vials, but in vials 1, 2, 3 only counted the pupae that were circled for GFP

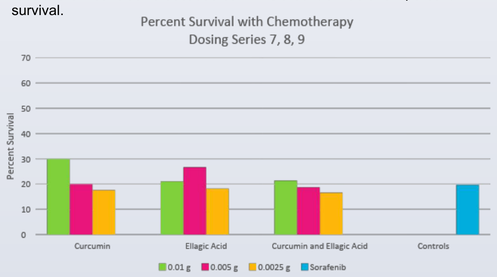
Results



Dosing series one, two and three Irradiated larvae were given different doses of curcumin, ellagic acid, and a combination of both. Determining the effects of curcumin and ellagic acid in combination with radiation led to inconclusive results, because in all vials, including the DMSO negative control, there were no alive *Drosophila Melanogaster*.



Dosing series four, five and six The effects of curcumin and ellagic acid in combination were tested on non irradiated larvae. Results show that there is a potential combinatorial effect of curcumin and ellagic acid. However, the results are inconclusive because our DMSO control had a low percent survival.



Dosing series seven, eight and nine The effects of curcumin and ellagic acid in combination with the chemotherapy sorafenib on non irradiated larvae. There is no significant difference between the percent survival from larvae that were given sorafenib and from larvae that were only given curcumin and/or ellagic acid.

Conclusions

For the first three dosing series with irradiated larvae, the survival rate of our negative control, DMSO, was zero percent, which led to inconclusive results. When the Drug Discovery class tested DMSO, the survival rate was 55 percent. This leads us to believe something in the irradiated larvae caused all of the Drosophila larvae to die.

Data from the next three dosing series suggest that there is a potential effect of using ellagic acid and curcumin in combination. However, again, the results for the DMSO control lead to our results being inconclusive.

Data from the last three dosing series does not support our original prediction that the survival rate of the *Drosophila melanogaster* would decrease when given curcumin and ellagic acid with chemotherapy. Rather, the graphs suggest that using ellagic acid and curcumin both in combination and alone decrease the effectiveness of the chemotherapy. The data is not statistically significant, so this conclusion cannot be made.

One limitation of our experiment is that the *Drosophila melanogaster* proved to be a difficult model to study. Very few of the larvae formed pupae, and the metabolism of the compound in larvae that formed pupae is nearly impossible to measure. These complications gave us limited and incomplete data.

Future Directions

For future reference, we propose the following:

Perform this same experiment again with two adjustments. The first is to run the entire experiment two times to help reduce potential procedural errors from compound mixing, vial preparation, and radiation of larvae. The second is to include a second group of irradiated larvae to reduce errors stemming from the radiation process itself.

Acknowledgments

We would like to thank Dr. Tin Tin Su, the Principle Investigator of our sponsor laboratory for allowing us the opportunity to do research on her project. Thank you to Dr. Pamela Harvey, our instructor, for inspiring confidence and providing the tools and resources necessary to complete our project. We would like to extend our thanks to Jack Schutz, Katie Franks, Oula Kareen, and Erin Kneeskern for mentoring us throughout the process. And finally, we would like to acknowledge The Howard Hughes Medical Institute, the University of Colorado Boulder's Molecular, Cellular, and Developmental Biology Department and Biological Sciences Initiative for the funding and continued support of undergraduate research.

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Effects of Coffee on a *Drosophila melanogaster*

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Abstract

Cancer is a devastating disease that affects millions and researchers across the world are in search for new drugs and therapies to combat cancer. *Drosophila* flies acted as model tumors in this experiment and were treated with varying dosages of caffeinated coffee, decaffeinated coffee, and pure powder caffeine. The flies will not only be given coffee as part of their diet, but they will be irradiated as well. This will act as dual treatment just as humans are treated with radiation and chemotherapy. We expected the survival rate of the larvae to be lower with larger dosages of coffee and have a higher survival rate with lower doses of coffee. The final results can be viewed on the supplemental data set.

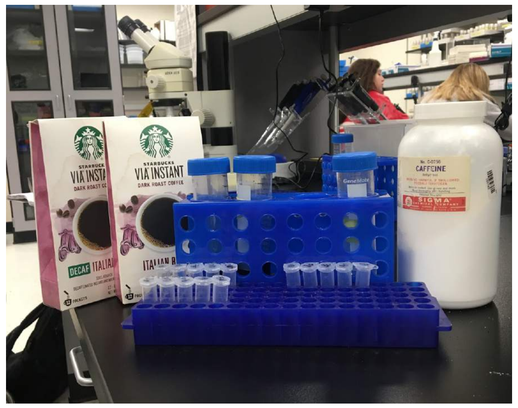
Introduction

In the experiment, *Drosophila melanogaster* are used as tumor models because of their quick reproduction rates and production of stem cells. Tumors are rapidly dividing groups of cells that can spread throughout the body, thus it is important to ensure that the correct treatment is administered to prevent further spreading of cancer. Chemotherapy is normally used as an adjunct to surgery to eradicate the mutated cells. This is an effective form of treatment along with radiation and a combination of drugs. However, there are risks involved when treating cancer. The body becomes incredibly weak during treatment and the patient runs the risk of becoming resistant to the drugs. Multidrug resistance is a serious problem that needs to be addressed because the cancer can disappear for a short amount of time, then return more aggressively. By testing different drugs, caffeine in our case, we can find other medications and drugs that have the potential to prevent cancer from spreading or from resistance.

Caffeine is a compound often associated with the popular morning drink, coffee. Coffee is a readily available and common drink all around the world. In an eight ounce cup of coffee, there is approximately 95 mg of caffeine. Studies have shown that caffeine, that is tested through coffee, can have a beneficial effect against cancer. Although previously thought to be potentially negative, more and more studies have come out to support the benefits of coffee. It has been shown that caffeine suppresses the IGFR-pAkt signaling pathway which is a pathway that typically leads to increased cell growth and can aid in tumor survival (Rosendahl et al., 2015). When this pathway is blocked, the cancer cells cease to divide and eventually die. By changing the pathway, tumors have a higher affinity to accept other drugs and medicines which can lead to cancer cell death. A higher consumption of coffee is also associated with smaller tumor growth and a lower risk than low coffee consumption (~1cup/day). Growth suppression of estrogen receptor-(+) breast cancer cells correlates with moderate to high consumption of coffee (two to over five cups a day) due to caffeine's effect of reducing estrogen receptor and cyclin D1 levels in these cells. Estrogen receptor-(+) tumors, or hormone receptor-positive, account for 80% of all breast cancers. Even though coffee shows promising results in treating cancer, it has not been used as a chemotherapy because it has been inconclusive as to which component helps in reducing tumor growth. This experiment aims to determine if coffee and/or caffeine consumption will impact cancer cell survival in a *Drosophila melanogaster* model.

Hypothesis

Coffee has shown to help reduce tumor proliferation by altering the IGFR-pAkt signaling pathway for cancer cell growth. By adding coffee to the food source of growing *Drosophila* larvae, survival rate will decrease. It is expected for the survival rates to decrease with both caffeine and coffee, however the drop in the rate for coffee should be more significant.



Methods

1. **Dilute -** An initial dilution was created using 10 mL of water and 100 mg of the solute (instant coffee, instant decaf coffee, or pure caffeine). These solutions were vortexed to ensure proper mixing. Serial dilutions were then performed with each compound to achieve concentrations of 10 mg/mL, 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL, 0.001 mg/mL of solute to solvent (water). It is important that all vials are properly labeled according to their solute and dilution. (See Table 1)

2. **Drug -** Using 48 vials all labeled according to their diluted substance and triplicate number, 3 mL of food will be added along with 3 uL of the diluted drug. This experiment took place in 23°C.

3. **Irradiate –** Irradiate *Drosophila* flies with 4000 rad. Irradiated larvae will be added to each vial in order to mimic real life procedures.

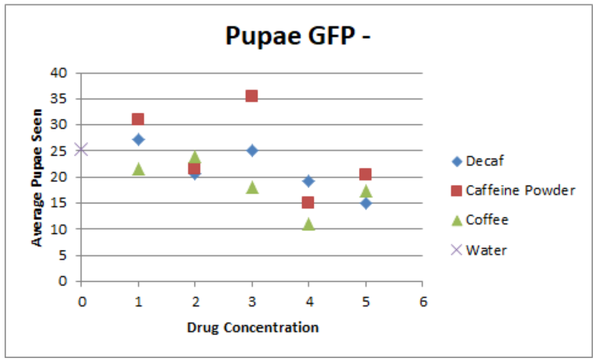
4. **Quantify -** After the larvae have grown to pupae they will be marked to determine if they are GFP-negative or GFP-positive based on if they will glow under a blue light. The homozygous GFP-negative flies will not glow and are the ones used in this experiment. This mutation mimics mutations found in human tumors that disrupt cell cycle checkpoints. It allows the cells to be more susceptible to radiation. Once the flies are grown the circled pupae will be marked if they were dead or alive (full or eclosed). Note how many pupae survived and died for GFP-positive and GFP-negative flies.

5. **Analyze -** Compare the amount of fly deaths to the amount of deaths in the positive control (colchicine) to determine efficacy of each solution. The total time for the experiment is three weeks.

Tube	Amount of Previous Solution (µL)	Amount of H2O (µL)	Drug Concentration (mg/mL)
1	100*	0	10.0
2	10	90	1.0
3	10	90	0.1
4	10	90	0.01
5	10	90	0.001

Table 1: Dilution Table
*Initial Dilution does not include any previous solution

Results



The survival data was collected after the poster was printed and can be viewed in the supplement. There is not enough information from the counted pupae to count survival rate of GFP - flies. However, based on the hypothesis the greater the concentration of the drug the lower the survival rate with caffeinated coffee having the lowest survival rate. This is what is expected and will be proved or rejected in the supplemental data set.

Conclusions

In our initial test, we expected the survival rate of the *Drosophila* larvae to drop when coffee was added to the food source. We expected to see a lower survival rate with a higher dose of coffee in the food and a higher survival rate with a lower dose of coffee. By experimenting with normal coffee, decaffeinated coffee, and pure caffeine powder, we can determine if caffeine is an important compound for reducing cancer cells.

If coffee is shown to be an effective chemotherapy, then we will have determined if it is the caffeine or another compound within the popular drink that produced these results. As one of the most popular drinks in the world discovering if it is an effective cancer treatment would benefit a large population. Through understanding the compounds in coffee that are or are not effective to kill cancer cells further research can be performed to produce a more efficient chemotherapy.

We hope to make further claims regarding the efficacy of coffee and caffeine after data is collected. This additional information will be available in our supplement.

Future Directions

Data from this experiment suggests possible future research including:

1. Testing coffee's antioxidant properties. These antioxidant properties can be tested to observe whether coffee is more beneficial in the prevention of cancer rather than killing cancer cells once already present.

2. Testing coffees possible effects on inhibiting angiogenesis. An in vivo study using test subjects such as mice could be done to test whether coffee has an effect in inhibiting angiogenesis, or the development of new blood vessels, which is essential for cancerous cells to secure oxygen and nutrients.

Acknowledgments

We would like to thank Dr. Tin Tin Su for providing resources and carrying out research work. Also we would like to thank Dr. Pamela Harvey for providing guidance and support throughout our research process. Finally, we would like to acknowledge our TA's Jessica Westfall. The funding for this course and experiment was provided by the Molecular, Cellular, and Developmental biology department at the University of Colorado- Boulder. Funding has also come from the Howard Hughes Medical Institute and the Biological Sciences Initiative.

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Identification of the Modulatory Effects of Resveratrol on Chemotherapy

University of Colorado Boulder

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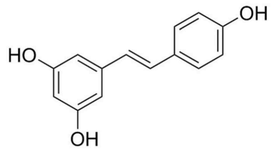
Abstract

The cure for cancer through treatment like chemotherapy has been sought since the dawn of the disease's incursion. But there is still no known method to treat all types of cancer sufficiently and permanently. This experiment looks at the dosing series effect of the drug compound resveratrol as well as the toxicity impact resveratrol has in accordance to colchicine as a potential chemotherapy. Resveratrol is a natural antioxidant found in foods and other derived products that have been observed to have additional chemotherapeutic potential in humans. Resveratrol is known to mediate inflammation and tumorigenesis, increase cell lifespan, and possess cardioprotective properties.

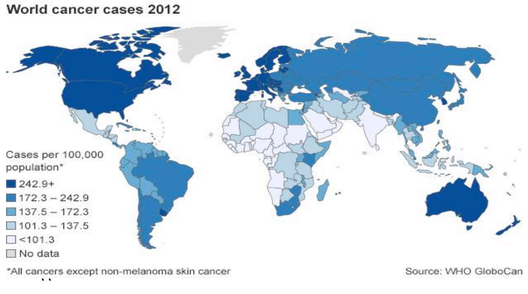


We introduced increasing concentrations of resveratrol in vials containing Drosophila melanogaster, a model of head and neck cancer tumors, third instar larvae without the grapes and GFP gene. In a second trial, we had a series of vials with a constant dose of resveratrol with decreasing concentrations of colchicine, a highly toxic chemotherapy. The negative control was dimethyl sulfoxide (DMSO), a compound known to have no effects on fly growth. We quantified the amount of alive and dead flies in each test vial after eclosion.

Although colchicine is known to be effective at lowering fly survival and resveratrol has been found to have positive effects on human cancer cells, the tested concentrations of resveratrol were ineffective at significantly reducing fly survival and only the 1 µM colchicine in 10 µM colchicine samples resulted in notably lowered fly survival. The dosages used in this study may not be sufficient to produce substantial results, and further testing at higher doses of resveratrol is needed to support the study.

Introduction

Cancer remains extremely prevalent among the world population, with approximately 14 million new cases in 2012, with this number expected to increase by 70% over the next two decades. In 2015, cancer accounted for 8.8 million deaths, or about 16% of the world total, with 70% occurring in low-income countries. Though the economic cost of cancer treatment and prevention (approximately US$1.16 trillion in 2010 in the US alone) increases every year, there is still no completely successful method for treating and preventing cancer.

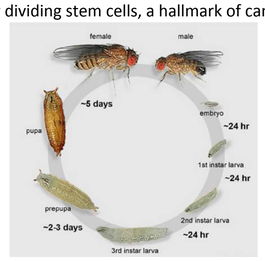


The traditional methods of treating cancer include surgery, chemotherapy, and radiotherapy, but none of these methods have a high success or survival rate and those who do survive often suffer from debilitating side effects for the rest of their lives. Worse, many therapies lose effectiveness over time as cancers change, meaning that there is never any guaranteed "cure" for any type of cancer. In addition to traditional therapies, due to the fact that 90-95% of cancers are attributed to lifestyle, including exercise and diet, herbs and other natural products are often used as well. Though in most cases they cannot replace surgery, chemotherapy, or radiotherapy can serve as supplementary therapy and cellular signaling agents.

Introduction

One of these natural therapeutic compounds that has received increasing attention over the last two decades is resveratrol, a phytoalexin antioxidant found in grapes, berries, peanuts, and related plants, in addition to derived products such as red wine and grape juice. The anticarcinogenic potential of resveratrol was first discovered in 1997, and since then it has also been found to mediate inflammation and tumorigenesis, confer cardioprotective effects, and increase cell lifespan. Resveratrol, both in vitro and in vivo, has been found to have positive effects on multiple types of human cancers. In addition, resveratrol has been noted to have potential when used as a combined therapy alongside other anti-cancer drugs.

Drosophila melanogaster, or the common fruit fly, , is a commonly used model organism because of its short life cycles and ease of breeding and care as well as possessing many homologous genes to humans. Third instar (15-20 mm long) *D. melanogaster* larvae are especially useful for testing potential chemotherapies as the pupae that they metamorphosize into possess rapidly dividing stem cells, a hallmark of cancer.

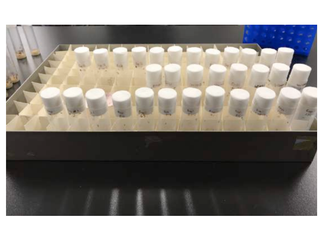


Hypothesis

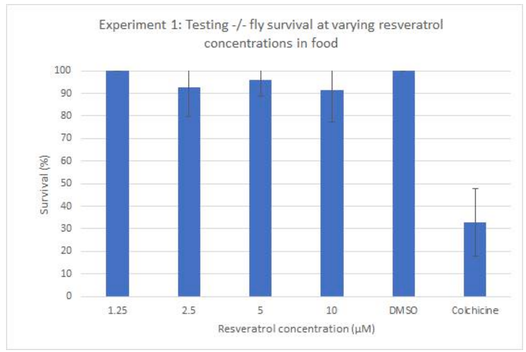
Hypothesis: Due to resveratrol's potential as a chemotherapeutic agent, it was hypothesized a dosing series of resveratrol added to fly food would increase fly death at increasing concentrations and that a constant concentration of resveratrol would decrease the concentration of colchicine needed to achieve significant fly death compared to colchicine by itself.

Methods

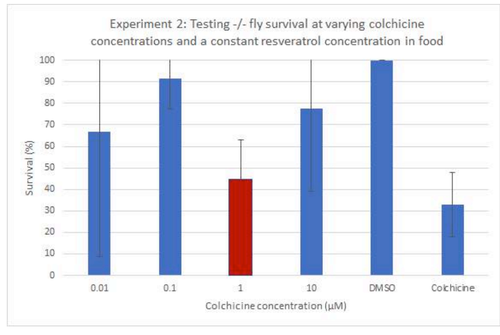
1. Prepare fly vials with two dosing series, the first with 10, 5, 2.5, and 1.25 µM concentrations of just resveratrol and the second with 10, 1, 0.1, and 0.01 µM concentrations of colchicine and a constant resveratrol concentration of 10 µM. Prepare 3 vials of each dose, each containing 3 mL of food in addition to the drugs.
2. Prepare 3 vials each of DMSO(Dimethyl sulfoxide) and colchicine controls, and four additional water controls containing 300, 30, 3, and 0.3 µL of water.
3. Add non-irradiated third instar larvae to each vial.
4. Mark GFP-negative larvae on each vial.
5. When adult flies have hatched, count alive/dead and quantify survival in each vial.



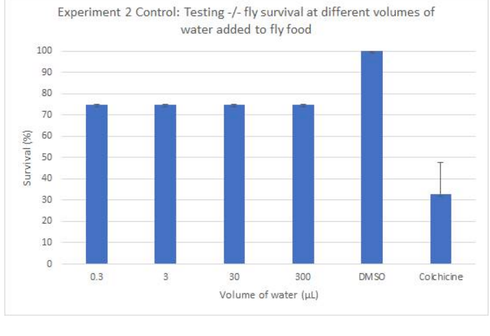
Results



Effect of a resveratrol dosing series on *D. melanogaster* survival
Initial concentration of resveratrol (10 µM) was diluted by half for 3 serial dilutions. Colchicine was used as a positive control and DMSO was used as a negative control. There were no results significantly different from the DMSO survival rate.



Effect of a colchicine dosing series in a constant resveratrol concentration on *D. melanogaster* survival
3 µL of 10 µM resveratrol was added to each vial, then different volumes of 10 µM colchicine were added to each vial to adjust colchicine concentration. Colchicine by itself was used as a positive control and DMSO was used as a negative control. The red bar indicates a result that was significantly different from the DMSO survival rate.



Effect of increasing volumes of water in food on *D. melanogaster* survival
Increasing volumes of distilled water were added to 3 mL fly food, starting at 0.1 µL and increasing tenfold with each vial until 300 µL.Colchicine was used as a positive control and DMSO was used as a negative control. This was done because experiment 2 used volume to vary colchicine concentration rather than serial dilutions.

