The Effect of PTU on the Development of Zebrafish Embryo

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Abstract

The survival rate, hatching rate, and pigmentation percentage of zebrafish embryo raised to a 0.005% PTU solution was examined in comparison with zebrafish embryo raised in an instant ocean solution (control group) in order to find potentially harmful properties of PTU (phenylthiourea). In this experiment, it was expected that the exposure of PTU would lead to a smaller pigmentation percentage because of its property of inhibiting the enzyme tyrosinase, a key component of melanin synthesis. Zebrafish embryo were divided into 6 wells, with three exposed to PTU, and the quantities of how many were hatched, alive, and pigmented were recorded once each day until 96 hours post-fertilization. Wells treated with PTU had no hatched or pigmented fish by 96 hours, as well as a higher mortality rate than the control. An unpaired t-test determined that there was a statistically significant difference in hatching percentage and pigmentation percentage between the two groups of zebrafish (p-value < .0001), but the difference in survival rate was not found as significant (p-value = .1081). The results support that PTU's property of inhibiting tyrosinase has a significant effect on developing zebrafish embryo, including the potential development of albinism and harmful developmental defects suggested by an abnormally longer hatching period and lack of pigment.

Participants

Students of AP Biology at Greendale High School ran experiments to see the effect of chemicals on zebrafish embryos. Zebrafish which were bred from The School of Freshwater Sciences, at the University of Wisconsin Milwaukee. We received them within a couple hours of post-fertilization and began our experiment on the same day.

Introduction

For this experiment, the experimental variable was the exposure to a concentration of 0.005% PTU. PTU inhibits melanogenesis, or the process that produces melanin. Melanin is known as the protein responsible for pigmentation in animals, so therefore, by inhibiting its production PTU stops the creation of pigment, leading to the disorder known as albinism (Hall, A., & Orlow, S.).

Zebrafish were used as our model organism because of their genetic similarities to that of a human being, as well as their translucent bodies/eggs offering accurate observation of pigment. The results of experimentation on zebrafish translate well to human beings because of our genetic similarities, including the fact that we share roughly 70 percent of the same genes (Burke, 2016).

It is known that PTU inhibits functions such as the production of pigmentation; however, there may be underlying issues that have yet to be discovered, such as secondary effects from albinism or unknown effects from PTU exposure. Through the usage of zebrafish as a model, we investigated some potential harmful effects of PTU exposure during a developmental period.

Hypothesis:

If zebrafish embryo are exposed to PTU, known information on its effects suggest that the fish will develop as albino due to the inhibition of tyrosinase. Additionally, we hypothesize that the presence of PTU will have enough of a harmful effect to lower hatching rates and survival rates of the embryo because it could negatively impact many undetermined but necessary components in development.

Materials and Methods

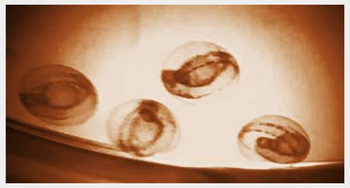
Materials:

- **Zebrafish Embryo** bred from The School of Freshwater Sciences at the University of Wisconsin Milwaukee
- **Instant Ocean solution**
- **0.5% mg/mL PTU solution diluted down to 0.005% mg/mL PTU Solution**
- **12 Well Plate**
- **Stereo Microscope**
- **Pipettes**

Independent Variable: Presence of PTU in solution
Dependent Variables: Survival rate of fish, the percentage of fish that hatched, percentage of fish that developed pigmentation.
Controls: Temperature (Same temperature exposed outside/inside incubator), amount of fluid in each well (2-3 mL), length of time between solution renewal (24 hours).

Procedure:

Zebrafish embryo were divided into wells and observed once a day for 96 post-fertilization. The first column of wells (3 total) were labeled as "control", and the second column of wells (3 total) were labeled as ".005% PTU", and the solutions were put in their respective markings.Each day, the older solution in each well was replaced with fresh solution of its designated type. The microscope was used to document the total number of eggs in each well, the number pigmented, the number of eggs that were dead, and the number of eggs that were hatched. Dead eggs were removed from the well when documented, and the eggs were put into an incubator overnight. Pigmentation was determined by our definition: "containing significant dark coloring disregarding the eyes or yolk." A hatched fish was determined by our definition: "at a minimum, the tail was completely outside of the eggshell."



