The Journal of Biological Sciences

Effects of Caffeine on the Lifespan of E. coli Infected Drosophila melanogaster

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Abstract

The field of longevity is an an essential area of research in the pursuit of improving the quality of human life. One way that helps increase longevity is strengthening the immune system by incorporating a healthy diet, thus boosting longevity. A healthy diet significantly influences an individual's immune system response when microbes threaten an individual's biological defenses which often leads to the development of an infection. Caffeine is a compound that is consumed daily in an individual's diet in moderation and can provide beneficial effects on the human body. In addition, caffeine affects the nervous system which causes an individual to be more alert and focused. It accomplishes this by blocking adenosine receptors in the brain and enabling caffeine to have antibiotic effects against infections. A common infection such as E. coli is another important variable in this study. D. melanogaster is the model organism and was used to study our research question. It is known E. coli decreases the lifespan of a fruit fly. Nevertheless, how caffeine affects the body against bacterial infections is still unknown. The experiment studies the effects of an E. coli infected D. melanogaster on a caffeine diet for an extended time. In this study, we investigate how caffeine will affect an E. coli infected fly's lifespan in comparison to an infected E. coli fly given no caffeine. The infection was introduced to the flies via septic injury using a very fine needle. We determined that E. coli infected fruit flies with a supplemented diet of caffeine will increase survivability than infected fruit flies with a non-supplemented diet. Our results showed insignificant statistical testing due to procedural error. We hope this experiment will inspire future research in enhancing the knowledge of caffeine and its antibiotic effects when an organism is exposed to a bacterial infection.

Introduction

The immune system is significantly important for all living organisms and continues to be impacted by various factors. Some factors include genetics, environment, and infectious pathogens which stimulate an immune system response. Immune systems provide a biological defense against foreign invaders when an individual is exposed to harmful pathogens or toxic substances. Individuals who have a weakened immunity or living with incurable autoimmune diseases are prominently at risk of acquiring an infection. Medications such as nonsteroidal anti-inflammatory drugs have been used to treat inflammation and other symptoms. Compounds found in these medications include acetylsalicylic acid, which helps regulate pain and inflammation by inhibiting the production of prostaglandins (Flower, 2003). Acetylsalicylic acid is one of many compounds utilized to help manage pain and provide support for the immune system. Another compound found and studied is propionic acid, which is an ingredient in Ibuprofen. This compound is essential for providing regulation of numerous inflammatory cytokines and chemokines, which help regulate immune response and control immune cell movement as well as the organization of immune organs (Borish and Steinke, 2003). Its properties can also have beneficial effects on visceral adipose tissue by reducing obesity-related inflammation and increasing lipogenesis and glucose absorption (Al-Lahham et al., 2012). Caffeine is another compound of significant interest and plays a key role in other compounds as well as drugs. It is a stimulant found in a variety of natural products consumed by humans. Furthermore, caffeine found in coffee is rich in antioxidants and prevents damage to immune cells (Azam et al., 2003). These natural antioxidant nutrients strengthen the development of tolerance and regulation of inflammation which corresponds with the immune system mechanisms and how an infection can impact an individual's immune system (Puertollano et al., 2011). Caffeine is effective since it functions with nonsteroidal anti-inflammatory drugs because it significantly increases the antinociceptive effects of these drugs (Granados-Soto and Castañeda-Hernández, 1999). The natural properties of caffeine provide a boost in the immune system function since it contains antioxidants and prevents possible susceptibility to infections.

A healthy immune system and rich antioxidants are vital for promoting strong immunity. Various types of infections can threaten immunity which can increase susceptibility and decrease longevity for an individual. Infections happen when microbes such as protozoa, helminths, fungi, viruses, or bacteria enter a host. When a host becomes infected with a living and replicating microbe, this causes damage and induces disease. The immune system will fight off the infection with the support of white blood cells and antibodies to eliminate the pathogenic invader. Nevertheless, once the cells are damaged because of the infection, as a result,