Conclusions

In experiment 1, the results didn't show any significant difference from the DMSO negative control, leading to the conclusion that any dose of resveratrol below a concentration of 10µM is most likely ineffective as a chemotherapy. Due to the flies having higher survival rates than expected, no doses in this experiment would be considered a hit. However, as resveratrol has been noted to have positive chemotherapeutic effects in past studies, resveratrol in higher doses may lead to lower survival rates which could have then potentially be considered a hit.

In experiment 2, the results showed decreasing survival rates when resveratrol was paired with colchicine. The most promising trial was the pairing of 1 µM (0.3 µL of 10 µM solution) of colchicine with 3 µL of 10 µM resveratrol, resulting in significantly lowered fly survival compared to DMSO but still greater than the pure colchicine control. Considering the inconsistencies in the fly death from dosage to dosage, it may be difficult to determine whether the rate of fly death was due to the drug or rather due to chance. However, all dosages maintained a higher fly death rate than resveratrol alone, which does signify that when paired, the two compounds can lead to lower survival rates.

Limitations in these experiments include the small doses used for each compound. The control colchicine vials, which had 3 µL of 50 µg/mL of colchicine in each, had significantly higher concentrations than vials in either experiment, which were diluted to a maximum of 10 µM of the compound. The control doses of colchicine should have been reduced to the experimental concentrations in order to understand the effects of equivalent dosages. In addition, the experiment 2 vials were prepared by adding different concentrations of 10 µM colchicine solution rather than by serial dilution. This was controlled by four experimental water vials, which all resulted in 75% fly survival (compared to 100% for DMSO), meaning that the increased volume of liquid may have affected fly survival in the experiment 2 vials.

Future Directions

Data from this experiment suggests possible future research including:

1. Testing dosing series at higher compound concentrations. Though the results from these experiments suggest that doses of 10 µM and below are ineffective, higher concentrations may prove to significantly decrease fly survival.
2. Testing against third instar larvae that have been irradiated. Irradiation significantly increases the sensitivity of the larvae to potential chemotherapies, and also has relevance to human cancer testing as many cancers are treated by combination radiation and chemotherapy.

Acknowledgments

We would like to thank **Dr. Pamela Harvey** for serving as our research instructor, teaching assistant **Ben Huxley,** and **Dr. Tin Tin Su** for serving as the principal investigator and sponsor for our lab. We also acknowledge funding and **supporting organizations**: Howard Hughes Medical Institute, CU Boulder Molecular, Cellular, and Developmental Biology department, and Biological Sciences Initiative (BSI) at CU Boulder.

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The Efficacy of 1,2-Propanediol as a Chemotherapeutic with Adjuvant Propolis in *Drosophila melanogaster*

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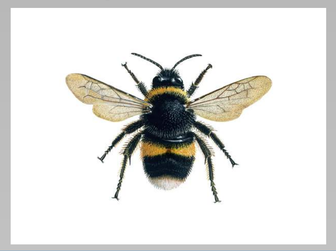
University of Colorado-Boulder

Abstract

Cancer is a disease in which abnormal cells divide uncontrollably and destroy healthy body tissue. The third instar larvae of *Drosophila melanogaster* make excellent cancer models due to their rapidly dividing cells. The *grapes* gene in *Drosophila* is the equivalent of the Checkpoint Kinase 1 gene in humans which, when mutated, is responsible for head and neck cancers.

Compound 1,2-Propanediol was tested and found to be hit as a potential chemotherapy. In addition to testing this compound, we chose to test Propolis, a resinous mixture that honey bees produce by mixing saliva and beeswax. Studies indicate Propolis as a potential adjuvant chemotherapeutic due to its ability to prevent drug efflux from cells (Vukovic). To test our compounds, we irradiated larvae at 4000 Rads and exposed them to 1,2-Propanediol alone and 1,2-Propanediol with Propolis. We used irradiated flies in conjunction with a drug in order to use our drug at a lower concentration and lower toxicity. Results have been inconclusive, as some of the flies have not eclosed yet. Preliminary results suggest Propolis increases survival among flies.



Introduction

Cancer is the rapid growth of abnormal cells in the body. In 2015, the World Health Organization reported that 1 in 6 global deaths were attributed to cancer (Cancer). Current treatment of the disease includes radiation therapy, chemotherapy, and surgery. Most chemotherapeutics only target DNA replication and remain extremely toxic. As the cancer is being killed, healthy cells are damaged as well.

Drosophila melanogaster are used commonly as cancer models because their third instar larvae undergo a period of rapid cell division which is similar to the nature of tumor cells. The flies share around 75% of their genes with humans. In our case, we look at the *grapes* gene which is the same as Checkpoint Kinase 1 in humans. This gene, in humans, is involved in the development of head and neck cancers if mutated. Using *Drosophila melanogaster* enables us to predict the efficacy of a novel chemotherapeutic when combined with radiation in humans.

The drug we decided to test 1,2-Propanediol was a “hit” when tested in the Drug Discovery Lab, indicating that it is a possible chemotherapeutic. We decided to test compound 1,2-Propanediol with Propolis, which is a resinous mixture that honey bees produce by mixing saliva and beeswax. Studies indicate that Propolis could be a potential chemotherapeutic adjuvant, as it prevents the efflux of drugs once they enter cancer cells. Further, several flavonoids within Propolis (luteolin and myricetin) exhibit the ability to induce apoptosis of cancer cell lines and inhibit cell cell growth (Vukovic). Propolis has had limited exposure in cancer trials, but preliminary results show promise for its ability to induce cancer cell death.

Statements of Hypothesis

- 1,2-Propanediol has the potential to be an effective chemotherapy treatment with increase in dosage and that the effect will be positively enhanced by a Propolis solution.

Methods

Raised and maintained adult *Drosophila melanogaster* and collected its third instar larvae

↓

Irradiated the third instar larvae

↓

Exposed third instar larvae to a dosing series with compound 1,2-Propanediol in 3 mL of fly food with positive and negative control.

↓

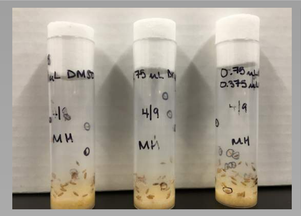
Exposed third instar larvae to a dosing series with compound 1,2-Propanediol and Propolis in 3 mL of fly food.

↓

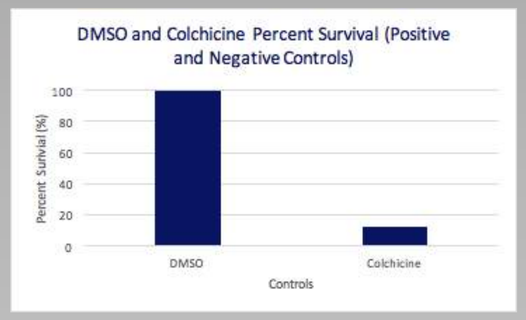
Marked GFP of third instar larvae after 48 hours of exposure to drug

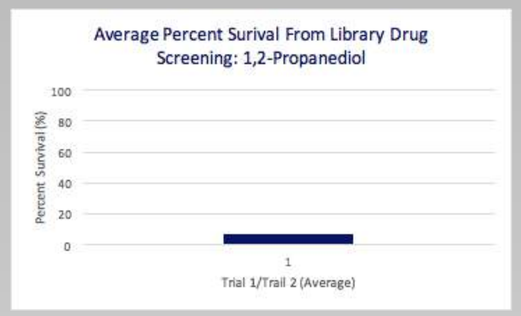
↓

Quantified survival of third instar larvae after 5-7 days



Results





We are still waiting for final results for our project. Due to a misplacement of our drug supply, we had to run drug vials on two separate occasions. Our second vials had larvae placed a few days after the initial set-up of the vials and we are still awaiting these results. The data and results recorded will be represented on a supplement to this poster.

During the initial library screenings in this lab, we tested 813 compounds, twice. The percent survival for 1,2-Propanediol, averaged between the two trials, was **7.14285714%**. In addition, all colchicine vials were correctly identified through the results obtained as well as the three known FDA-approved chemotherapies. DMSO showed a **100%** survival rate in the one trial taken into account in the figure and Colchicine showed an **11.96%** survival rate. Preliminary results, although not conclusive, suggest Propolis could increase survival among irradiated flies. Flies exposed to Propolis and drug had a **100%** survival rate, compared to a **96.8%** survival rate with drug alone.

Conclusion

We have been unable to confirm our hypothesis that Propolis decreases survival in conjunction with a chemotherapy because we are still waiting on some results. Preliminary results have been inconclusive about the efficacy of our drug to kill the third instar larvae, but some results suggest that Propolis increases the survival among the flies.

In the first set of vials, 100% of the flies lived in the drug/Propolis vial, compared to 96.8% in the drug alone vial. Although this contrasts with our initial hypothesis, these results could prove to be very important to cancer patients if Propolis helps to lower the potentially toxic doses of chemotherapies in conjunction with radiation. We would like to further our research with a revised hypothesis, being that Propolis helps lower effective dose quantities of chemotherapies when used with radiation.

Future Directions

Experimentation led to inconclusive results, so we would like to further experiment in order to test the effects of Propolis and 1,2-Propanediol.

1. Test Propolis with the positive control Colchicine in order to see if Propolis aids a known chemotherapy or increases survival among flies.
2. Test propolis alone without any drug compound in order to test whether or not Propolis increases survival of irradiated flies.
3. Test a full dosing series consisting of a wider variation of doses of 1,2-Propanediol and Propolis since we did not receive an adequate amount of the drug to perform all dilutions.

Acknowledgements

We would like to thank **Dr. Tin Tin Su** for providing us the opportunity to assist with her research through this course. We would also like to thank our Teacher’s Assistants, Katie Franks, Jack Shutz, Erin Kneeskern, Oula Kareen for their dedication to helping us with the research and learning involved with this lab. Thank you to **Pamela Harvey** for conducting the lab and giving us the opportunity to be introduced to undergraduate research. Thank you to the **Howard Hughes Medical Institute** and the **Biological Sciences Initiative** for funding the research done in this lab. Finally, we like to extend a large thank you to the **Molecular, Cellular, and Developmental Biology** department at the University for supporting the lab and our participation in it.

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The effect of sulforaphane, broccoli sprout extract, and raw broccoli on *Drosophila melanogaster* third instar larvae

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Abstract

Roughly 1 of 2 Americans will suffer from cancer during their lifetime. Due to tumor heterogeneity, drug resistance and negative side effects of strong chemotherapies, new anti-cancer compounds must be constantly developed.

In this study, *Sulforaphane (SFN)* is analyzed in an isolated environment as the primary compound. Additional analysis of broccoli sprout extract and raw broccoli explore the synergistic potential of metabolizing SFN in conjunction with natural Brassica compounds.

Drosophila melanogaster third instar larvae were exposed to 40µM SFN. Half of the larvae were irradiated to explore interaction between radiation and compound.

Combination therapies can decrease effective dose while increasing potency of chemotherapeutic compounds, protecting overall patient health. Lower doses of chemotherapy minimize healthy cell death and reduce negative side effects. Compounds like SFN may improve potency of other anti-cancer treatments or limit cancer cell proliferation. Results were inconclusive.

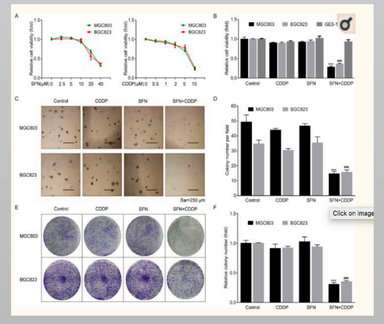
Introduction

Each case of cancer exhibits unique factors that make developing a single treatment challenging. Recent research suggests that a class of compounds called phytochemicals possess chemotherapeutic properties by inducing apoptosis and preventing cell proliferation.

Among these phytochemicals, compounds in the *Brassica* family demonstrate the ability to attack multiple hallmarks of cancer at a time. *Sulforaphane (SFN)* ($C_6H_{11}NOS_2$) demonstrates ability to reduce the viability of gastric carcinoma in human cell lines. SFN was effective when paired with other anti-cancer compounds in adjuvant therapy.

D. melanogaster serves as a practical model organism in this experiment because they are inexpensive, have 10 day life cycles, and exhibit cell signaling pathways similar to cancer.

D. melanogaster share 75% of their genes with humans and display under-regulated growth factors resulting in high cellular proliferation.



Hypothesis

Food + capsule or raw broccoli:

	Sulforaphane	BSE	Broccoli
Irradiated	*Promote death*	*Promote life*	*Promote life*
Non-irradiated	*Promote death*	*No effect*	*No effect- balance of negative and positive compounds. NOTE: possibly a compound that reduces cell proliferation if they die.*

Drug ethanol extract + food

	Sulforaphane	BSE	Broccoli
Irradiated	*Promote death*	*Promote life*	*Promote life*
Non-irradiated	*Promote death*	*No effect*	*No effect*

There is no expected difference between the survival rates of pure food dissolution and ethanol extract vials because the powders are already purified forms of each compound. Broccoli in its pure and extracted forms should promote cellular growth.

Purpose

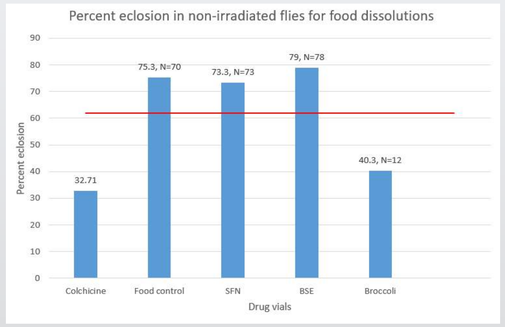
- Determine whether SFN is useful in killing rapidly dividing cells
- Compare the effects of isolated SFN to synergistic effects of compounds found in broccoli

Methods

1. **Breed** *D. melanogaster* in population cages, sync lifecycles, isolate 3rd instar larvae by sieving, collect larvae in containers for irradiation
2. **Mix** food and compounds
   - Food sets: mix SFN, BSE, and raw broccoli into fly food
   - SFN: 40uM, BSE: 8.929mg/mL, raw broccoli: 0.9g/3mL
   - Ethanol extraction: combine SFN, BSE, and raw broccoli with ethanol to extract biologically active compounds, mix 3uL of extract with 3mL of food for each compound.
   - SFN, BSE: combine 1 capsule with 3mL ethanol
3. **Irradiate** half the larvae with 4000 Rad
4. **Expose**
   - Add 50-100 irradiated and non-irradiated larvae separately to the food and ethanol extraction sets.
5. **Quantify**
   - Mark GFP+ (grp+/grp-) periodically after pupae begin to form
   - Mark eclosion 9-14 days after combing the larvae and food mixed with drug
6. **Identify hits**
   - Compare eclosion rates of SFN, BSE, and raw broccoli (mixed with food and ethanol extraction) to negative controls and colchicine data

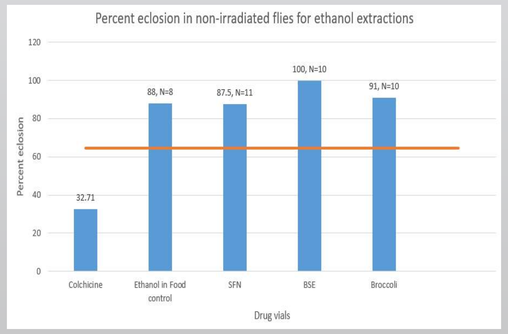
Results

Results were inconclusive. While raw broccoli mixed with food produced an eclosion rate similar to that of Colchicine, vials containing raw broccoli had significantly lower populations than other vials, producing inconsistent results. All compounds derived using ethanol extraction yielded results that suggest these compounds are not effective anti-cancer drugs alone at this concentration.



Food Dissolution

Raw broccoli mixed with fly food produced percent eclosion similar to that of Colchicine, but also had significantly lower larvae populations.



Ethanol Extraction

None of the compounds processed using ethanol extraction yielded eclosion rates within a 95% confidence interval of known chemotherapy, Colchicine. None of these compounds are effective anti-cancer compounds at given concentration, alone.

Conclusions

Irradiated fly data was unavailable. Due to this only non-irradiated fly data can be analyzed. Based on the food dissolution graph, raw broccoli was the only compound group with an eclosion percent outside two standard deviations of the negative control. This suggests raw broccoli has chemotherapeutic potential. However, the average population for this triplicate was 12, compared to 70, 73, and 78 for the other compounds, meaning that this anomaly could be attributed to chance.

Based on ethanol extraction data, all 4 compound groups fall above two standard deviations of the negative control. This suggests that none of these compounds are likely to possess chemotherapeutic potential. As with the raw broccoli data, the average populations of the ethanol extract vials are between 8-11, which suggests that these values could be attributed to chance.

Future Directions

1) Combine SFN with a known chemotherapy and observe the interaction. Analyze potential for combination therapy.
2) Conduct dosing series of SFN on *Drosophila melanogaster.*
3) Test SFN in human cancer cell lines.
4) Test SFN as an anti-cancer drug in mice.

Acknowledgments

A special thanks to principle investigator Dr. Tin Tin Su, Dr. Pamela Harvey, teaching assistants Katie Franks, Erin Kneeskern, Oula Kareen, and Jack Schutz. Also a special thanks to our funding sources: Molecular, Cellular, and Developmental Biology, Howard Hughes Medical Institute and Biological Sciences Initiative, without which the research would not have been possible.

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The Effects of Mistletoe Extract on Ribosomal Function and Ribotoxic Stress-Induced Apoptosis in Third Instar *Drosophila* larvae

University of Colorado Boulder

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Abstract

Cancer is the second leading cause of death within the United States, contributing to **one of every four deaths**. In 2014, 1,596,486 Americans received a new cancer diagnosis and **591,686 died from cancer.** The direct medical costs for treating cancer in 2014 was $87.3 billion (Center for Disease Control and Prevention, 2018). Most common treatments for cancer currently include chemotherapy and radiation treatments. Drug-resistance in tumor cells to common therapies presents a roadblock in treating cancer, and viable **alternatives continued to be researched**. In our experiment, we looked at the effect of **Mistletoe extract (ME**) on Drosophila larvae survival rates and impact on ribosomal RNA.

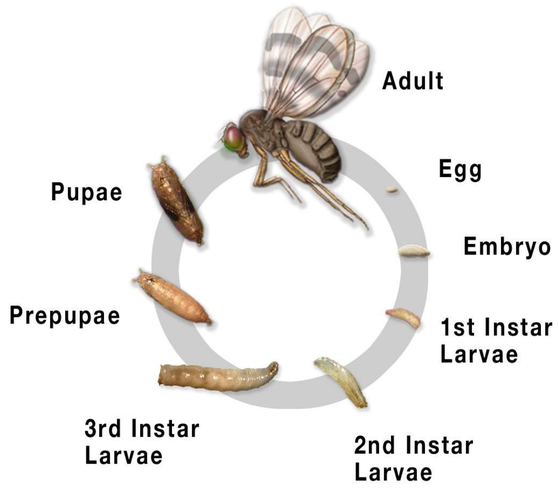
Introduction

Mistletoe extract has been regarded as a holistic treatment for cancer. Current research has isolated mistletoe extract **lectin** (ML-1) as a potential cancer treatment due to its cytotoxic properties. (Gamerith et al. 2017; Trefzer et al. 2014). ML-1 is a class II **ribosome-inactivating protein** that presents a B-chain that binds to over-expressed CDC75s in tumor cells, allowing for cellular uptake of ML-1 (Bergmann et al. 2008). The A-chain on ML-1 cleave 28s ribosomal RNA, **preventing global protein synthesis** (Narayanan et al. 2005). ML-1 also promotes an **immune response**, and is associated with **increased cytokines** IL-1b, TNF-a and interferon gamma.