Pigmented Embryo in Control Well

Embryo Without Pigment in PTU Exposed Well

References

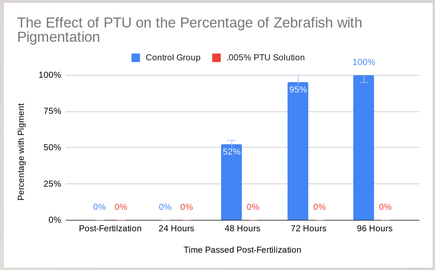
Burke, E. Why Use Zebrafish to Study Human Diseases? (2016, October 14). Retrieved from https://irp.nih.gov/blog/post/2016/08/why-use-zebrafish-to-study-human-diseases.

Hall, A., & Orlow, S. (2005, April). Degradation of tyrosinase induced by phenylthiourea occurs following Golgi maturation. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15760341

Results and Statistical Findings

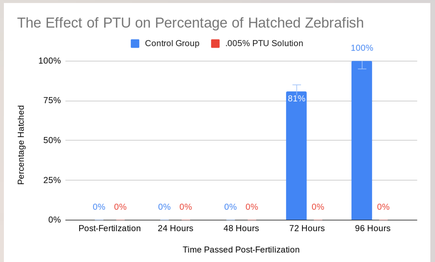
Pigmentation:

Our data shows that the percentage of pigmented zebrafish decreases if a population is exposed to PTU, supporting our hypothesis. An unpaired t-test determined that there was an extremely statistically significant difference in pigmentation percentage between the two zebrafish populations (p-value < .0001).



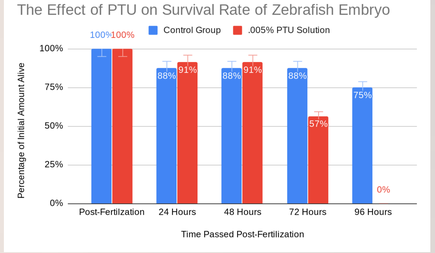
Hatching Percentage

Our data supports our hypothesis that PTU would decrease hatching percentage, as we had no fish exposed to PTU hatch.
An unpaired t-test determined that there was an extremely statistically significant difference in hatching percentage between the zebrafish populations. (p-value < .0001).



Survival Rate:

Despite drastic differences in the end result for survival rates, it was not found as statistically significant (p-value at .1081) most likely due to a small sample size of only 6 populations. If there were trials performed in the future, the difference in survival rate would likely come out statistically significant if it followed the same trend as our data.



Discussion

The results of the experiment suggest that PTU effectively inhibits the production of melanin, as no fish treated with the PTU solution developed pigment. Melanin, with its property of protecting cells from UV rays, is a essential protein for protection against skin cancers. Experiments like these open up the potential risk of chemical exposures during embryonic development, as PTU exposure as little as a 0.005% solution has a statistically significant connection to the lack of pigment, or the disorder albinism. Further understanding of the tolerable amounts of exposure to PTU and related chemicals during embryonic development can help us better protect the melanogenesis pathway and build a preventative to the secondary effects of lack of melanin, such as a higher risk of skin cancer.

This experiment also shed light on other potential risks of PTU exposure during the developmental period. The exposure to PTU led to a statistically significant decrease in hatching rate during the first 96 hours post-fertilization, which upon further investigation may draw connections to currently unknown consequences of this chemical on the body. Genetic similarities between humans and zebrafish also suggest that these harmful effects may extend to humans, and a more thorough understanding of the chemical can help us prevent any health concerns it may provide.

This experiment may have inaccurate data, since current accepted toxicology charts don't support the severe mortality rate for the concentration of PTU used. More trials and a repeated experiment would be necessary to support the validity of these results.