multiple symptoms will occur. Symptoms include fever, headache, weakness, urticaria, etc. These pathogenic bacteria interrupt cell function by multiplying rapidly, harming cells and tissues, producing toxins that destroy cellular metabolic processes, and can inspire autoimmunity (Drexler and Medicine (US), 2010) Bacteria are a microbe of interest because of their diverse genetic material and ability to evolve very quickly. Escherichia coli, otherwise known as E. coli, is a bacterium that can cause an infection. In the United States, E. coli is a frequent infection that is transmitted to humans via the consumption of contaminated food or water. Some E. coli are classified as pathogenic because these specific types of strains cause mild to life-threatening infections (Lim et al., 2010). Immune systems are significantly important for survival and are needed to maintain a healthy life. To survive a mild or chronic bacterial infection, treatment is required if a host's immune system cannot fight the infectious pathogen. There are different ways to maintain and strengthen immunity such as plenty of sleep, daily exercise, and practicing a healthy diet. All of these important strategies greatly help promote the immune system and therefore positively affect longevity.

Caffeine, also called trimethylxanthine, is a central nervous system stimulant found in plant alkaloids which affect about 20% of plant species (Ain et al., 2016). Some of these plants such as coffee beans, cacao beans, and teas are some of the most commonly consumed plants ingested. Caffeine can be isolated into a bitter white solid that is added to many foods and drinks to help with taste and for its use as a stimulant. Since caffeine is a stimulant, it provides an increase in arousal and alertness thus allowing an individual to feel more energized (Sutphin et al., 2012). A few other effects of caffeine include heightened awareness and increased speed of focus and retention. These effects make caffeine desirable which is most commonly consumed in the use of coffee. Caffeine further affects the body in that it causes an increase in blood pressure, heart rate, and urination. The effects of caffeine last for about a few hours. About 180-190 mg of caffeine are ingested by adults each day (Jahrami et al., 2020). Consuming caffeine every day allows for the body to build tolerance causing the body to need more caffeine to have the same effects. Having too much caffeine can also affect the body in that it causes blood pressure to drop, headaches, nausea, and anxiety (Richards and Smith, 2015). However, caffeine in moderation has shown to be useful in preventing neurodegenerative disease (Sutphin et al., 2012). Caffeine has recently been more useful in drugs such as NSAIDs and is now being further investigated for its effects on the bacterial infection (AL-Janabi, 2011).

Caffeine is metabolized in the body and broken down into three primary metabolites paraxanthine, theobromine, and theophylline. Each one affects the body differently as paraxanthine improves performance by elevating fatty acids and glycerol to fuel muscles, theobromine increase oxygen and nutrients to the brain as well as increase urine output, and theophylline increase heart rate and relax the smooth muscle of the bronchi which has also been proven to have anti-inflammatory effects (Cappelletti et al., 2015). Caffeine further affects the body in the brain as it easily passes the blood-brain barrier by being water and fat-soluble. Caffeine is similar in structure to adenosine allowing for the caffeine to bind to the adenosine receptors. Adenosine slows the nerve cell activity and when the caffeine blocks its ability to do its job it causes nerve activity to increase allowing for the body to get the stimulating feeling as well as raising dopamine levels. Caffeine blocks adenosine receptors mainly in the A1 and A2A subtypes and inhibits cyclic adenosine monophosphate-phosphodiesterase (cAMP-PDE) and thus increasing concentrations of intracellular cAMP (Lorist and Tops, 2003). Adenosine is a strong immunomodulator by suppressing immune cells and cAMP is also an immunomodulator it allows the immune system to work better against disease and infections by suppressing the effects of the inflammatory and normal immune response (Horrigan et al., 2006). A study done on mice looked at the immunomodulatory effects of caffeine and found that immunomodulatory actions of caffeine are mediated by inhibition of the cAMP-PDE and that caffeine exposure to the A2A receptor can increase anti-inflammatory actions. They also found that caffeine increases anti-inflammatory effects in other drugs such as aspirin (Horrigan et al., 2006). Caffeine's effect on the body makes it helpful in having antibiotic effects and increasing the effects of other antibiotic drugs. Against bacteria such as Escherichia coli (E. coli), caffeine can degrade the strain and cause filamentous growth which stops cell division and allows for the bacteria to be vulnerable to further damage (Whitney and Weir, 2015).