Clinical trials are currently testing ML-1 as an immunotherapy, though trials have not tested for ME's immunotherapy potential in **head and neck cancers**. To model head and neck cancers, we used third instar Drosophila larvae because of their cellular similarities to cancer. However, Drosophila lack the adaptive immune system present in humans and the immune response mediated by ML-1. Thus, we are interested in **ME's potential to induce immune response-independent cytotoxicity** within our Drosophila model.

Hypothesis

We predict that Mistletoe Extract **decreases cell viability** in irradiated third-instar Drosophila larvae in an immune-system fashion **through ribotoxic stress-induced apoptosis**.



Methods and Experimentation

Vial Preparation: For mistletoe extract (ME) mixed into food vials, we used **Mistletoe Liquid Extract**, Organic Mistletoe Tincture Supplement (Hawaii Pharm, Hawaii). Fly food vials were prepared according to Discovery Based Laboratory II (MCDB 2151) Fly Food Protocol (See Supplemental Methodology). Based on previous calculations, (see Supplemental Data) ME volumes added were **3.0ul,1.5ul,0.75ul,0.375ul, and 0.1875ul**. ME dilutions were performed according to MCDB 2151 Pipetting Exercise using the Bradford Assay protocol (see Supplemental Methodology). **Vials were prepared in triplicate for each dose**, for 15 vials total.

Positive control: **Colchicine** is used as our positive control because it is a highly lethal compound and is currently used as a chemotherapy. **3ul** of Colchicine dissolved in DMSO (50ug/mL) will be added to fly food vials in triplicate. 3 total vials.

Negative control: **3ul** of **Glycerol** or **DMSO** will be added to two separate sets of fly food vials in triplicate. Glycerol is a protic polar solvent in which ME was dissolved in. DMSO is the solvent for colchicine and has been established to not have any effects on drosophila viability. DMSO is also used to compare for potential effects of glycerol on drosophila viability. 6 total vials.

Gel Electrophoresis: 6 fly food vials were prepared with 3.0ul,1.5ul,0.75ul,0.375ul, and 0.1875ul of ME added. Approximately 75 larvae were added to each vial. See Supplemental Methodology for gel electrophoresis procedure

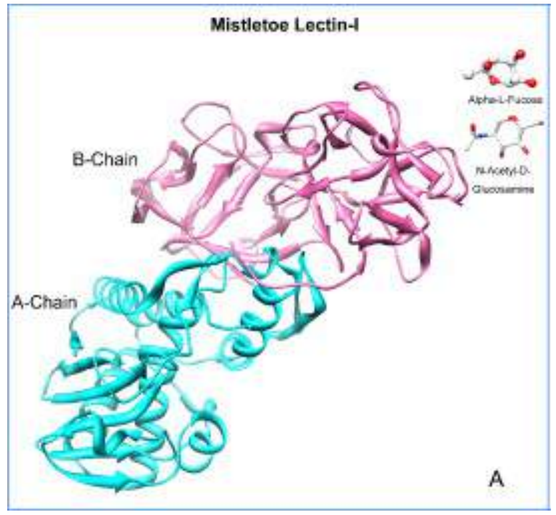
Results

As a group, **our hypothesis was** that the amount of surviving flies would decrease with the addition of Mistletoe Extract. The vials containing Colchicine were known to be lethal was used as the positive control. The vials containing DMSO and Glycerol were used as negative controls because they were the vehicle for our compound.

The vials containing Mistletoe Extract were predicted to be lethal. As the dosage of ME increased, the lethality of the compound should have become more apparent. The greatest amount of flies in vials containing the dosage of 0.1875ul and the least in vials containing the dosage of 3.0ul.

The actual results were inconclusive. In order for results to be viable, 10 Drosophila must make it to the state of eclosion.

The reason for the lack of eclosion could be due to many factors**. We hypothesize our inconclusive results are due** to not enough moisture in the food or an overcrowd of larvae added into the vials.



The p53 gene is a tumor suppressor gene. Over 50% of human cancers have a mutation in the p53 gene (Nozaki & Nakagawara, 2011). It is therefore also in our interest to **investigate p53-independent cancer treatments**. Recombinant mistletoe lectin (rML) has been demonstrated to induce p53-independent, dose-dependent apoptosis in-vitro in irradiated mouse embryo fibroblasts, likely through apoptosis-associated factor 1 (Apaf-1) (Hostanka et al. 2003). A drosophila homologue for Apaf-1, ***dark***, has been identified (Rodriquez et al. 1999). As dysregulation of apoptosis has been identified as one of the major physiological mechanisms through which conditions such as **cancer, AIDS, neurodegenerative disorders, and autoimmune diseases** proliferate their effects. The potential apoptotic mechanism of *dark* may **serve to be a path towards uncovering future therapies** and treatments (8).

Conclusions

Our results were **inconclusive**, therefore we were not able to draw a cohesive conclusion from our results. After further examination we decided that it would be best to prepare our next vials with food that contained **more water**, since most of the food became too dry for the larvae to eat. We still have confidence that Mistletoe Extract could have positive results, but we would need to **redo the experiment** in order to quantify results to prove our hypothesis.

Future Directions

If we could be in this lab for an additional semester with unlimited funding, we would **first repeat our experiment**. Because we believe our results were do to inadequate food and an overcrowd of larvae per food vial, we would create the same experiment, but creating triplicates in different food consistencies and with less larvae within each. **Testing ME on the wild-type, as well as conducting this experiment without radiation**, would produce differing results and further our knowledge on ME within Drosophila. If our results consistently yielded the desired more than two standard deviations away from the mean survival of DMSO, ME would become a **hit compound** and continue onto pre-clinical trails.

Acknowledgments

We would first like to acknowledge The **Howard Hughes Medical Institute** and **The Biological Sciences Initiative** for the funding and supporting the research done by the Discovery Lab, along with the **Molecular, Cellular and Developmental Biology Departments** at the University of Colorado at Boulder. The main experimental components come from the Lead Investigator **Tin Tin Su**'s lab and experimental approach. We would like to thank Principal Investigator, **Dr. Pamela Harvey**, along with teaching assistants **Jess Colmenero, Ryan Fleischer and Jack McLeod**, for their contributions and expertise.

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Eugenol as a Potential Chemotherapy

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Abstract

As cancer is second leading cause of death in the world, the demand for more research on new treatments has increased significantly. Current treatment options are limited in their effectiveness of treating cancer and are often quite toxic to the human body, causing patients to experience painful side effects such as easily bruising and bleeding, hair and nail loss, fatigue, anemia, loss of appetite and ulcers. Our goal in Discovery Lab is to identify potential chemotherapies that have lower toxicities in combination with radiation therapy that can cure a disease that kills 7.6 million people every year.

In this study, the effects of eugenol, a common compound found in herbs such as cloves, are being studied on Drosophila larvae. Eugenol was chosen for its antioxidant, antiviral, anti-inflammatory, and anti-bacterial properties. To study eugenol, we obtained clove oil and clove powder and used varying concentrations of oil and powder an d tested them on irradiated and non-irradiated *Drosophila* larvae. We expected an indirect relationship between concentration of eugenol and fly survival.

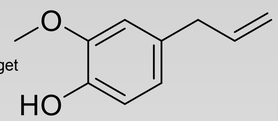
Cloves
https://www.healthline.com/health/clove-oil-toothache

Introduction

Cancer is the second leading cause of death globally with nearly 8.8 million in the year 2015. It has no preference and claims the lives of all ages from children to the elderly. And this number is only expected to increase. Many of the current treatment options are limited in their ability to combat this disease as cancer has the ability to resist many of the chemotherapy currently on the market. In addition, cancer is unique to people as different genes are able to mutate and impact different parts of the body. All of this has increased the demand for more research on new chemotherapies.

Drosophilia melanogaster, specially third star larvae, is used as the model for cancer in this experiment because it mimics cancer by its rapidly dividing nature.

Eugenol is an extremely common compound found in all types of herbs ranging from cinnamon to bay leaf to cloves. Eugenol extracted from clove is used in this experiment. Clove is known for its spicy smell. Eugenol has been claimed to have numerous healing properties, which include anticancer, anti-inflammatory, antibacterial, and claimed to cure and prevent an abundance of different diseases. It is most commonly used in the dentist to decrease the swelling in the gums.



Eugenol: Target Molecule

There have been several studies conducted about eugenol and its potential as treatment for cancer. It has been linked to causing apoptosis in cancer cells, evading p53, a commonly mutated gene found in cancer. Eugenol also was seen to inhibit several other genes, resulting in apoptosis of cancerous cells.These results have been seen in lung, colon, and breast cancer cells.

Statements of Hypothesis

Out of the 900 compounds tested in our two drug screens, we identified 32 hits. While these were fairly successful drug screens, our group hypothesized that another compound, eugenol, will provide a more effective in killing the larvae.

Methods

Two different methods of Eugenol were tested: clove oil and eugenol extracted with ethanol from clove powder. Each are tested with irradiation and without to determine eugenol potential effectiveness by itself. Ethanol and coconut oil are used as negative controls, which are made by adding 3 uL into 3 mL of agar. Ethanol is solely for the clove powder. While coconut oil is for both. The temperature is kept constant throughout the experiment at 25 °C.

Clove powder:

- Eugenol is extracted from clove powder by adding 5 mL of ethanol to 100 mg of powder.
- The concentrations used were 2 uM, 1 uM, and 0.5 uM, which is done by a 1:2 series dilution.
- 3 uM of each concentration is placed into 3 mL of agar.
- 3 vials are set up for each concentration.

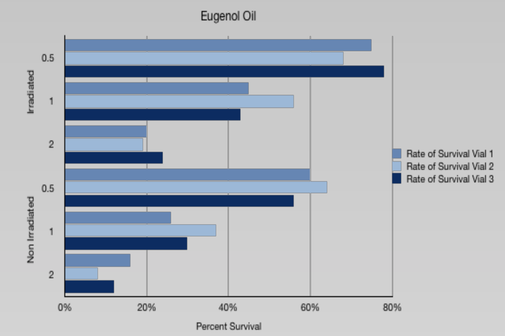
Clove Oil:

- 3.2 microliters of 6.33 M Eugenol is mixed with 10 mL of coconut oil for the stock solution
- For the 10% by volume, take 0.3 uL of the stock solution and place into 3 mL of food.
- For 8% by volume, take 0.24 uL of the stock solution. 0.18 uL for 6% by volume, 0.12 uL for 4%, and 0.06 uL for 2%.

For both:

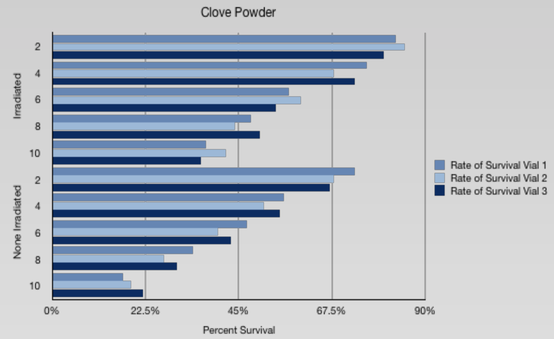
- Third instar larvae of *Drosophila melanogaster* are collected and placed into each of the vials.
- One set is irradiated at 4000 rad and another set is not.
- Allow the
- Circle GFP -/- pupae, normally .
- After an incubation period of 10 days, the rate of survival was calculated.

Anticipated Results



However, in the vials that contained irradiated larvae, we had nearly no pupae formation. This was consistent among other lab groups as well, therefore, our results will be inconclusive for the irradiated set due to experimental error. We predict to have conclusive results for the non-irradiated larvae as we had expected pupae formation. Our results were not ready by the time the poster needed to be printed, so please see our results on the supplement.

Our experiment using eugenol shows its effectiveness as a possible chemotherapy for cancers. We conducted an in vivo experiment using a dosing series and irradiated and non-irradiated larvae. We predict that there will be an indirect relationship between concentration of eugenol and survival rate of Drosophila. So, as the concentration increases for both clove oil and clove powder, the survival rate of the larvae decreases.



Anticipated Conclusion

The goal of this experiment is to determine the potential of Eugenol as a future chemotherapy drug. Clove oil and clove powder were tested at different concentrations. Each set was tested with and without being irradiated at 4000 rad. The similarities between *Drosophila melanogaster* and cancer cells allow the possibility of eugenol as a chemotherapy, determined by the results. If the results are correctly predicted, then the higher concentrations of eugenol in both the clove oil and powder will provide a statistically significant difference between the survival rate of those concentration to the respective negative controls. This would indicate more testing should be done to determine its complete potential for chemotherapy. This predication is supported by other experiments performed with eugenol and cancer.

The irradiated set would be retested in the future to determine if combining eugenol with radiation will have a larger impact on larvae survival. This may show that eugenol has more potential as a chemotherapy as it meets the drugs intended purpose.

Future Directions

- Test all concentrations of oil and powder again to confirm results
- Using pure clove leaf rather than a powder or ethanol extraction
- Test on another animal species to see if eugenol is safe in mammals
- Retest the irradiate larvae to determine if radiation has an impact on larvae survival

Acknowledgements

We would like to thank Dr. Pamela Harvey,, Marisa Martin-Wegryn, Jessica Westfall, Jesse Kurland, and Sam Gendelman for making our experiment possible and helping it run smoothly. Moreover, would like to thank the MCDB department, Howard Hughes Medical Institute, and Biological Sciences Initiative for providing the funding necessary for this lab. Finally, we would like to thank Dr. Tin Tin Su for making this lab possible and giving students the opportunity to participate in chemotherapy research.

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Examination of Grape Seed Extract on Head & Neck Cancer Models

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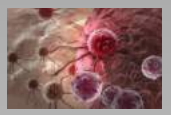
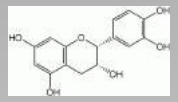
Abstract

Chemotherapy in combination with radiation is the common treatment for all varieties of cancer. In reality current chemotherapies on the market have proven to be insufficient and newer approaches are a necessity. Studies show that Grape Seed Extract contains properties that exhibit selectivity towards cancer cells. These anticancer properties were the rationale for observing Grape Seed Extract as a potential chemotherapy. Our objective here is to determine whether GSE has an effect on the cancer model, and if so, at what dosage and method (powder dissolution versus stock solution) display the most desired results? We performed a dosing series on Drosophila and tested different methods of distributing the GSE through both a powder dissolution and a stock solution (GSE dissolved in water). The data we obtained indicate that vials treated with powder and stock solution presented similar effects in reducing percent survival of the Drosophila. Despite variation in our data, our results for all concentrations of both set of vials with powder and with stock solution were outside the threshold of two standard deviations from the average negative control percent survival, considering them a hit. However, majority of vials contained less than 10 pupae making the totality of our data inconclusive.

Introduction





Chemotherapy is often viewed as a cure-all solution for cancer to many individuals. In reality however, for the 50% patients who undergo chemotherapy, only about 10-15% of them are cured by this treatment. This demonstrates the staggering need for more effective cancer treatment options, as this disease impacts the lives of many individuals. Cancer treatment has faced many obstacles because cancer is a multifaceted disease embodying the different hallmarks of cancer. For example, a treatment that targets cancer's ability to avoid apoptosis likely won't influence the cell's angiogenesis ability, and therefore wouldn't be effective alone.

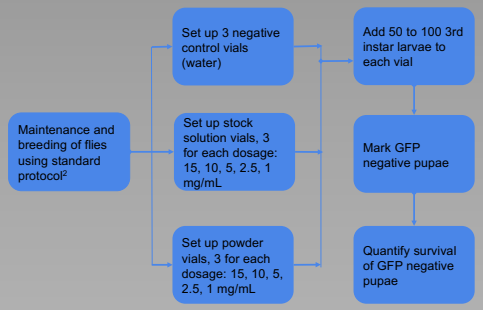
The compound that we are testing is Grape Seed Extract (GSE), which is primarily >95% proanthocyanidin (an antioxidant/free radical scavenger) and also consists of a slew of gallic acid, catechin, and epicatechin. Grape seed extract is a dietary supplement often taken regularly by many. Other phytochemicals (a larger group which Proanthocyanidins fall within)are highly abundant in many fruits and vegetables. Studies done previously of GSE on cancerous tumors has shown promising results. GSE has been shown to inhibit pancreatic cancer cell growth in vitro and in vivo through induction of apoptosis and the targeting the p13K/Akt pathway[1], GSE has shown to decrease cyclin B1 expression and an increase in cdc25C phosphorylation leading to cell arrest in head and neck squamous cell carcinoma(HNSCC)[4] GSE has shown to activate DNA damage sensors and checkpoints in division leading to cell cycle arrest in HNSCC[4], and GSE has shown that it can induce double stranded breaks in HNSCC leading to cell cycle arrest[4].

For this lab we are testing GSE through Drosophila(fruit flies) which acts as the model organism for our experiment. They are a holometabolous insect that develop from a larvae into pupae before adulthood in a process spanning seven days. Drosophila have a life span of ten weeks during which adult female flies can lay about 50-70 eggs per day. It takes about three days after fertilization for flies to develop into third instar larvae, which is used in this experiment. Drosophila serves as a good model organism as it is cheap to maintain compared to other experimental subjects like mice. Additionally, the Drosophila we are selectively choosing display a mutation in the *grapes* gene, which is equivalent to Checkpoint Kinase I in humans. The *grapes* mutation in flies causes less protein (Checkpoint Kinase 1/*grapes* protein) to be made, therefore induces less checks for DNA damage which models head and neck cancers. Tested larvae will undergo a compound treatment with radiation first. Radiation will also induce DNA damage at a higher rate to further unchecked mitotic divisions.

Statement of Hypothesis

If Drosophila is treated with a high concentration of GSE (distributed through a stock solution) then the GSE will have a greater effect on irradiated larvae because the homogeneity of the stock solution will distribute the GSE more efficiently to Drosophila and the higher concentration will allow for the anticancer properties of the extract to be more effective.

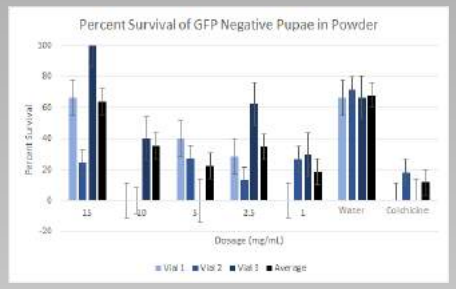
Methods

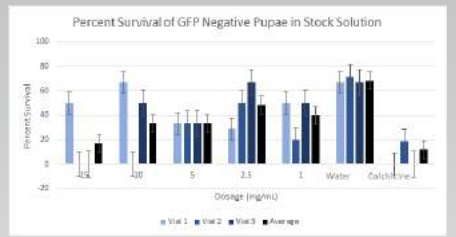


Dosage (mg/mL)	Powder/Stock	GSE (mg)	Water (mL)	Fly Food (mL)
15.0	Stock	600.0	1.00	3.0
10.0	Stock	400.0	1.00	3.0
5.0	Stock	200.0	1.00	3.0
2.5	Stock	100.0	1.00	3.0
1.0	Stock	40.0	1.00	3.0
15.0	Powder	45.0	0	3.0
10.0	Powder	30.0	0	3.0
5.0	Powder	15.0	0	3.0
2.5	Powder	7.5	0	3.0
1.0	Powder	3.0	0	3.0
Control	Negative	0	1.00	3.0

Results

The general trend of the data shows that vials with powder produced similar effects in reducing the percent survival of the flies in comparison to the stock solution. The most notable difference is between the dosage of 15 mg/mL of stock solution (yielded lowest percent survival) and the 15 mg/mL of the powder (yielded the highest percent survival). Despite the similarity between the stock solution and powder results, all dosages except 15 mg/mL of powder landed outside two standard deviations of the mean survival of the negative control, indicating a hit. The mean of the percent survival of the water is 68.25% and the standard deviation is 3.88, creating an expected standard bell curve ranging from 76.01% to 60.49%.





Conclusion

The experiment produced inconclusive results many vials contained less than 10 pupae, therefore we can neither deny or accept our hypothesis with strong conviction.

Vials with powder and stock solution performed similar results with only a slight difference and there was no correlation between the different dosages. In contrast, when comparing the data with the mean percent survival of the negative control the data shows that GSE does have an effect on the larvae's percent survival. This effect is perceived throughout all methods even with difference in dosage. As aforementioned the results were inconclusive, however various studies showed positive results of the use of GSE with cancer cells making it promising as a future prospect in chemotherapies.

Future Directions

Data from this experiment suggests that more research on GSE is warranted. Below is a list of possible experiments to use in future testing:

- Further testing of GSE to investigate the most effective dose in various models with radiation as a neoadjuvant treatment and without radiation.
- Testing to explore GSE and its ability to selectively target cancerous cells as previous studies have presented this possibility.[1]
- Testing the use of GSE as a chemoprevention in comparison to its use as a chemotherapy.[3]
- Testing to explore GSE and its ability to reduce toxicity when administered in combination with chemotherapy as previous studies have presented this possibility.[5]

Acknowledgements

We would like to thank Dr. Tin Tin Su for serving as the principal investigator and sponsor this lab. We would also like to thank the Howard Hughes Medical Institution, CU Boulder Molecular, Cellular, Developmental Biology department and Biological Sciences Initiative (BSI) at CU Boulder. We want to especially thank Dr. Pamela Harvey for her amazing assistance and her enthusiasm for this class. Dr. Pamela Harvey provided us with extended time and unbounded patience, which we greatly appreciate. This project would never be complete without our lovely TA/mentor Jess Colmenero for her profound guidance and availability.

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The Effect of Serial Doses of Cannibigerol in *Drosophila melanogaster*

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Abstract

Cancer is a fatal disease internationally--with many different characteristics making treatment difficult. Combination therapy--using varying combinations of chemotherapy, radiation, and surgery--currently works best at degrading cancers. Roughly half of all cancer patients use chemotherapeutic treatment--the use of cytotoxic drugs targeting cancer cells--however, current chemotherapies damage the body, only cure 10-15% of patients, and can fail from innate or acquired cellular resistance. Cannabigerol (CBG) is a non-psychotropic cannabinoid found in cannabis that has shown increased apoptosis induction through activation of cannabinoid and vanilloid receptors in cancer cells. CBG already demonstrates success in reducing tumor growth and progression in colorectal cancers, and it is believed to not negatively affect the body.

In this study, CBG was examined as a potential chemotherapy in head and neck cancers. *Drosophila Melanogaster* third instar larvae modeled tumors since they rapidly divide like cancer cells. The larvae were *grp+/grp-* for susceptibility to irradiation--modeling radiation treatment. A CBG dosing series examined the compound's efficacy on inhibiting growth of both irradiated and non-irradiated third instar larvae. Due to inconsistencies with the negative control used (coconut oil), the results were inconclusive. However, cannabigerol shows potential as a hit chemotherapeutic, and further experiments should be conducted in the future.

Introduction

Cancer is classified as a disease in which cells divide uncontrollably and destroy body tissue [1]. Due to several different abnormal mechanisms, cells will rapidly divide into large masses--known as tumors, and can eventually develop new blood vessels as well as migrate to various other regions in the body. Cancer can be caused by three main factors: random genetic mutations, hereditary genetic mutations, and environmental factors--such as diet, smoking, and exposure to radiation or other known carcinogens. The disease is one of the leading causes of death worldwide. The National Cancer Institute estimates that there were about 1.7 million new cases of cancer [3], and that approximately 600,000 people will die due to it in 2017 [2]. Although it can affect all significant organs, its major forms include lung, breast, prostate, and colorectal cancer.

Cancer is a unique disease, as no form of it is ever the same. This is due to the disease's six major identified hallmarks and mechanisms by which it operates: self-sufficiency, insensitivity to anti-growth signals, avoidance of apoptosis, the ability to acquire an independent blood source, telomerase reactivation, and metastasis [11]. Each of these hallmarks contribute to the difficulty of curing the disease, as each present new and difficult challenges for researchers to overcome.

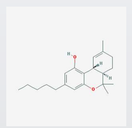
Currently, all forms of cancer are incurable. However, there are treatments that prove successful in cases that are diagnosed early. The most successful treatment methods today include radiation therapy, surgery, and chemotherapy. Radiation therapy destroys cancerous cells and tissues through high-energy radiation. Surgical cancer treatment involves removal of tumors and surrounding cancerous tissues. Chemotherapeutic drugs destroy cancerous cells and tissues by blocking or altering their cellular processes. Since a single chemotherapy usually only targets one cancer hallmark, it is now common to take a varying combination of chemotherapeutics and/or use chemotherapy with other therapies. Traditional chemotherapeutics are used on at least 50% of all cancer patients, yet they only cure about 10-15% of patients [12]. Since chemotherapies are such universally popular cancer treatments, it is necessary to find agents that can successfully target and kill more cancer cells. Additionally, cellular resistance to chemotherapeutics is a rising problem, so new promising chemotherapies are in high demand. Cancer cells can have innate resistance to certain chemotherapies--making the drugs immediately ineffective in treatment. Also, if the cancer returns in patients, it can develop acquired resistance to the previous chemotherapy the patient was treated with; cancer cells develop more efflux pumps--pumping the drugs out of the cells and preventing them from reaching a toxic concentration in the cells, so the cancer survives despite its previous susceptibility to that chemotherapy.

Cannabigerol (CBG) is a non-psychotropic cannabinoid extracted from cannabis. In high doses, it shows potential increase in cellular apoptosis [5], and it may be an effective chemotherapeutic. One study discovered taht CBG was successful at reducing tumor growth and progression of colorectal cancers [6]. CBG and its sister chemicals cannabidiol and cannabichromene induce apoptosis through the activation of cannabinoid and vanilloid receptors present on cancer cells. CBG activates CB2 cell receptors, which modulate diverse signaling targets and induce apoptosis, cell migration, and immune suppression [7]. CBG also blocks transient receptor potential (TRP) channels, which are important in modulating ion entry and transport machinery. These TRPs additionally interact with other proteins--forming signaling complexes. When TRPs' expressions are altered, cancer cells can utilize these intricate signaling complexes to spread uncontrolled growth signals--leading to carcinogenesis [7]. By blocking TRP channels, CBG may effectively reduce tumor growth and metastasis. Equally as important in CBG's chemotherapeutic potential is its anti-inflammatory ability [8]. As cancer is a unique type of inflammatory disease, anti-inflammatory compounds such as CBG may slow the proliferation of cancer growth.

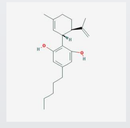
Hypotheses

Since CBG shows involvement in inducing apoptosis and reducing cancer cell growth and proliferation, CBG will greatly decrease *Drosophila* third instar larvae survival and act as an efficient chemotherapy both traditionally and combined with radiation. This is supported by findings from Cresco Labs [5] highlighting CBG blocking TRP proteins and interacting in other cell signaling mechanisms, research in *Carcinogenesis* [6] discussing CBG reducing colon carcinogenesis, and the study in *Biochemical Pharmacology* [8] analyzing anti-inflammatory mechanisms in CBG. Due to CBG's chemical structure and no research indicating CBG as an antioxidant like its sister chemicals--cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) [9]--CBG likely is not an antioxidant, and it will efficiently work as a chemotherapy combined with radiation.

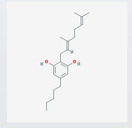
This is supported through research from the National University of Singapore [10] concluding compounds with more stabilizing functional groups produce higher antioxidant activity through their hydrogen-donating and radical stabilizing abilities. While both THC and CBD compounds include cyclohexene and alkyl groups contributing to charge delocalization and resonance, CBG only has two alkyl chains attached to a diol--making it less stable than its sister compounds. With less stability, CBG likely does not donate hydrogen protons in antioxidant mechanisms as frequently or stabilize free radicals as efficiently as CBD and THC. Since radiation therapy kills cancer cells through oxidative stress, CBG must not interfere with the free radical oxidative mechanisms enough to produce any antioxidant effect--making CBG a potentially effective chemotherapy combined with radiation.



Delta-9-tetrahydrocannabinol

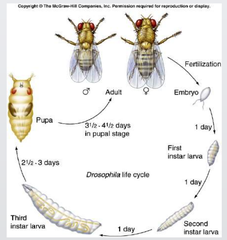


Cannabidiol



Cannabigerol

Drosophila Melanogaster Life Cycle



Purpose

1. Determine whether or not cannibigerol is a possible 'hit' for treatment in head and neck cancers.
2. Further experiment with the range of concentrations of cannibigerol to see what is the most effective dose.

Methods

1. Grapes mutant larvae were collected.

2. The larvae were sorted through a 600 µm sieve to collect the third instar larvae.

3. Larvae were put into irradiation chamber and irradiated at 4000 R. They were set aside

4. 6 vials were labeled with 10%, 8%, 6%, 4%, 2% and 0%.

5. Then 5 vials were labeled "neg. control 10%", "neg. control 8%", and so on ending in "neg. control 2%".

6. 3 mL of food were added into each vial and in the vials labeled with just percentages, that percentage of CBG oil was added to the vial (ex. 10% = 300uL of CBG oil). Oil was mixed into the food with the tip of the pipette.

7. In the "neg. control" vials the percentages listed for the coconut oil (ex. neg. control 10% = 300 µl of coconut oil) were added into the food. Oil mixed in with the tip of the pipette.

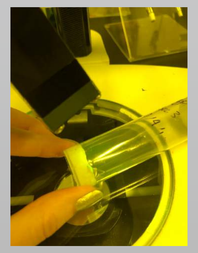
8. After the food hardened in the vials, 50-100 irradiated larvae were added to each vial and flugged.

9. After 5-7 days GFP+/- pupae were marked under the microscope.

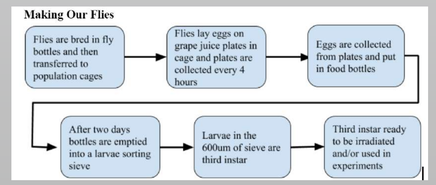
10. Survival of pupae was then quantified after an additional 5-7 days.

*When performing the antioxidant trials, just non-irradiated larvae was used

Quantifying GFP+/-



How We Make Our Flies



Results

Due to time constraints, not all the results from this study could be attained on the poster. See supplemental evidence for CBG Trial 4, CBG Trial 5, and CBG Dosing Averages data. CBG Trial 3 results were not obtained due to a temporary larvae shortage delaying the trial past the time allotted.

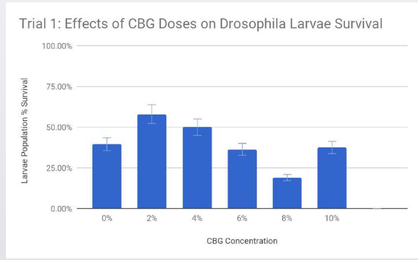


Figure 1: Survivability of third instar larvae is compared to the CBG dose they received. The highest CBG concentrations (6-10%) produced the lowest larvae survival rates--indicating that high dosing is necessary for proficient treatment. Due to the volume and consistency of coconut oil, all larvae in the negative control vial died likely from drowning, and its data was inconclusive. This was apparent since no larvae were able to climb up the negative control's vial to pupate, and the vial surface appeared slick from the oil. This result led to using a negative control dose series with identical concentrations as the CBG series in future trials to analyze how coconut oil affects larvae and create a more consistent comparison between the negative control and CBG. Additionally, despite having no drug present in the population, the 0% vial's survival rate was abnormally low; there were no factors explaining how this happened, but the result led to more scrutiny and caution in vial handling, drug administration, and food preparation during the other trials.

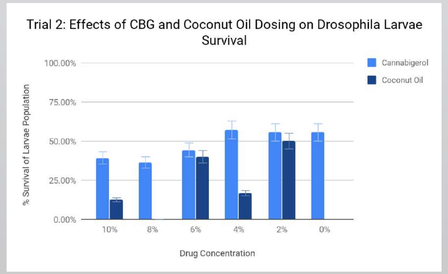


Figure 2: The comparison of larvae survival to doses of CBG or coconut oil. Once again, the coconut oil's consistency and volumes prevented larvae from climbing up vials to pupate and likely drowned them--leading to abnormally low negative control % survival rates. Despite the abnormal data from the negative control, % survival trends in larvae treated with CBG remained consistent with data from Figure 1. Higher concentrations of CBG still produced the lowest survival rates--with 8% and 10% concentrations killing the most larvae. Thus, CBG likely needs to be administered in high doses for efficient treatment. While the 0% concentration vial's survival rate increased between trials 1 and 2, there were still dead flies and larvae found in the center of the vial--likely resulting from asphyxiation or starvation, so this led to more frequent vial examination during the next trials.

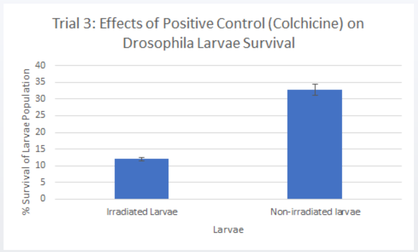


Figure 3: Colchicine was our positive control because it was a chemotherapy that was previously used to be used to treat cancers before it was found that it was extremely toxic and dangerous to patients. It demonstrates the percent survival that is expected of actual chemotherapies, but the efficacy of our compound is only determined by comparing the percent survival of our negative control (coconut oil) to our compound's survival rate.

Conclusions

At this time, results of further drug set vials as well as antioxidant vials are still pending. One thing that does carry over, however, is the problems encountered by our negative control, coconut oil, and when future trials are conducted there are a few things to do differently as discussed in future directions. We were also unable to do "hit" calculations due to having only one set of drug vial data at this time. There are low survival rates for some doses of the cannibigerol in the first drug sets shown in the results section which is promising due to the fact that it is killing most of the larvae in doses such as 8%. Results gained from our last set of drug vials should hopefully add to our data and give us a little bigger of a sample size.

Future Directions

For future experimentation, we would like to combine both cannabigerol and cannabidiol, to see if they are more effective as chemotherapies when taken together. Equally, because of the difficulties presented with coconut oil, our negative control, it would be wise to test CBG in a different medium, such as a solid state, to explore potential efficacy differences. Of course, given the time and funding, an *in vitro* study on an immortalized cell line would do wonders for our data. Being able to see the potential that CBG has on human cancer cells would answer many questions that our data presents. If such a test were to be successful, a future *in vivo* test on a mouse or rat would be optimal, to determine potential toxic and effective doses of CBG in a full body system.

Acknowledgments

A special thanks to Dr. Tin Tin Su for allowing our lab to adopt her experimental ideas and for giving her expertise to use in our research. We thank the Howard Hughes Medical Institute, Biological Sciences Initiative, and the Molecular, Cellular, and Developmental Biology Department at University of Colorado Boulder for their continued support and advice throughout the Discovery Lab. Lastly, a huge thank you to Dr. Pamela Harvey, Katie Franks, Jack Schutz, Alia Alsaif, and Erin Kneeskern for all of their time, dedication, and hard work put into orienting the mechanics of our research.

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Cellular Arrest via Inhibition of NAD Biosynthesis Indicates the Molecule's Potential in Combination Therapy Cancer Research

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Abstract

Roughly 1 in 3 people will get cancer in their lifetime and although scientists have been studying and treating cancer for decades, there is still no cure nor a safe treatment method with high success rates. In our study, we focused on *Drosophila melanogaster* as a model organism for head and neck tumors, as they have rapidly dividing cells that mimic that of tumors, as well as genes that are comparable to human genes. In our research, we found that there was promise that the inhibition of the biosynthesis of NAD/NADH could lead to cellular apoptosis and produce a possible, less toxic chemotherapy. FK866 is an inhibitor of NAD/NADH biosynthesis. Doses of FK866 were tested at 10 μM of the drug being administered at 3 different volumes and fed to our irradiated larvae and their survival was quantified. Although one of our negative controls demonstrated no developed flies, the vials that contained the drug showed the desirable, negative effect on the percent survival of the *Drosophila melanogaster*.

Introduction

Extensive research in previous years has revealed the importance of Nicotinamide Adenine Dinucleotide (NAD or NADH) in maintaining the stability of DNA, and most importantly, cellular metabolism. Through the consumption of proteins, sugars, and fats (Figure 2), AcetylCoA is formed and initiates the citric acid cycle, the biosynthesis of NAD. Nicotinamide phosphoribosyl transferase (NaMPRTase) forms nicotinamide mononucleotide (NMN) from a precursor molecule, then NMN is used in an ATP consumption reaction to form NAD. This NAD undergoes redox reactions (Figure 1) and, combined with the oxidative phosphorylation of ADP, ATP is formed.

As we know the importance of ATP in cellular metabolism, we can inhibit this citric acid cycle, so the cancerous cells perform apoptosis. We achieve this by the inhibition of NaMPRTase because it is crucial in the formation of NMN from a precursor molecule. Now, the cell should not be able to complete the citric acid cycle and would produce dramatically less ATP, triggering apoptosis. FK866 Hydrochloride Hydrate shows potential in being an NaMPRTase inhibitor. If the administration of this NAD inhibitor in a cancerous cell is successful, it can be used as a less toxic chemotherapy in sending tumors into remission as there have been studies performed demonstrating that this is true. However, our group studied its effectiveness not only as a chemotherapy, but as a chemotherapy used in combination cancer therapy specifically in head and neck tumors.

In this study, we use the model organism *Drosophila melanogaster* that produces offspring with a radiosensitive phenotype to assist in selecting which larvae in our drug vials are considered a "hit" for our compound. An engineered balancer chromosome that allows the flies to produce Green Fluorescent Protein (GFP), and a mutation in the fly's checkpoint kinase 1 [CHK1; also known as the grapes gene (*grp*) in *Drosophila*]; (Figures 3 and 4) gene allows us to determine which are most susceptible to radiation. Since the balancer chromosome is dominant and has a natural tendency to be lethal in a case of homozygous dominant, 66% of our fly population will glow green with a lesser susceptibility to radiation, and 33% of our fly population will have no GFP while being more susceptible to radiation. Thus, once this generation of offspring is radiated and administered our compound in a drug vial, we can mathematically determine a "hit" in our compound if 50% or more of non-GFP are dead in that one vial.

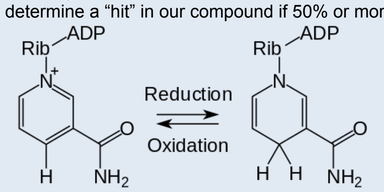


Figure 1: Redox reactions and equation of nicotinamide adenine dinucleotide.

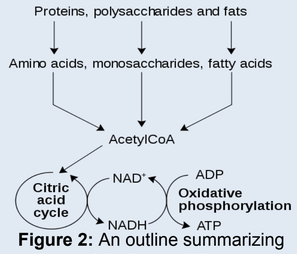


Figure 2: An outline summarizing redox metabolism and the relationship NAD has with the citric acid cycle.

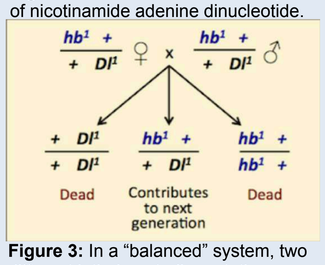


Figure 3: In a "balanced" system, two different lethal or sterile mutations on homologous chromosomes ensure that only those progeny heterozygous for both mutations survive. Our engineered balancer chromosomes contain the gene for GFP.

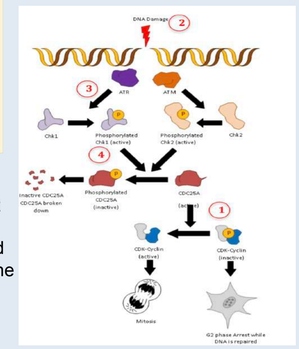


Figure 4: The function of checkpoint kinase 1 (CHK1) is shown to phosphorylate CDC25A and degrade it. CDC25A acts as a phosphatase in the event of DNA damage to lock the cell in G2 phase arrest while the DNA is repaired.