We designed an experiment to test if caffeine improves immune system function and utilized D. melanogaster. The immune system was challenged by exposing the D. melanogaster to E. coli and performing septic injury. Our septic injury protocol involved a fine needle that was submerged into the E. coli bacteria and then inserted into the thorax of the D. melanogaster. The data was acquired by using longevity as a measurement of how a fly's immune system reacted to the bacteria and caffeine. Based on previous research studies, E. coli will decrease the lifespan to 12 days. In contrast, caffeine was found to increase the lifespan of D. melanogaster when studied independently from bacteria (Ramanavièienë et al.). We hypothesize the flies exposed to caffeine will have an increase in longevity because of the antibiotic effects of caffeine on the immune system and E. coli infection.

Materials & Methods

Experimental Design

Oregon R wild type, *D. melanogaster* was used as the model organism. Each group was fed 3.00 grams of Ward's Drosophila Medium white dry food, mixed with 8.5mL of water. The *D. melanogaster* was provided with the necessary environment, so no other variables interfered with the data. There were 5 groups in this experiment. Group 1 was a control group with *D. melanogaster* under normal conditions. Group 2 was another control group of *D. melanogaster* with a septic injury, but no *E. coli*, allowing us to ensure the pricking alone is not what caused the flies to die. Group 3 was *D. melanogaster* with caffeine, another control to make sure the caffeine did not play a role in the slowing survival rate. Group 4 has only *E. coli.* Lastly group 5, *D. melanogaster* with caffeine and *E. coli*.

Escherichia coli Preparation

K-12 strain was used for this experiment. The *E. coli* was ordered and provided from Carolina Biological Supply Company, which also included a 10mL rehydration buffer solution. The buffer solution was mixed with dehydrated *E. coli* and incubated at 37 °C. About 1.25 g of LB powder and 50.00 mL of distilled water was mixed until LB powder dissolved before it was further prepared in the autoclave for 30 minutes. The final optical density had a reading of 0.79 that was kept constant through all trials.

Septic Injury

The *D. melanogaster* was infected with *E. coli* by pricking the side of the thorax below the wing. Each fly was pricked with the needle approximately 2 mm into the thorax for an efficient direct exposure. The needle was dipped in *E. coli* before use. This specific technique enabled for direct exposure of *E. coli* into the body of a *D. melanogaster* without the application of an injection needle. Every fly was pricked in the same location, with about the same pressure applied, and depth. To reduce variability, the same individual performed the septic injury. The vial was positioned on its side until all flies recover to eliminate the possibility of a fly being trapped in the food. The protocol followed for this experiment can be further reviewed (Khalil et al., 2015).

Caffeine

Caffeine was acquired from Ward's Science. The *D. melanogaster* consumed the caffeine from it being mixed into their food and water from a calculated ratio. The *D. melanogaster* was given 0.625mg/mL of caffeine, which was then determined based on the calculated food and water ratio (Nikitin et al., 2008). A 50mL stock bottle of 10x concentrated caffeine water was made that will keep that ratio more consistent between trials. This stock bottle will be used to draw out the 0.85mL of caffeine water with 7.65ml of regular water to dilute to the needed concentration.

Results

To test if caffeine increases the longevity of *E. coli* infected *D. melanogaster*, we performed an average percent survival of all five groups across the six trials conducted. The data was recorded on days 1, 5, 8, and 12 to show the average percentage of survival over time. Day 12 shows the final average survival for each group collected. The lowest average percent survival on day 12 was 75% from the group that was pricked without *E. coli,* followed by 82% from the group with *E. coli* and caffeine, 85% from the group with *E. coli*, and 97% from both the control group with no *E. coli* or caffeine and the group only given caffeine (Figure 1). The principal difference is best illustrated between all the groups on day 1 to the group pricked with no *E. coli* from day 5 to day 12, which had a p-value < 0.05. Additionally, all the groups on day 1 and the group with *E. coli* and caffeine on day 12 had a p-value < 0.05. Between the other groups on different days the p-value > 0.05, which is not significant. The lowest survival rates were from the groups that received a prick from the needle. Moreover, the flies pricked with and without *E. coli* decreased in survival rate in contrast to flies that were not pricked. Overall, the data was insignificant and caffeine shows no effect on the survival rate.