Hypothesis

We hypothesize that with the inhibition of NaMPRTase, precursor molecules can no longer assist in the biosynthesis of NAD. Thus, ATP cannot be generated, cellular metabolism will be halted, and the cells within our tumor model used in the experiment will be targeted for apoptosis.

Methods

Culturing Drosophila

Before Day 1 of our experiment, we spent three-weeks culturing our flies. As a class, we prepared fly food in a flask of 500mL of water and 85g of Nutri-Fly Bloomington's Formulation to pour in 25mL bottles. Grape juice plates containing yeast and water acted as an egg-laying setting for the female flies in a population cage. Once embryos developed on these plates, they were collected and cultured in prepared food bottles. During the third week, a substantial number of larvae grew in these food bottles and were then collected using sieves to be prepared for irradiation.

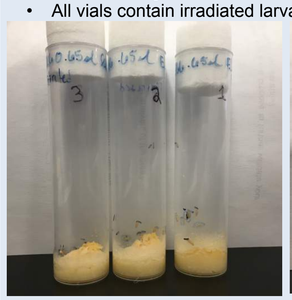
Larvae Irradiation

We specifically used third instar larvae for this experiment since they represent a cancer cell after irradiation. They were collected from the sieves during the third week of experimental preparation and irradiated in a machine at 4000 Rad.

Drug Vial Preparation

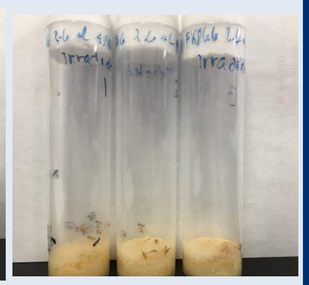
This part of our experiment is when we were able to culture the irradiated larvae in our compound and analytically determine if the population of irradiated third instar larvae were affected. We tested our compound (FK866 Hydrochloride Hydrate) in triplicates with our positive control (50 μg/mL colchicine) and our negative control (water). Our stock solution was diluted from its original concentration of 10M to 10μM before being administered to the drug vials. The following were prepared:

- 3 vials containing 2.6 μL of 10μM FK866 Hydrochloride Hydrate with 3 mL of food
- 3 vials containing 1.3 μL of 10 μM FK866 Hydrochloride Hydrate with 3 mL of food
- 3 vials containing 0.65 μL of 10 μM FK866 Hydrochloride Hydrate with 3 mL of food
- 3 μL of water in 1 drug vial with 3 mL of food
- 3 μL of 50 mg/mL colchicine in 1 drug vial with 3 mL of food
- All vials contain irradiated larvae.



Drug vials containing 0.65 μL of 10 μM of FK866 Hydrochloride Hydrate with 3 mL of food

Drug vials containing 1.3 μL of 10 μM FK866 Hydrochloride Hydrate with 3 mL of food



Drug vials containing 0.65 μL of 10 μM of FK866 Hydrochloride Hydrate with 3 mL of food

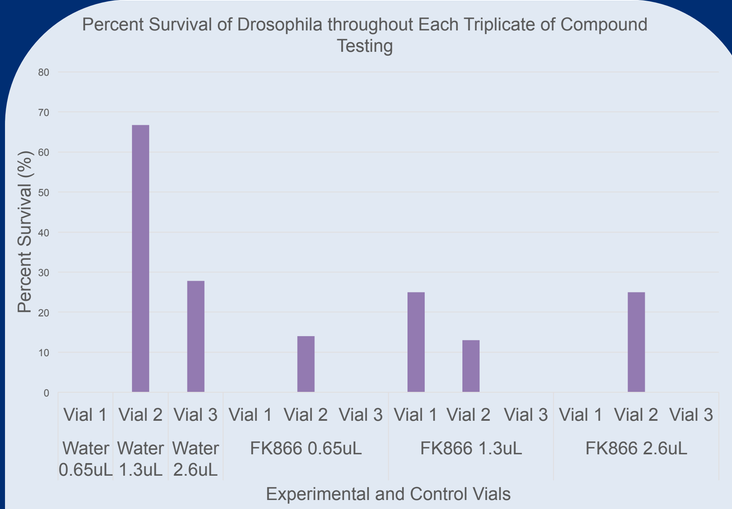
Results

FK866 Proves to be a Possible Chemotherapy for Head and Neck Cancers

Our experiment using the nicotinamide phosphoribosyltransferase inhibitor, FK866, shows its effectiveness as a possible chemotherapy in head and neck cancers. The *in vivo* experiment that we conducted by using a dosing series in our discovery-based laboratory showed a negative effect on the percent survival of the developing *Drosophila*. However, where we had expected developed *Drosophila* in our negative control, we obtained results that indicated that the majority of the fly population that was cultured did not survive. With our negative control (water), the flies were expected to live and develop normally, but instead we saw a similar trend in percent survival as compared to that of the vials containing our drug. Therefore, our results remain inconclusive on the basis of experimental error.

FK866 May Lead to ATP Depletion within Developing *Drosophila melanogaster*

Within the course of a normal compound screening, you will find that the third instar larvae crawl up along the sides of the drug vial before entering their pupae state. Something abnormal to report in our experiment is the lack of this behavior, as we observed many larvae entering the pupae state while still stuck in the food or had just barely moved up the side of the vial. This behavior could be explained since our compound is expected to inhibit the formation of ATP. Additionally, we found that the larvae in the majority of our water vials did exhibit the natural behavior of crawling up the side of the vials.



Conclusions

Although our negative control had been flawed leading to inconclusive results, FK866 proves to be a possible chemotherapy for head and neck tumors, as it impacted the percent survival at a 10 μM concentration. Based off the data in our dosing series, the relative data surrounding each volume of FK866 administered shows there is no differing toxicity.

Future Directions

Future experiments could include:

- Administering our compound and then determining the survival of the larvae into the pupae stage rather the survival of the larvae from the pupae to the adult stage
- Test compounds that inhibit ATP production in a way different from FK866
- Investigate compounds that similarly inhibit NAD production through inhibiting NaMPRTase that are similar to FK866
- Reconduct the experiment with proper negative controls so our data is more reliable

Acknowledgements

Our group would like to thank our principal investigator of our sponsor laboratory, Tin Tin Su, Ph. D. as well as our professor Dr. Pamela Harvey. We would like to thank Howard Hughes Medical Institute and the CU Boulder MCDB department for our funding. Finally, we would like to acknowledge Biological Sciences Initiative (BSI) at CU Boulder for their support.

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Further Information

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Effect of Chaga Mushroom (*Inonotus obliquus)* on *Drosophila melanogaster* Survival Rates

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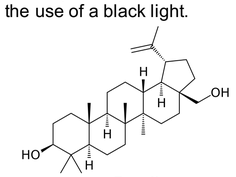
MCDB 2171

Abstract

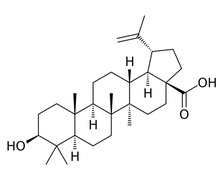
The majority of current cancer treatments have limited efficacy and severe side effects. The Chaga mushroom (*Inonotus obliquus*), a parasitic fungus found on softwood trees, has an extensive history of use in folk medicine. In this study, fruit flies (*Drosophila melanogaster*) were used as a whole-animal in vivo model for head and neck tumors, to investigate the possible cytotoxic properties of the Chaga mushroom. Third instar larvae were exposed to 4000 rad of ionizing radiation, and then incubated in vials containing a dosing series of different concentrations of a Chaga aqueous extract mixed in with a cornmeal agar food. Absorbance values were taken to standardize the dosing series concentrations. Distilled water was used as the negative control, while Colchicine, a known chemotherapeutic was used as the positive control. After metamorphosis, the survival rate of the flies was quantified. The data shows a downward trend in survival rates when flies are given higher doses of Chaga extract in their food. While not all of the data was statistically significant, the data for the full strength concentration of Chaga extract was outside of two standard deviations from the mean of the negative control, and is therefore statistically significant. Testing isolated Chaga constituents and stronger doses could yield further significant results.

Introduction

Cancer involves abnormal cells dividing uncontrollably and continues to be one of the leading causes of death throughout the world. The majority of current chemotherapies have limited efficacy, are very expensive, and cause severe side effects for the patient. The goal of this study was to find an alternative chemotherapeutic drug that, when combined with radiation, is more effective at promoting apoptosis in cancer cells without causing harm to the rest of the body. Third instar larvae of *Drosophila melanogaster* (fruit flies), the model for this study, were cultivated to have the *grapes* negative double mutation. The *grapes* gene in fruit flies, equivalent to the checkpoint kinase 1 gene in humans, can be mutated to produce flies which are more susceptible to radiation because the *grapes* gene is essential to a pathway which halts the cell cycle in response to DNA damage. In this study, *grapes* mutant flies were used as a model for tumors. These flies were identified using an engineered chromosome that included a GFP (green fluorescent protein) marker in addition to the *grapes* mutation. The GFP negative larvae (mutant) can be differentiated from the GFP positive larvae with the use of a black light.



Betulin

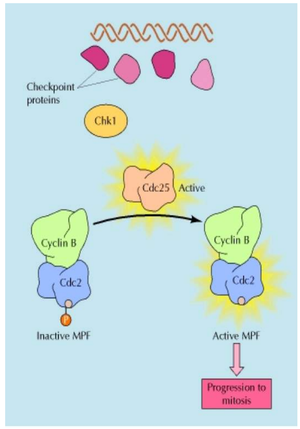


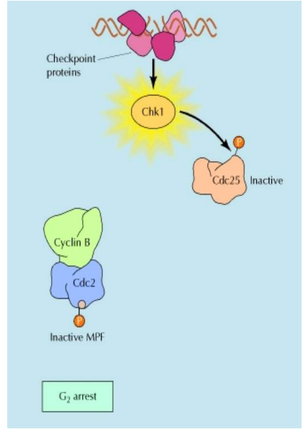
Betulinic Acid

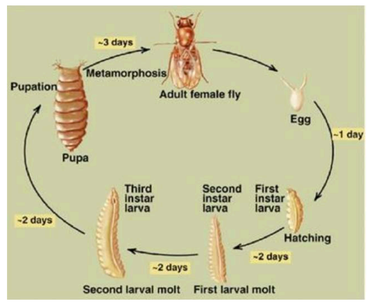
Inonotus obliquus, commonly the Chaga mushroom, is a parasitic fungus found growing on the bark of birch and some other softwood trees. The fungus has an extensive history of use in folk medicine in Russia and other Northern European countries, and some scientific evidence suggests several of its constituents have potential to be incorporated into cancer treatment. The most common method of ingestion is to grind the hard sclerotium and create a hot water extraction. This extraction is often diluted and consumed as a tea. Our research will test both a hot water extract of the raw fungal sclerotium as well as isolated betulin and betulinic acid, two compounds present in the extract with some known cytotoxic activity. By radiating the third instar *Drosophila melanogaster* larvae before allowing them to ingest Chaga extract in their food, a decrease in survival would indicate a mechanism resulting in cytotoxicity, and the potential for Chaga in chemotherapy development.

Hypothesis

When irradiated, the *Drosophila melanogaster* treated with Chaga mushroom extract will have a lower survival rate than the flies not treated with Chaga.







Methods

Chaga Extract Procedure

1. Grind Chaga sclerotium into 8oz of a fine powder
2. Add powder to 1L of hot water just below boiling
3. Allow extraction to occur uncovered over the course of 48 hours maintaining temperature between 90 and 95 degrees Celsius. Periodically add water when the water level is low to keep from drying out or burning.

Lab Procedure

1. The initial concentration of the Chaga extract was determined by examining its absorbance (see figure 2). Using distilled water, a 50%, 25%, 12.5%, and 6.25% concentration of Chaga extract was made from the full strength extract.

2. Four vials of each dose, including a full strength dose and the positive (Colchicine, 50µg/mL) and negative controls (DI water), were used for this study, resulting in a total of 24 test vials.

3. Three milliliters of fly food were mixed with 30 microliters of the Chaga extract solution, in the aforementioned concentrations.

4. All of the 3rd instar larvae used for this experiment underwent radiation prior to placement in the vials with Chaga extract. Only grp/grp larvae were used. This dosing series was run twice.

5. At the end of the fly life cycles, survival rates of the flies in all 24 test vials were compared to examine efficacy.

Results

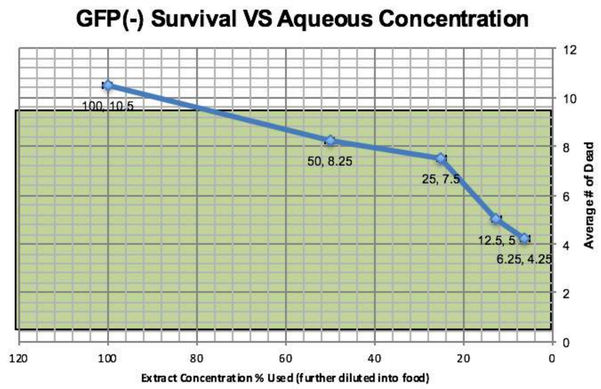


Figure 1: Shaded green area represents the mean plus two standard deviations (0.33- 9.17 flies) for the negative control (water). Data points outside of green box indicate statistically significant results.

This experiment yielded an overall negative trend in survival rates of drosophila exposed to increasing concentrations of chaga extract. GFP negative flies were present in every vial, and most vials had some living subjects. Our hypothesis stated that flies exposed to chaga extract would have lower survival rates than flies not exposed to chaga extract.

The calculated mean death value for flies exposed to water, our negative control, was 4.75 flies. This value had a standard deviation of 2.21, with a range of 0.33 to 9.17. Anything within this range would be statistically insignificant and anything outside of this range would indicate a "hit".
The chaga concentration values were then plotted against the average number of dead flies in each concentration group (100%, 50%, 25%, 12.5%, and 6.25%). The average number of dead flies in the 100% chaga concentration group was 10.5 which is above the range, making it statistically significant. Chagas extract at this concentration is therefore a "hit". The other chaga concentration values indicated statistically insignificant results.

Chaga Extract Analysis

Figure 2: Absorbance Results

Measurement results				
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Wavelength: 405 nm				
% Concentration	1	2	3	Average Abs
100%	3.931	4.248	4.42	4.200
50%	2.043	2.03	2.264	2.112
25%	1.314	1.395	1.39	1.366
12.50%	0.781	0.789	0.753	0.774
6.25%	0.435	0.453	0.455	0.448
0%	0.043	0.052	0.077	0.057

Conclusions

An aqueous Chaga extract was further diluted into fly food (30µL of an extract of varying concentrations / 3mL of food) and used to perform a dosing series. Each extract concentration was tested on *grapes* mutants, irradiated third instar larvae, a model for cancerous tumors, and survival was quantified. Since production of aqueous extract involves continuously adding water, concentrations were standardized using absorbance measurements (Figure 2). The concentrations tested were 100%, 50%, 25%, 12.5%, and 6.25%.

Higher concentration extracts were consistently more successful at killing the larvae than lower concentrations. At the highest concentration, when combined with radiation results showed statistically significant success higher than two standard deviations above the mean of the negative control (Figure 1), indicating a "hit."

There were many limitations to this study. First, each extract concentration was tested on only four vials of larvae. In a repeat of this study, more vials would need to be tested to improve the integrity of the experiment. Second, since the higher concentrations led to lower survival, more concentrated extracts could be made in the future using both water and organic solvents, as the literature suggests active constituents betulin and betulinic acid are more highly soluble in DMSO than water. Additionally, in vitro studies using human cells should be done to investigate any further hits.

Future Directions

Based on our results, we would like to re-attempt this study using stronger Chagas concentrations, to determine a more effective dosage curve.
Furthermore, we would like to investigate the active compounds, betulin and betulinic acid, to ultimately study how these compounds interfere with the biochemical mechanisms associated with induced apoptosis. Beyond this experiment, we would like to perform in vitro studies using malignant human epithelial cells to study the lipid thermodynamics, pharmacokinetics, and the overall cell growth inhibition of different concentrations of Chagas extract.

Acknowledgments

We would like to thank our principal investigator, Dr. Tin Tin Su and our research instructor, Dr. Pamela Harvey. We would also like to thank our teaching assistants, Isabella Shelby, Ben Huxley, Julianna Rohn, and Alia Alsaif. We would like to acknowledge our funding sources, the Molecular, Cellular, and Developmental Biology Department at CU Boulder, Howard Hughes Medical Institute, and the Biological Sciences Initiative at CU Boulder.

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Photos courtesy of google images

Sanguinaria Canadensis (Bloodroot) Inhibits the Growth of Cancerous Cells in a Dose-dependent Manner

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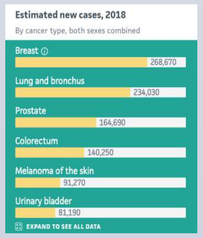
Abstract

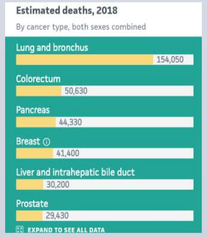
Cancer affects approximately 38.4% of people throughout their entire lives in the United States. As people are living longer, cancer becomes increasingly prevalent due to increased rates of cell degradation and mutations in older populations. Radiation can be used in conjunction with chemotherapies to kill cancer cells, with hopefully minimal side effects to the patient. The Bloodroot plant, or *Sanguinaria Canadensis,* may have chemotherapeutic effects due to the pro-apoptotic properties of the Sanguinaria compound found within it. Our experiment included two different forms of the compound, Bloodroot extract (a liquid) and Sanguinaria-specific pellets. We tested our compound on Drosophila Melanogaster in varying concentrations by placing 3 µL of our diluted extract or dissolved pellet into food vials. Irradiated third-instar fly larvae where then placed in the vials and were marked for Green Fluorescent Protein (GFP), a protein that makes the flies glow. A lack of GFP implies that the fly possesses two mutant copies of the grapes gene which makes them more susceptible to radiation. The Bloodroot extract contains other ingredients which could be more effective in decreasing the larvae survival rates. By testing both Sanguinaria pellets and extract and quantifying dead and alive flies, the chemotherapeutic effect of both can be compared to one another and an effective concentration can be evaluated.

Introduction

Different forms of cancer are amongst the top ten leading causes of death worldwide. While the origin and progression of cancer cells vary, all types involve rapid cell division and metastasis to surrounding tissues. For the year 2018, there's an estimated 1,735,350 new cancer cases with a predicted 609,640 deaths within the United States. This is just a fraction of the over 15,000,000 affected worldwide. This lab focuses on head and neck cancers which involve the mouth, throat, nose, sinuses, larynx, or salivary glands. More than 5 million people are affected by head and neck cancer globally, with more than 300,000 dying. As more people become diagnosed with cancer, the race for treatments increases exponentially. While preventative methods are taking place, cancer is always changing, progressing, and in some cases becoming resistant to treatments. By targeting cancer cells' abilities to evade apoptosis, influence growth factors, and undergo metastasis, it's possible to find future therapies.

Sanguinaria Canadensis, also known as Bloodroot, is a flower that Native Americans believed was a "cure all" medicinal herb. It contains Sanguinarine and Chelerythrine which are known alkaloids that contain antioxidants which have the potential to block proliferation of cancerous cells and induce apoptosis. To determine Bloodroot's effectiveness as a chemotherapy, we used Drosophila Melanogaster to represent cancerous cells. Due to their rapid growth of stem cells during metamorphosis, they mirror the formation of cancer cells. In addition, 75% of Drosophila genes are similar to humans including the grapes mutation (Checkpoint Kinase 1 mutation in humans) making results comparable to an extent. Flies that have the grapes mutation are more susceptible to radiation which allows for greater results when in combination with a potential new drug. This mimics combination therapy which has been proven to be more successful than single treatments. Sanguinaria Canadensis is highly toxic; however, by testing multiple concentrations in addition to radiation, a synergistic effect could occur which could make low dosages effective.





Acknowledgments

We would first and foremost like to thank Pamela Harvey for her guidance and expertise in the Drug Discovery lab. We would also like to thank our TAs Jessica Westfall, Jesse Kurland, Marisa Martin-Wegryn, and Sam Gendelman for their dedication to our section and for helping us every day in lab. Our research would not be possible without funding from (not sure). There is also huge thanks to be given to Tin Tin Su for allowing us to continue her research and learn from her techniques. Finally, we would like to thank the Molecular, Cellular, and Developmental Biology department for making this class possible.

Purpose

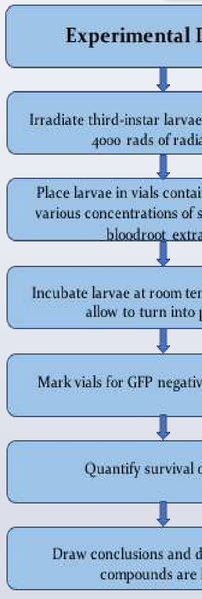
1. To investigate how Sanguinaria Canadensis effects rapidly growing cells by quantifying percent survival of Drosophila.
2. To compare the results between Bloodroot extract and Sanguinaria Canadensis pellets.
3. To experiment with varying concentrations of Bloodroot to identify which is most effective.
4. To observe the effects of radiation in combination with Bloodroot to obtain a synergistic outcome.
5. Be able to identify flies with the grapes mutation and understand its significance.

Hypothesis

Combining the processes of irradiating flies and utilizing various concentrations of Sanguinaria is an effective method in representing the process in which humans with cancer are treated in hospitals with radiation and chemotherapy treatments. It is predicted that the highest concentrations of Sanguinaria *canadensis* will be the most effective in causing apoptosis in the drosophila. However, lower dosages will also be effective because radiation is also being utilized. Combination therapy with the Sanguinarine compound and radiation will show a synergistic effect and will allow for a lower dose of each form of therapy.

This is a critical factor when thinking about treating humans because by administering lower doses of the drugs, it will lead to less incidences of side effects. All drugs have a therapeutic index which is a ratio comparing effective doses of the drug to lethal doses of the drug (does more harm than good). The side effects resulting from using the compound can be more harmful to the patient which should be avoided at all costs.

Methods



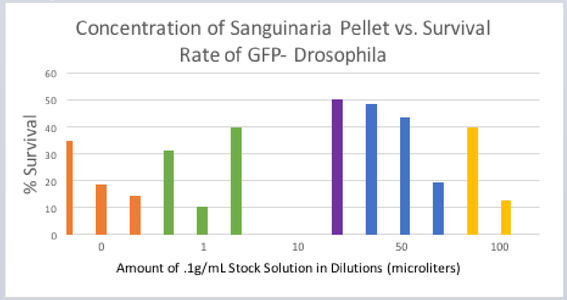
Vial Number	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5
Stock concentration	1g/10mL 1 gram of Bloodroot pellet to 10 milliliters of water				
Concentration	100 µL stock 0 µL water (3 µL into food) **1:1**	10 µL stock 90 µL water (3 µL into food) **1:10**	1 µL stock 99 µL water (3 µL into food) **1:100**	50 µL stock 50 µL water (3 µL into food) **1:2**	100 µL water (3 µL into food) **Negative Control**
Amount of Food	3 mL	3 mL	3mL	3mL	3mL
Additional water added	100 µL	100 µL	100 µL	100 µL	100 µL
# Vials	triplicates	triplicates	triplicates	triplicates	triplicates

The experiment was performed in a laboratory environment. Before the experiment began, populations of flies were maintained in order to have irradiated third instar larvae for the procedure. Bloodroot (*Sanguinaria Canadensis)* was obtained in pellet form as well as an extract. The concentrations of the pellets were determined in a dosage series. After discovering that 1 gram of the pellets were soluble in 10 mL of water, they were further diluted to account for the drug's toxicity (view the chart above). Regarding the extract; we lacked information about how much of the compound can be measured in a person's blood. Therefore, we made the starting point a 10% dilution because it's similar to taking 10% of an individual's blood and replacing it with the compound. Any higher concentration would be unnecessary. The dilutions then decreased by an increment of 2% which allowed us to test how different concentrations affect percent survival of the drosophila.

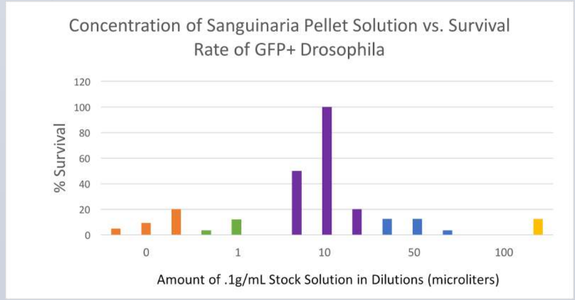
Green Fluorescent Protein (GFP) serves as a marker to identify whether the Drosophila are heterozygous for the grapes mutation. A mutation in the grapes gene signifies that the fly cannot sufficiently repair damaged DNA which causes the fly to be more susceptible to radiation. Flies that have GFP only have one copy of the grapes mutation. These flies maintain the population because they are fertile. Flies without GFP have two copies of the grapes mutation which are the flies being studied in this experiment.

Results

Graph I.I



Graph I.II



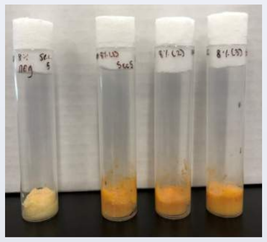
The results of the graphs above portray the percent survival of the two different strains of flies at the five concentrations. Each color resembles a set of triplicates for a certain concentration. Percent survival was determined by creating a ratio of dead flies to the number of total flies in the vial. The results from the GFP- and GFP+ graphs depict that there isn't a large correlation between an increase in dose and a decrease in fly survival; however, it is evident that the drug does possess potential as fly survival numbers are low. Graph I.I shows percent survival of flies with two grapes mutations while graph I.II shows percent survival for flies heterozygous for the grapes mutation (marked with a balancer chromosome containing GFP). Do note that the 1:10 dilution lacked a large amount of pupae on the walls making quantification of dead and alive flies insignificant. The other vials had over 15 larvae on the walls to quantify percent survival, while the 1:10 concentration contained less than ten. The 1:10 vials were dismissed when formulating a conclusion about the efficacy of Bloodroot as chemotherapy.

Conclusions

The results obtained for this experiment were inconclusive due to the observations from the negative control. Theoretically, since the negative control contained only water, all flies should have survived. However, low survival rates in the negative control suggests an outside factor could've affected whether or not the flies were dead or alive. The low percent survival could be due to a number of factors: improper environment for the larvae to develop, water could've stripped away the nutrients in the food, contamination to the water, or the flies were overly susceptible to radiation.

While the information was inconclusive, the experiment did demonstrate that Bloodroot could have an effect on killing Drosophila. The dosing series demonstrates that the compound is effective at various concentrations so when in combination was radiation, a lower dosage could be administered. Since the triplicates were inconsistent, further experiments would need to take place to confirm the efficacy of the compound.

Our results demonstrated a lower percent survival in the flies that were GFP positive. This did not correlate to what we expected. GFP negative flies are more susceptible to radiation, but since percent survival was lower in GFP positive flies, there could be an unknown link between the effect of Bloodroot and flies with only one grapes mutation and a GFP balancer chromosome.

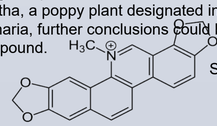


Results regarding the Bloodroot extract remained inconclusive. For this experiment, coconut oil was used as the negative control; however, results weren't comparable because both the negative control and the extract lacked pupae. A possible explanation could've been that the coconut oil was too thick and the larvae couldn't swim through which disabled them from forming pupae on the walls. The extract, also oil based, demonstrated similar results and pupae couldn't form. In the vials containing the extract, the food appeared more dry which could have also contributed to the incomplete results.

Future Directions

Data from this lab could be used to further investigate Sanguinaria Canadensis as a possible chemotherapy or provide insight in conducting research.

1. Larvae cannot swim through oil. When using extract that has an oil-based consistency (or when using coconut oil as a negative control) mix the compound with Ethanol. Ethanol can separate the contents from the oil which can then be mixed with the food and tested for percent survival. This will allow for the pupae to attach to the walls.
2. Complete another trial of this experiment and analysis the results. If possible, conduct an in vivo experiment by testing the drug on mice. The compound can be injected or taken orally. Mice resemble humans more closely than flies do which could lead to more accurate results.
3. Medicinal chemistry: Bloodroot contains the compound Sanguinaria which is the predicted element that directly affects the spread of cancer cells. By further investigating compounds similar in structure to Saguinaria (such as Eomecon Chionantha, a poppy plant designated in China) or compounds containing Sanguinaria, further conclusions could be made regarding the efficacy of the compound.



Structure of Sanguinaria Compound

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BOSWELLIA SERRATA POWDER EXTRACT (FRANKINSENCE OIL) ON GROWTH OF DROSPHILA MELANOGASTER

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Abstract

Cancer is the uncontrolled rapid division of abnormal cells in the body. In this experiment, we used Drosophila Melanogaster in the third instar larvae stage of development as a model of cancer due to the rapidly dividing stem cells. In this study, we tested the effects of Boswellia Serrata extract on larvae survival. Our dosing series included 4 different concentrations of our compound, which were mixed with the cornmeal-agar food vials. The concentrations ranged from 1 microliter per ml of food to .0001 microliter per ml of food. We determined these concentrations from previous experiments used to identify hit compounds. The survival rated were compared to a negative control, DMSO, known to have no effect on survival was dissolved with and a positive control, colchicine, a known chemotherapy. We were able to identify our compound as a hit as the average percent survival was more than two standard deviations lower than the average DMSO control.

Introduction

Cancer is defined as a growth or tumor that is caused by the rapid division of abnormal cells. The goal of an effective chemotherapy is to destroy the cancerous cells while maintaining the relative health of surrounding, non cancerous cells, and keeping the patient at a comfortable state. About 1.7 million new cancer cases are expected to be diagnosed in 2018 and about 610,000 Americans are projected to die of cancer. In our research we found that Frankincense oil is an essential oil, that is extracted from Boswellia trees, is commonly known for its anti inflammatory properties. Several studies have already been conducted that show promising results in Frankincense's ability to inhibit growth and induce apoptosis in pancreatic and bladder cancer cells when tested through in vitro methods. In our initial testing of Frankincense essential oil, we are using the third instar larvae of Drosophila Melanogaster as a model for rapidly dividing cancer cells, we plan to see how the extract can combat cancer alone as well as its ability to reduce cancer cells when combined with radiation.

Hypothesis

The Boswellia Serrata Extract will show anti-cancer properties and kill a significant amount of the drosophila melanogaster and prove to be a "hit" for a chemotherapy in cancers similar to that of pancreatic and bladder cancers.

Objectives

Our objective is to test the different dilutions of the Boswellia Serrata powder in hopes that it provides a significant death rate in our fruit flies.

Materials

Boswellia Serrata Powder

Frankinsence Oil

Methods

1.Collecting Third Instar Larvae

In order to test our compound on the third instar larvae, we first had to breed and collect embryos. For the process, we. first transferred adult flies to fresh culture bottles with yeast, then transferred the adults into a population cage. We then collected embryos from the grape juice plates in the population cage and transferred them to fresh food bottles without yeast. Next, we collected larvae between 600 and 850 micrometers, then irradiated the third instar larvae. <u>These third instar larvae are what will be placed into our vials containing our food-drug mixture.</u>

2. Drug Screen

To perform our initial drug screen <u>, we made 4 different dilutions using the powdered form of Boswellia Serrata Extract.</u> Our first dilution, also used as the stock contained 100mg of extract per ml of solution, diluted with water. Our second dilution was a 1:10 dilution of our first, 1 ml of stock solution per 10 ml of diluted solution. Our third dilution was a 1:10 dilution of our second, making it a 1:100 dilution of the stock. Our fourth and final dilution was a 1:10 dilution of the third, making it a 1:1000 dilution of the stock. We took 3 microliters of each dilution and mixed it with 3 mL of food and placed it in vials where the third instar larvae were placed once the food. solidified. We repeated this for six different sets of vials. We repeat these same steps with a negative control, water and a positive control, colchicine to increase accuracy of our results.

3. Irradiation

Since radiation is often used in conjunction with many chemotherapies, we decided to test half of our drug vials, <u>three of the sets of the different dilutions to test if the effects of our drugs were enhanced by radiation.</u>

4. Quantifying Survival

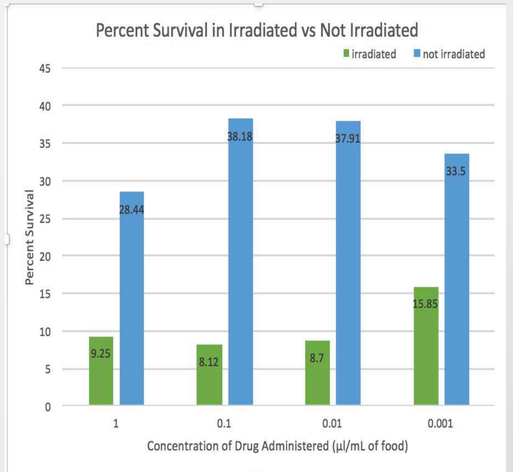
To quantify survival, we first marked the flies that did not express the green fluorescent protein(GFP) gene. Once enough time for viable flies to eclose had passed, we quantified survival in the GFP negative flies by observing them over a light <u>box to see how many flies remained in pupal cases (dead) and flies that have eclosed (alive).</u>

Test concentrations on third instar larvae

Irradiate half of our larvae to understand correlation of drug and irradiation

Identify % of eclosion to quantify survival of our larvae (hoping for small survival rate)

Results

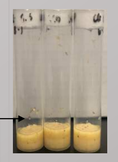


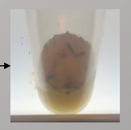
This graph shows the percent survival of the flies treated with the different dilutions of the Boswellia Serrata powder. It also compares the percent survival when the flies were irradiated and when they were not. It is evident that we had more success when we combined the drug and irradiation. We saw a significant amount of death in our flies when looking at the irradiated flies. The most successful dilution was the .01 (μl/mL of food) combined with irradiation. This yielded 8.7% survival, the lowest of all of our trials.

The picture to the right is one of our vials that was not irradiated, there was an abundant amount of flies that had eclosed and were alive, which was not what we wanted to see. However in comparison to the irradiated vials, there was a significant amount more living flies in this vial, which further solidifies that mixing the drug with irradiation is more effective.

Discussion

While we did test the Boswellia Serrata powder during the majority of our experiment, we did put together some vials with the oil itself. With the fear of it degrading our vials, we were not sure the oil would be able to produce viable results. While the oil did not degrade the vials, it did seem to drown the flies and we were not capable of quantifying any survival or lack there of. We also irradiated half of the vials that had the oil, but the remaining flies seemed to be burned by the irradiation and were stuck in the food and oil mixture, again not allowing us to quantify the percent survival.





Future Directions

Future experiments can include:

1.**Conducting** another dosing series since the percent survivals do not seem to strongly correlate with the concentrations of the drug administered.

2.**Conducting** the same experiment several more times to ensure the compound is a hit.

3.**Testing** the compound on other organisms such as mice to observe metabolism, toxicity and symptoms.

Acknowledgments

We would like to thank Dr. Tin Tin Su for allowing us to use her research and for forming a solid ground for our own research. We would also like to thank Dr. Pamela Harvey for teaching this course and for her constant support. Our TA Jesse Kurland is also to thank for his hands on help in lab and for his patience through out the semester. This would not have been possible without our funding sources, The Howard Hughes Medical Institute, the Biological Sciences Initiative and the MCDB department at the University of Colorado-Boulder

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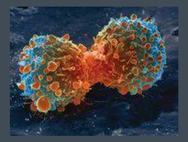
The Effect of Topical Tretinoin on *Drosophila* Larvae Survival and Chemotherapy Research



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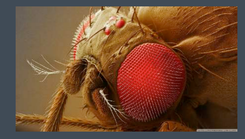


Abstract

In the past year, over 1.5 million new cancer cases have been diagnosed and over 500,000 deaths have come from this disease in the United States. Worldwide, cancer is known to be the leading cause of death, and within the next couple decades, there will be about 22 million new forms of cancer discovered. Researchers are trying to find cytotoxic chemotherapy drugs to combat the proliferating cells associated with cancer development. We experimented with a compound we believe has the potential to be used in battling this disease.

Tretinoin is an ingredient in topical cream used to treat acne. Retinoids such as Tretinoin are active regulators of cell reproduction, proliferation, and differentiation. In this experiment, we tested the effectivity of Tretinoin as a new form of chemotherapy. This compound has been a part of similar experiments with promising results, but we tested topical Tretinoin specifically to observe it as a possible hit in fighting tumor growth. To represent a tumor composed of uncontrollably dividing, mutated cells, we used irradiated *Drosophila* and administrated the drug by feeding them different concentrations of Tretinoin in their food. By quantifying survival rate after the larvae's metamorphosis into an adult fly, and by comparing these results to an already approved drug in the market for chemotherapy, we were able to see whether Tretinoin has the capacity of developing into a chemotherapy drug in the future.

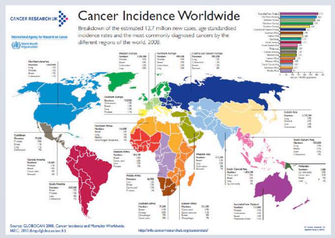
Introduction

Cancer is the direct consequence of a series of mishaps at the molecular level that fundamentally alter the normal function and ability of cells. The conventional control systems found in healthy cells that prevent cell overgrowth and the invasion into foreign tissues are inhibited. These cancerous cells no longer require specific signals to induce cell growth and division. The irregularities found in cancer cells often result from mutations in the protein-encoding genes that control cell division. These mutations cause a chain reaction of more mutated cells, as more cells mutate the routine DNA repair proteins stop regularly functioning as well, consequently creating more and more mutated cells and daughter cells.

Acute promyelocytic leukemia (APL) is a is a unique subtype of acute leukemia, in the United States, "APL accounts for 5-15% of all adult leukemia. " APL directly affects the bone marrow, which produces blood cells. In healthy bone marrow, stem cells produce erythrocytes (red blood cells) that carry oxygen, leukocytes (white blood cells) that protect the body from infection, and platelets (thrombocytes) that are involved in blood clotting. In acute promyelocytic leukemia, white blood cells build up in the bone marrow. The congestion of white blood cells leads to a shortage of healthy cells found in our blood, which is the direct source of many of the signs and symptoms of APL.

Traditional and efficient treatments for cancer are a combination of treatments. Radiation wipes out cancer cells with high-energy rays targeted directly to the tumor. It primarily damages DNA and prevents its replication. Radiation does not only kill the cancer cells, but it also kills some normal cells, specifically the cells that are dividing. Also, chemotherapy drugs are used simultaneously to eradicate all the cancer cells effectively. Chemotherapy contains toxic compounds that target the uncontrollable growing cells. Chemotherapy and radiation are used collectively to combat cells that are resistant or become resistant to the drugs. If chemotherapy or radiation were used exclusively, the amount needed for lethality needed to kill all the mutated cancer cells the levels of the toxicity in the body would be fatal.

Tretinoin is an ingredient in topical cream used to treat acne. It is a naturally occurring by-product of vitamin A (retinol). Retinoids such as Tretinoin are active regulators of cell reproduction, proliferation, and differentiation. Tretinoin binds to receptors responsible for the development of acute promyelocytic leukemia; the exact process is unknown but is found to be effective nonetheless. We will be testing if the topical cream Tretinoin could be used to combat forms of cancer.