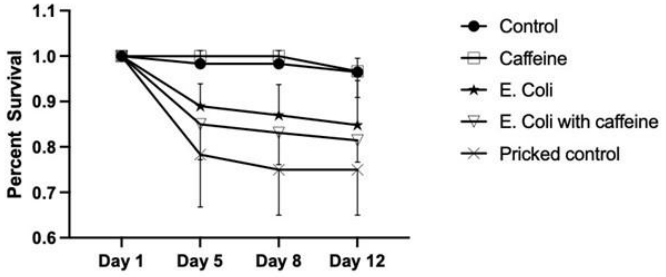

Figure 1. The average percent survivability of *D. melanogaster* between six trials. Five groups (flies with *E. coli*, flies with *E. coli* and caffeine, flies with only caffeine, control flies with nothing, and control flies that are only pricked with no *E. coli*) are compared on Day 1, Day 5, Day 8, and Day 12. A line graph is shown with standard error bars that represent the standard deviation of the averages between the six trials.

Discussion

We tested if caffeine increased the lifespan of an infected *D. melanogaster* with *E. coli* and our data revealed there were no statistically significant differences within the five groups. Our data indicates that caffeine does not significantly affect survival and the *E. coli* exposure was not successful. The septic injury control group was supposed to eliminate the

variability of injury caused by actual needle injection, but had the opposite effect and reduced the lifespan of *D. melanogaster.* It has the lowest survival rate as shown in the line graph (Figure 1). It was concluded there was an error in the septic injury protocol. *D. melanogaster* dying due to a faulty protocol and as a result the data remains inconclusive. Despite our initial hypothesis not being supported, the future directions of this experiment can be investigated with the septic injury assay and challenge survivability rate. Other factors than caffeine can also be surveyed in conjunction with septic injury to observe and test if the specific factor of interest can impact lifespan.

When statistically analyzed, there was no significance behind the data found when given *D. melanogaster* caffeine to treat an *E. coli* infection. Although when compared to the group of *D. melanogaster* that was not fed caffeine, there was a slight difference in the survival rate. There was a potential difference, which could be further explored in an improved design of the experiment or tested in a new one. If the experiment was to be replicated in the future, the procedure of exposing the *D. melanogaster* to *E. coli* should be modified because the *E. coli* was unable to affect the immune system as significantly when infecting the flies with septic injury. A possible solution would be to use a needle and inject the flies as opposed to pricking them to ensure the bacteria is entering the fly and therefore affecting their immune system. The method of infection can also be adapted to allow for a longer period for the trials and result in more significant data being developed. Unfortunately, the septic injury alone caused the *D. melanogaster* to die, so there could be an alternative approach for exposure instead of a physical injury. The *D. melanogaster* can be exposed and infected with *E. coli* via their food or water supply. Another modification to improve the experiment could include challenging the immune system by using more infectious bacteria that could decrease the lifespan of the flies quicker. To further challenge the immune system, additional work can be produced for the immune system to determine if caffeine will combat the infection and increase longevity by acting as an antibiotic.

Despite the data not being statistically significant to test and support our initial hypothesis, the experiment was educational in providing further knowledge in the pricking method and expanding our understanding of caffeine and its effect. Prior studies tested with caffeine have found the compound to aid in immunity and fighting infections by increasing the potency of the anti-inflammatory drugs, but further testing against these infections with caffeine itself can prove to be valuable. Caffeine is consumed regularly every day by many people and with further studies on its effects on immunity and other diseases, it can be a new and inexpensive aid that is accessible to everyone.

Acknowledgements

We express our deepest appreciation and gratitude to all of our professors who provided us with endless insight and encouragement. This experiment would not be possible without the efforts of Dr. Kwangwon Lee, Dr. Nathan Fried, and Professor Adam Poff. We also want to sincerely thank Rutgers University- Camden for the opportunity to conduct our research in their laboratory facility.

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