The Problem

A high dosage of 0.1% Tretinoin cream was tested twice daily in a large blind, randomized trial for the prevention of nonmelanoma skin cancer back in 1998. Of the 566 patients tested with Tretinoin over a one-year period, 135 patients died in the two to six year study period after Tretinoin was first applied which is 22%. Though, 16% of the patients who had taken the placebo also died in the study period. The cause of the deaths were most likely due to a poor trial design in consideration to outside variables not taken into account such as elderly age and smoking habits. Further trials were terminated until more data can be obtained of the drug.

Hypothesis:
The testing of 0.05% Tretinoin through the methods of pipetting and dilutions can lead to finding a minimal concentration of the drug that is able to effectively kill a tumor while avoiding a lethal dosage to the body.

Purpose

1. Determine whether Tretinoin is a "hit" in effectively killing tumor cells as modeled by the *Drosophila* larvae.
2. Investigate the 'hits' that occur and validate their effectiveness.
3. Further experiment with the range of concentrations of the drug by comparing their effectivity to each other and positive and negative control groups.

Methods

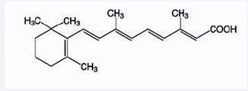
Creating the Solution
The testing will begin by diluting 0.2 mL of 0.05% Tretinoin in 800 uL of water, to make a 1,000 uL solution. This creates a 0.01% concentration of Tretinoin in solution. We chose this concentration as our starting point because we know that the human recommended dose for treatment is 430 mg/m^2, and our starting concentration is estimated to be in the range of this value in comparison to a fly.

The Independent Variable
From there, we will be diluting the solution by a ratio of 1:2 for a total of three times by taking 500 uL of the previous solution of drug and water and adding this volume to 500 uL of water. In total, we will have four different concentrations consisting of 0.01%, 0.005%, 0.0025%, and 0.00125% Tretinoin in water.

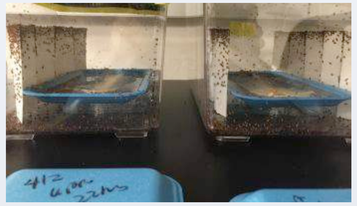
Application
Once the solutions have been prepared we will insert them into the fly vials by pipetting 3 uL of each of the four drug solutions into 3 mL of food. Three vials will be prepared for each of the four solutions. Three vials will also be used to test our negative control, water, and three more vials will be used to test our positive control, 50 ug/mL colchicine. From there we will insert the third instar larvae that were irradiated at 4,000 Rad into each prepared vial, record which flies are GFP positive or negative, and allow them to go through metamorphosis. Then we will record the amount of dead and live flies for each phenotype after pupation, allowing us to determine how effective the
Tretinoin was in killing the flies. The GFP-negative flies are what we are looking for, and the ratio of dead-to-alive flies of this phenotype will show how effective the Tretinoin was.

Comparison
Each group member will have tested one vial of each of the four drug concentrations as well as the positive and negative controls. The fly survival results of each member will be compared to check for precise results. The solutions will also be compared to each other's toxicity and effectivity as well as the positive and negative controls in order to determine which dosage of Tretinoin, being the least toxic and most effective, is more likely to become an effective chemotherapeutic drug.

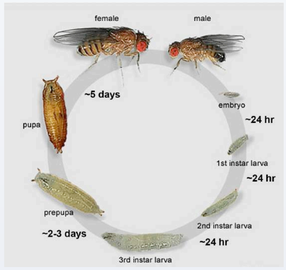
Data



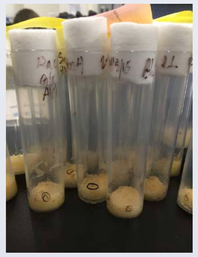
Molecular structure of Tretinoin $C_{20}H_{28}O_2$



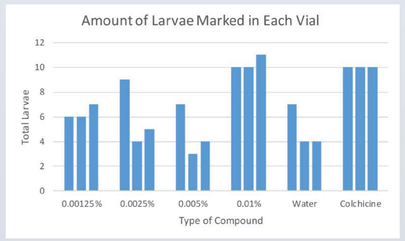
***Drosophila* mated in population cages. Females laid their eggs on the grape juice plates in the cage where eggs were then collected to hatch the larvae used for the experiment.**



***Drosophila* life cycle:**
Larvae were irradiated at the 3rd instar stage and survival quantification occurred at pupae eclosion



Drug/food mixtures were placed in vials along with irradiated larvae. Larvae would crawl up the vial in order to pupate. The vials on the left show larvae in the process of crawling up the vial. GFP negative larvae were circled as they are the targets for the drug due to the fact they are more susceptible to radiation from being homozygous for the *grapes* mutation.



The total amount of larvae which crawled upwards to proceed to metamorphosis in each vial was recorded. As seen on the graph, there is no correlation of drug concentration and amount of larvae recorded, showing that Tretinoin so far has no immediate effect on *Drosophila* survival. We predict to see results when quantifying after metamorphosis.

Conclusions

Based on our preliminary data, we expect to see a decrease of survival of *Drosophila* larvae as the compound concentration increased in each vial. A negative correlation between drug concentration and fly survival is anticipated. This is because we expect the Tretinoin to act as a chemotherapy drug which will kill the larvae that represent cancer tumor cells due to the fact that Tretinoin is a drug which is created to prevent cell proliferation and reproduction and has been previously been used in clinical trials against cancer,

As seen in the total larvae present in each vial, the amount of larvae which crawled up each vial to pupate did not exhibit a strong correlation with the amount of drug in each vial. We expected the more the concentrated drug vials to show less larvae crawling up the walls of each vial to pupate. From this information, we can infer that the Tretinoin compound may have a later effect in fly survival after eclosion quantifications. The compound most likely will be effective in later stages of the *Drosophila* life cycle than when we marked the larvae for GFP.

If the increase of drug concentration does not display a decrease fly survival even in eclosion, we can infer that the drug concentration used in the vials was too little to indicate drug viability.

Future Directions

Future Directions:

- If no effect of drug concentration on survival rate is detected, repeat the procedures using a higher concentration of drug in each vial
- Test whether topical administration of Tretinoin is more effective than oral treatment
- Find the most effective percent survival rate of larvae correlated with the lowest concentration of Tretinoin
- Conduct blinded and randomized testing to prevent any biased results
- Test the drug with other analogs such as actual cancer cells or more complex *in vivo* studies to see its effectivity in a more human biological model

Acknowledgments

We would first like to thank Dr. Tin Tin Su for her work as our Principle Investigator of this Discovery Lab. Her work setting up this lab and helping it run smoothly was invaluable. Another thanks goes to Dr. Pamela Harvey for her teaching, mentorship and knowledge that made this research possible. We would also like to thank the Molecular, Cellular and Developmental Biology Department and the Biological Sciences Initiative here at CU for their support and funding of this research and the Discovery Lab.

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Dose-response relationship of two potential chemotherapeutics in *Drosophila*

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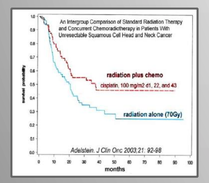
University of Colorado - Boulder

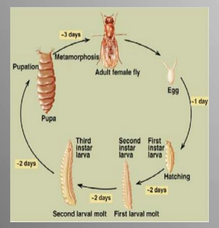
Abstract

During metamorphosis, Drosophila pupae become a mass of rapidly-dividing stem cells that can be an effective model for a cancerous tumor. The Drug Discovery Lab has identified many potentially effective radiation sensitizers by screening chemical libraries in this model. We investigated the chemotherapeutic efficacy of two compounds that showed promising results in a previous term of library screening, NSC56737 ("Compound A") and NSC679527 ("Compound B"). The chemical libraries are initially screened only in irradiated larvae, so positive results can indicate both compounds that work independently as cytotoxic chemotherapies and compounds that have synergistic effects with ionizing radiation. We tested the lethality of various doses of Compound A and Compound B to Drosophila pupae that were not irradiated, in order to determine if they functioned as cytotoxic drugs and identify potential effective doses. Our experiment was ultimately inconclusive, because very few larvae reached the pupal stage and the resulting sample population was too small to describe the drugs' effects. Additional testing in the Drosophila model is needed to validate the initial results and determine if these compounds can be effective without radiation.

Introduction

Conventional chemotherapeutics, while beneficial in the treatment and survival of cancer patients, have severe cytotoxic side effects that are becoming increasingly apparent as cancer patients survive longer (Corrie, 2007). Many of the long term adverse effects of chemotherapies are still being elucidated. Additionally, the majority of current anticancer drugs have very narrow therapeutic ranges, which limits their effectiveness (Eder, 2010). This experiment aims to find the lowest dose at which checkpoint mutant Drosophila, a model for cancer, respond to potential new chemotherapies.



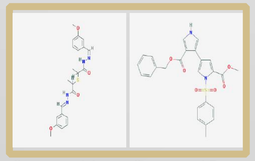


During metamorphosis, the imaginal disc cells in Drosophila pupae proliferate very rapidly, making checkpoint mutant larvae an effective animal model for rapidly growing tumors with checkpoint mutations (Su, 2011). This experiment measures the effective doses of two potential small molecule chemotherapies (Compound A (NSC56737) and Compound B (NSC679527) from Mechanistic Set III from the National Cancer Institute) in *grapes* mutant Drosophila without ionizing radiation. These compounds were originally identified as potentially effective radiation sensitizers in the same fly model. Although they were originally screened as multimodal therapies, our experiment aimed to determine whether these compounds were also effective as chemotherapies without radiation.

Hypotheses

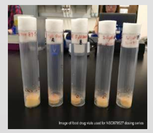
The primary hypothesis of this experiment is that there will be a dose response curve, meaning survival rates of larvae will increase as concentration of compounds decreases, when two compounds previously identified as "hits" are tested in larvae without radiation.

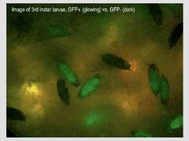
The secondary hypothesis of this experiment is that three compounds with structures similar to the structure of hit a compound, will also be hits. In other words, those drugs, when given at the same concentration, would lead to a significantly lower survival rate when compared to DMSO, and therefore could be considered potential candidates for new chemotherapy drugs.



Compound A Compound B

Methods





1. **Culture** a population of 3rd instar larvae

Rationale: 3rd instar larvae of Drosophila melanogaster serve as a useful analogs to human cancerous tumors, *grapes* mutants used for radiosensitivity as well as for relevance to human cancers which contain checkpoint kinase 1 (Chk-1) mutations (i.e. head and neck cancers). Method of cultivation used to sync Drosophila life cycles, ensuring that larvae are compatible developmentally, food used is nutritionally sufficient for developing larvae and can evenly distribute drug for roughly equal consumption by larvae.

2. **Expose** non-irradiated larvae to varying dosages of compound

Rationale: 10uM validations done in order to confirm that the drugs are "hits", dilution amounts derived based on amount of drug available (only 10uL total for each drug) and desire for redundancy in data at lower dosages (5uM and 2.5 uM). Positive control (colchicine) and negative control (DMSO) run for purposes of comparison.

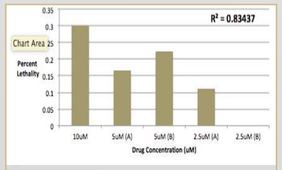
3.**Quantify** survival

Rationale: While none of the flies were radiated, and therefore balancer chromosome (GFP) presence theoretically should not matter, GFP was still recorded for each vial so that it can be incorporated into data analysis. Survival quantification indicates lethality of each dose.

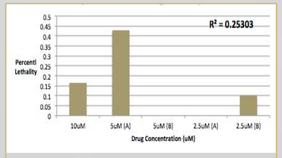
4.**Compare** survival rates at each dosage and across GFP designations

Rationale: Determine existence of dosage curves for each compound, qualifying them as potential medication or simply as a poison.

Results



Percent lethality per concentration of Compound B (across GFP designations)



Percent lethality per concentration of Compound A (across GFP designations)

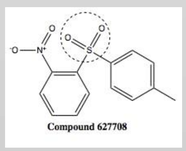
Percent lethality versus decreasing drug concentrations conducted in non-irradiated drosophila larvae.

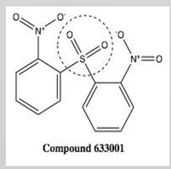
Dosing curve for Compound B (NSC679527) demonstrated to be partially representative of the expected estimate regarding the strength of the relationship between dosing and survival (R^2= 0.83). Compound A (NSC56737) did not demonstrate any strength in the relationship between drug dosing and percent survival (R^2=0.25) as there are no visually identifiable pupae at 5 or 2.5uM.

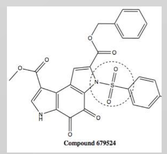
**Percent lethality is not considered significant in either experimental drug as the n values (ei number of visually identifiable pupae) were too small for either drug across concentrations.

Discussion

Neither compound demonstrated (to a statistically significant extent) dose dependence. Both compounds were, however, confirmed as potential chemotherapy hits at the standard 10uM concentration as initially tested by the students of the Discovery Lab. Compound B (NSC679527) demonstrated the expected dose dependent curve, albeit not significantly. The lack of significant dose dependence of lethality in B is most likely due to a very small sample size. Compound A (NSC56737) did not demonstrate any dose dependence, which could be due to the small sample size or could indicate that this compound acts more as a poison than drug. Based on these results, compounds from Mechanistic Set with similar functional groups as Compound B were chosen to examine under the second hypothesis of the experiment. Given the potential for dose dependence and the additional verification of Compound B as a potential chemotherapy, it is predicted that these three additional compounds of similar functional groups will also be considered new, emergent chemotherapies.





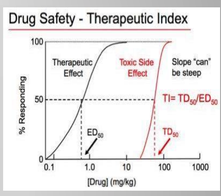


Future Directions

Re-test compound dosing series in order to accurately confirm existence or absence of dosing curves for Compound A (NSC56737) and Compound B (NSC679527). These experiments would test a greater number of non-irradiated 3rd instar larvae per food drug vial, and would include a greater number of replicates at each concentration. If drug curves do exist, preliminary therapeutic indices would be calculated in order to determine potential medical viability for each compound.

If the compounds have confirmed drug curves, we would conduct the same dosing series on irradiated larvae and compare the results of compound alone vs. compound + radiation. We can then identify whether additive or synergistic effects occur which would dictate the administration of the compounds in human clinical trials.

If compounds NSC627708, NSC633001, and NSC637914 demonstrate lethality comparable to colchicine, we would then test to see whether drug dosage curves exist for each of them. Additional tests would study the compounds' mechanism and see if the SO_2 bridge structure is causing lethality, or whether another aspect of these structures causes cell death. Depending on mechanism, we would synthesize new similar compounds and test them for efficacy.



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Biological Sciences Initiative

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TWO TIME HITS, NEW POSSIBLE CHEMOTHERAPIES

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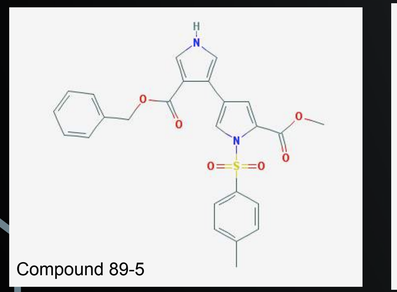
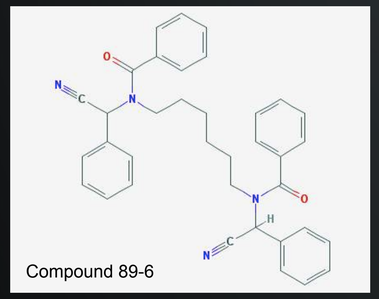
Abstract

Drosophila is a common fruit fly that is used a model organism because they have pathways that resemble humans just much more simple, they are easy to replicate and they are inexpensive to take care of. This is why we use them to test chemotherapies in our drug discovery lab. Cancer is a prominent disease that affects pretty much everyone. We have learned that finding chemotherapies is not the easiest thing in the world and definitely is not the cheapest. In our lab we tested 813 compounds and found 39 of those 813 to be hits. We decided to take two compounds that were identified as hits twice and test them at different concentrations. We believe that performing a dosing series will allow us to find the lethal dose for the subject. Due to time constraints we are still waiting for our results. After the results are obtained we believe that performing another experiment with those concentrations will validate the results. We hope that the results will show us the lethal dose. That way others can test the initial dosing concentrations as well as the new concentrations without irradiating the larvae to compare with combination therapy.



Compound 89-5



Compound 89-6

Introduction

According to the National Center of Health Statistics, cancer is the second leading cause of death in adults and number one leading cause of death in children. In 2017 there was an estimated 600,920 deaths that were cancer related (NIH 2017). Cancer is typically treated by surgery, radiation therapy, chemotherapies and different combinations of the three. Chemotherapies are not the most efficient treatment because they are expensive and toxic to the patient. The goal of our research is to find new potential chemotherapies that serve less as a threat to the patient.

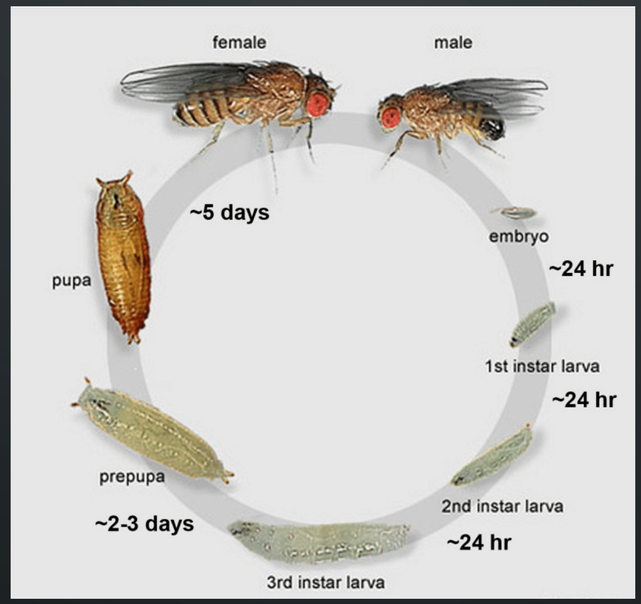
We tested two drug compounds that came from the Diversity Set IV. We selected compounds 89-5 and 89-6 because both times that they were tested at 10uM they came back as successful hits. We used a dosing series diluting our compounds in DMSO at two different dose levels 1.5uL and .75uL. We added the diluted compounds to commeal-agar food vials. We compared the compound vials to our positive control (50 ug/mL colchicine) and our negative control (DMSO).

In this lab we use the model organism Drosophila also known as fruit flies. Many of the genes that Drosophila have are conserved in humans. This means that the genes are similar enough that we can see the effects in the flies and compare them to humans. The flies have almost identical core signally pathways as human, yet are much more simpler making it easier to study. They are inexpensive to upkeep and have very short lifespans so we are able to examine and reproduce them in a short amount of time.

We believe that if we use a dosing series for compounds 89-5 and 89-6 we will be able to determine the lethal dose.

Hypothesis

- We will be testing two compounds from the Diversity Set IV at lower doses than they were tested before, we believe that by performing a dosing series we will be able to find the lethal dose.



Methods

Dosing Series

1. Transfer adults to fresh culture bottles with yeast
2. Transfer adults to a population cage
3. Collect embryos from grape juice plates
4. Transfer embryos to fresh culture bottles without yeast
5. Collect and sort larvae by size, collecting those that are 600-850um
6. Irradiate third-instar larvae
7. Set up 8 food vials and label them as followed: 89-5 Dose 1, 89-5 Dose 2, 89-6 Dose 1, 89-6 Dose 2, Colchicine Positive Control 1, Colchicine Positive Control 2, DMSO Negative Control 1, and DMSO Negative Control 2
8. Grab vials 89-5 Dose 1 and 89-6 Dose 1 and pipet 1.5uL of each compound in their appropriate vials
9. Grab vials 89-5 Dose 2 and 89-6 Dose 2 and pipet .75uL of each compound in their appropriate vials
10. Grab both Colchicine Positive Control vials and place 3uL of colchicine into each food vial
11. Grab both DMSO Negative Control vials and place 3uL of DMSO into each food vial
12. Transfer the third star larvae into vials containing food/drug mixture
13. Incubate culture vials for 10 days at 25ºC
14. Quantify survival of flies by counting empty and closed pupae

Expected Results

Unfortunately we did not get to add our results due to time constraints. However, based off the fact that we know these two compounds were hits we believe that performing the dosing series curve will allow us to find the lethal dose of both compounds.

Conclusion

Since we do not have our results from this dosing series we are unable to conclude what our findings mean. We do know that during our first initial testing we tested 813 compounds from Diversity Set VI. Out of the 813 compounds we identified 39 hits. Compounds 89-5 and 89-6 were identified as hits twice at the original 10uM. From these results we were able to justify the need for a dosing curve for these two compounds.

Future Directions

We believe that the following actions should be taken

- This procedure with the new doses should be tested again to validate the results
- Once the data is confirmed you can find the most effective and lowest possible dose of toxicity to give the subject
- All the doses from the first test and dosing series should then be repeated this time making sure not to irradiate the larvae. This way we can see if combined therapy is more effective or not.

Acknowledgements

We would like to give a big thank you to Dr. Pamela Harvey for her guidance through out our research experiment as being a wonderful instructor. Thank you to our teaching assistants Jess, Jack, and Ryan you guys were extremely helpful and patient with us during lab and this experiment. We would like to acknowledge Dr. Tin Tin Su for creating and sharing her Drosophila experiment with us. We are also grateful to the Molecular, Cellular, and Developmental Biology Department at University of Colorado Boulder for supporting this Discovery-based lab.

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The Use of Astragalus Extract Inhibits Cell Replication in Drosophila Melanogaster

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Department of Molecular, Cellular and Developmental Biology
University of Colorado Boulder

Abstract

According to the National Cancer Institute, approximately 39.6% of men and women will be diagnosed with cancer at some point in their lives. Furthermore, the NCI projects that worldwide cancer cases will increase by 50% by 2030. Despite extensive research, many cancers still do not respond to known chemotherapies, so it is important to continue researching and looking for new ones. Our team was interested in testing the effects of Astragalus extract on tumors. Essentially, by using Drosophila as models for tumors, we tested whether or not the Astragalus compound could positively affect them.

The Astragalus species is part of the Leguminosae family of Chinese medicine. There are approximately 200 constituents obtained from about 46 species of Astragalus.The most interesting part about Astragalus that makes it such a promising possible candidate for helping cure cancer, is its antioxidant properties. Antioxidant drugs or substances are substances that inhibit the oxidation of other molecules. Oxidation is a reaction which can lead to damaged cells due to its formation of free radicals. Free radicals are chemically dangerous reactions that can cause harm to cells, and the antioxidants can help neutralize the free radicals. Thus, by inhibiting the oxidation of molecules you're technically inhibiting the process of cell damage. Therefore, hypothetically, the Astragalus, as a major antioxidant, could aid in terminating cancer cells. By using it's antioxidative properties to help terminate reactions leading to damaged cells, it could prevent more cancer cells from becoming cancerous, or prevent cells from becoming cancer cells in the first place by helping them respond more effectively. In research done by University of Houston, cancer patients that took Astragalus supplements experienced a faster recovery and improved survival rates, as well as improved tumor fighting ability. By improving the immune system of cancer patients, it helped in conjunction with chemotherapies and improved the immune system's ability to fight cell mutation. With this background from the study, as well as Astragalus' known ability to provide antioxidants, we hypothesized that Drosophila co-treated with Astragalus and radiation (a dose of 4000 rads) will reduce cell mutations within the flies and aid in the process of tumor termination.

In order to continue with this experiment, we used specific doses of the medicine so that it could act like a drug that we used earlier in the semester when we were testing flies with different drugs. By testing different dosage amounts and concentrations, we developed a dose-response curve so that we could see the effects of various concentrations of the drug. The flies we used for our experiment had mutated *grapes* genes. In Drosophila, the *grapes* gene is similar to human Checkpoint Kinase 1, a gene that is often found to be mutated in head and neck cancer cells. A mutation in this gene allows cells to survive and continue replicating despite extensive DNA damage. We followed this mutation in our flies by tracking an engineered balancer chromosome called "green fluorescent protein" or GFP on chromosome 2. Flies that were homozygous negative for this gene have the were homozygous for the *grapes* mutation and were used to quantify survival, whereas flies that were heterozygous for the *grapes* mutation fluorescence green when they were larvae and were not used. Flies that were homozygous for the GFP gene did not survive into pupae because they did not have the necessary normal genes for survival. This type of flies, when they are 3rd instar larvae, have rapidly dividing cells and are very similar to cancer cells, so they serve as a good model to test compounds on. We chose to test Astragalus in conjunction with radiation because when the two therapies are used in conjunction, chemotherapies can be administered in lower doses and thus present a lower cytotoxicity to the patient. Astragalus dosage that could provide us with the results we want; dead flies.

Introduction

Astragalus Root

Hypothesis

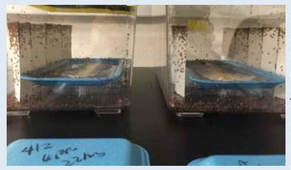
We hypothesized that Astragalus root extract would have a positive effect on the inhibition of eclosion of flies and could be a possible lead compound for head and neck chemotherapies. Due to its anti-oxidative properties as described above, we believe that the use of the Astragalus extract will prevent the pupae of the Drosophila Melanogaster from forming from the third-instar larvae.

Furthermore, our results should show high percentages of dead GFP+ and GFP- flies. If our results follow this hypothesis, than we can conclude that the Astragalus aided in the process of preventing cell replication,, and should require further research in order to see the complete extent of the Astragalus' effects against cancer.

Methods

To make a stock solution of 0.1 g/mL for the drug trials, 5 grams of dried astragalus extract was mixed with 50 mL of water until all of the extract had dissolved. Then, three serial dilutions were made from the stock solution using the molecular weight of Astragaloside IV (784.97 g/mol), which is the primary component of the Astragalus extract used. The first was a 1:2 dilution, and was made by adding 50 uL of the stock solution to 50 uL of water, making a 50 mM solution. The second was a 1:10 dilution and was made by adding 10 uL of the stock solution to 90 uL of water, making a 10 mM solution. The last was a 1:100 dilution and was made by adding 1 uL of the stock to 99 uL of water, making a 1 mM solution. Each dilution will be tested in triplicates against a positive control of colchicine and a negative control of water. After adding the dilutions to water, the concentrations will be diluted to 50 uM (1:2 dilution), 10 uM (1:10 dilution), and 1 uM (1:100 dilution) by mixing 3 uL of the drug with 3 mL of food. These dilutions were chosen because the represent a good range for the dosing series, from very concentrated to very dilute.

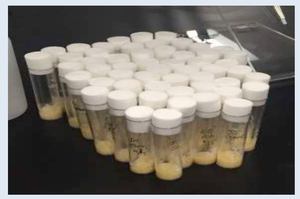
The larvae will be irradiated and then placed in the drug vials where they will incubate for a week, at which point GFP will be marked. After incubating for approximately one more week, fly survival will be quantified. Data will collected with regards to the positive control, 50ug/mL colchicine, and the negative control, water, and will be analyzed for astragalus' possible effectiveness as a chemotherapy by comparing survival rates to the controls.



Step 1:
Set up the population cage for the breeding of the Drosophila melanogaster (inside the population cages are grape juice agar plates that we made ourselves). The grape juice is used because the flies like sugary substances. The agar is used to encourage female Drosophila to lay eggs.

Step 2:
Collect the embryos from the grape juice agar plates

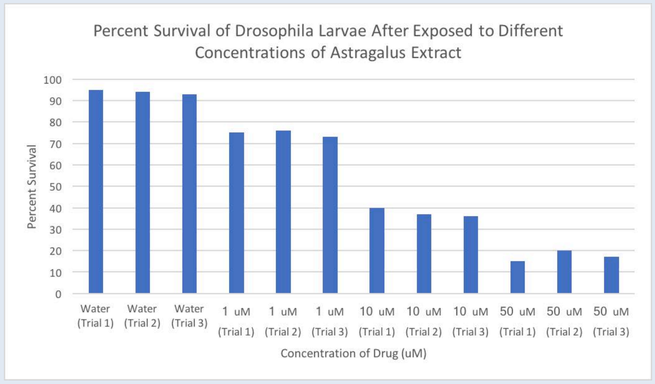
Step 3:
Separate the embryos from the agar plates and place them in cultured food bottles. Then, culture the embryos until they're third instar larvae, and irradiate them at 4000 Rads.



Step 5:
Place the irradiated flies into food vials. The vials were in groups of 3, with each group containing 3 identical concentrations. In the end, we had 9 vials with 3 different concentrations, and an extra 3 vials for a controlled set of flies.

Step 6:
Finally we quantified survival of the GFP+ and GFP- flies to see how our Astragalus reacted with the flies.

Expected Results



If the results come out as expected, the graph of percent survival of Drosophila larvae vs. concentration of astragalus used should look similar to this one. As the concentration of Astragalus increases, the percent survival of the Drosophila larvae should consistently decrease. The water control should have high survival rates of flies to show that it doesn't have an effect of the survival rate of the flies since the Astragalus was diluted in water.

Conclusions

Future Directions

Future Directions for testing Astragalus could include the following:

- Testing more diverse concentrations of Astragalus Extract
- Testing many different concentrations of Astragalus on human head and neck cancer cells.
- Testing the same experiment again except with pure Astragaloside IV instead of having it in a powder partially diluted with other polysaccharides.
- Testing the effects of exposing non-irradiated flies and irradiated flies to pure Astragalus extract.
- Test compounds that have a similar structure to Astragaloside IV
- Repeat the current experiment with many more trials to gain more accurate results.

Acknowledgements

We would like to thank Dr. Tin Tin Su, Pamela Harvey, PhD., Jessica Westfall, Jesse Kurland, Sam Gendelman, and Michaela Nelson for their help with our project. We would also like to thank the CU Molecular, Cellular, and Developmental Biology Department, the Howard Hughes Medical Institute, and the Biological Sciences Initiative for funding the class.

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Synergistic Effects of Ascorbic Acid and Radiation in *Drosophila melanogaster*, a Model of Cancer

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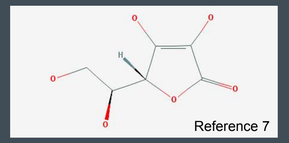


Reference 7

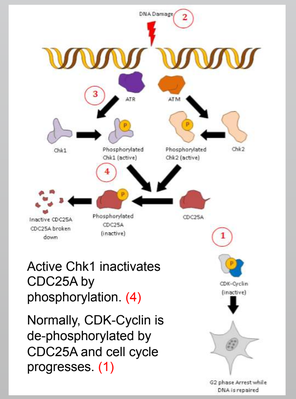
Reference 1

Abstract

Cancer cells are able to avoid apoptosis (programmed cell death) by ignoring signals sent through the extrinsic pathway or by inhibiting apoptosis through rebalancing the intracellular pro- and anti-apoptotic molecules (4). Thus, cancer cells are able to sustain DNA damage and will not go into cell cycle arrest, allowing them to survive and to proliferate quickly. The issue with chemotherapy is that it's a cytotoxic substance that creates problematic symptoms for the cancer patient, such as hair loss, myelosuppression, reduced fertility, and late effects such as cardiomyopathy (2). To combat this, a combination of drugs, each given at low doses, is administered to patients in order to create a higher cure rate (e.g. radiation and chemotherapy). Recent studies have shown ascorbic acid as a viable candidate to work alongside radiation in order to control the cancer cells' growth (10, 5). Using Ascorbic acid (L-AsA) can potentially lower side effects cancer patients receive since it is not a cytotoxic compound and may be equally as effective as combining chemotherapy with radiation.

This research will be investigating the effectiveness of radiation therapy in combination with L-AsA in *Drosophila*. Shinozaki *et al.* 2011 reported that radiation used in combination with L-AsA leads to increased apoptosis in the HL60 (Human Leukemia) cell line. An increase in apoptosis may be due to, in part, a disruption of the mitochondrial membrane potential by the presence of sodium ascorbate (a key mineral in ascorbic acid) that acts as a prooxidant which allows cytochrome C (CYT-C) to be released into the cytoplasm. CYT-C plays a key role in intrinsic apoptotic pathways helping to create an apoptosome, which actives caspase-9s (5), thereby amplifying recruitment of many more active caspases, resulting in cell death (3). These previous findings from cell culture tests suggest that exposure to high concentrations of L-AsA may help to control tumor growth. Our experiment aims to investigate this in a model organism using combinatorial treatment.

Introduction



Active Chk1 inactivates CDC25A by phosphorylation. (4)

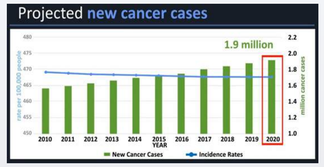
Normally, CDK-Cyclin is de-phosphorylated by CDC25A and cell cycle progresses. (1)

Reference 11

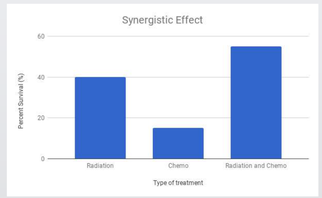
Drosophila melanogaster is an ideal model organism to conduct these tests with because it multiplies relatively quickly, and shares many genes with humans. This allows us to see an experiment's results and the progression of a disease through multiple generations faster than you could with humans. One such gene that is similar to humans' is the *grapes* gene (grp), the *Drosophila* equivalent of a human's Checkpoint kinase 1 (Chk1), which determines whether cells in the S phase that have just replicated their DNA can proceed to mitosis. If the grp gene is mutated in *Drosophila*, then it allows for cells to continue through to mitosis and proliferate uncontrollably, all the while acquiring DNA damage that remains unchecked. This kind of apoptosis evasion is exhibited most often in head and neck tumors and is why we are able to use this fly as a model for it.

Purpose

- In 2016, there were around **1.6 million new cases** of cancer in the US alone and this number only continues to grow. (Ref. 6)
- Current cancer treatments such as radiation and chemotherapy are **aggressive** and come with **adverse side effects** for the patients. (Ref. 2)
- Our experiment aims to identify compounds that work synergistically with radiation therapy in hopes of being able to **minimize dosage** of each therapy while keeping therapeutic effect, thereby **minimizing the side effects** that may kill the patient before the cancer does.



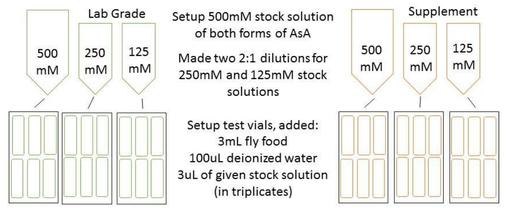
The number of new cancer cases expected to continue to increase over time. Reference 9.



Hypothesis

Previously, experiments have shown that treating cancer with radiation alongside with Ascorbic Acid has a positive effect in controlling tumor growth. If Ascorbic Acid has been successful in suppressing tumor growth and proliferation, then it may be useful in cancer treatment because it is not a cytotoxic compound, but rather, a water-soluble weak acid. This could help to limit any side effects it may cause while being just as effective at treating tumor growth as a cytotoxic compound in conjunction with radiation.

Methods

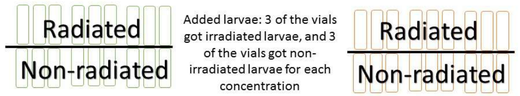


Setup 500mM stock solution of both forms of AsA

Made two 2:1 dilutions for 250mM and 125mM stock solutions

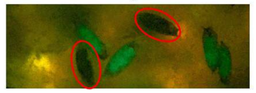
Setup test vials, added:
3mL fly food
100uL deionized water
3uL of given stock solution (in triplicates)

When in 3 mL of food, the stock solutions were diluted in 1:1000 ratio. This made 500 µM, 250 µM, and 125 µM of compound in vials

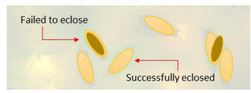


Added larvae: 3 of the vials got irradiated larvae, and 3 of the vials got non-irradiated larvae for each concentration

Marking GFP-

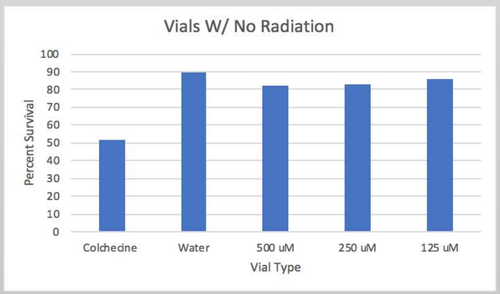


Quantifying Survival

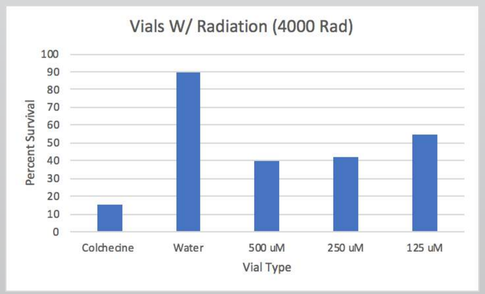


**We irradiated and treated 3rd instar larvae because it is at this point in their development that they have the highest amount of stem cells that are rapidly dividing and are highly resistant to breaks in their DNA, just like cancer cells.

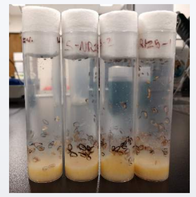
Expected Results

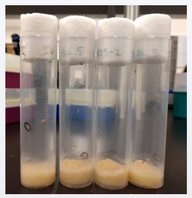


The graph to the left shows our expected results when ascorbic acid is added to vials with non-irradiated larvae. Since ascorbic acid is not a cytotoxic compound, we expect a high percent survival similar to our negative control.



The graph to the left shows our expected results when ascorbic acid works adjuvant with radiation. We expect to have a much lower percent survival. In humans, the maximum concentration intake of ascorbic acid is around 206 µM, so the vials with 500 µM and 250 µM will have similar percent survivals because they would exceed the maximum intake of ascorbic acid.





Left: Vials with non-irradiated larvae ready for quantification

Right: Vials with irradiated larvae, many did not make it to pupation. Results mostly inconclusive.

Conclusions

Future Directions

For the Library screening: Of the 813 compounds, the drug discovery lab collectively identified 32 hits, 5 of which were known chemotherapies. This data will be sent to Dr. Tin Tin Su's lab for further, in depth testing.

For Ascorbic acid: Approximately half of the flies did not reach pupation and as a result, a majority of the vials with irradiated larvae were not able to be quantified. Re-testing would be beneficial in order to collect data to see how radiation works alongside AsA. May need to do some troubleshooting and revise our hypothesis and/or the concentrations of Ascorbic acid we expose our flies to.

Acknowledgments

- A huge thank you to Dr. Tin Tin Su for allowing us to contribute to her research and adapt her methods to our drug discovery project.
- We would like to thank Dr. Pamela Harvey for her relentless support and dedication to coordinating our Discovery Lab.
- We would also like to thank our TAs Jessica Vu Westfall and Jesse Kurland for their patient guidance throughout this semester.
- This research could not have been possible without the help of the Howard Hughes Medical Institute, Biological Sciences Initiative, and Department of Molecular, Cellular and Developmental Biology for their generous funding and support.

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