Exploring the Effects of Tyrosinase Inhibitor on Skin Pigmentation and Development of Zebrafish Larvae

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Introduction

Each year, about 132,000 melanoma skin cancer cases are diagnosed globally, with one in five Americans at risk (World Health Organization). Melanogenesis is the body's protective process where melanocytes produce melanin in melanosomes to adapt to UV radiation (Maranduca et al., 2019). Skin pigmentation and development are essential aspects to be studied to understand the progression of melanoma skin cancer. In addition, this study will provide knowledge on skin pigmentation which influences a person's intersectionality, the interconnections of identities that determine factors of disadvantage and discrimination.

To investigate the mechanisms of development in humans, this study will focus on teh growth of embryonic stages in zebrafish. Zebrafish undergo seven developmental periods known as embryogenesis. The first stage is the zygote period (0 to ¾ hour) where fertilization occurs, triggering movement towards the animal pole. The blastodisc separates from the vegetal cytoplasm, leading to the first cleavage. The cleavage period (¾ to 2 ¼ hours) follows with six divisions at 15-minute intervals. The eighth zygotic cell cycle marks the blastula period (2 ¼ to 5 ¼ hours). During this stage, the midblastula transition (MBT) occurs, and the yolk syncytial layer (YSL) develops, initiating epiboly. The gastrula period (5 ¼ to 10 hours) sees further progress in epiboly, formation of primary germ layers, and the embryonic axis through cell movements. The segmentation period (10 to 24 hours) is the fifth stage with morphogenetic movements, somite formation, elongation of the embryo, and prominence of organ rudiments. The pharyngula period (24 to 48 hours) signifies the development of vertebrate structures such as bilateral body plan, notochord, and new somites. Hatching (48 to 72 hours) involves the embryo

breaking through the chorion. In the larval stage, the zebrafish undergoes fin transformation, melanocyte development, and body morphology changes while tripling in size (Kimmel et al., 1995).

Skin pigmentation begins to develop during the larval stage at the 24 post-fertilization hour mark, where melanocytes travel from the neural crest to its skin (D'Costa and Shepherd, 2009). Melanocytes emerge from small amounts of bipotent melanogenic progenitors derived from the neural crest. The first stage of melanocyte development in zebrafish larva gives rise to four stripes: the dorsal, lateral, ventral, and yolk sac (Mort et al., 2015). The number of melanocytes in zebrafish larvae significantly increases during the development stage, making it an ideal system to see the effects of tyrosinase inhibitors (Compound P) on melanocyte production to further investigate the factors that affect production of melanin.

Tyrosinase is a key enzyme that catalyzes a rate-limiting step of melanogenesis, completed with other tyrosinase-related proteins: tyrosinase-related proteins-1 and tyrosinase-related proteins-2 (Niu and Aisa, 2017). When these tyrosinase activities are suppressed by inhibitors, melanin synthesis is reduced. In a 2011 study, they found that phenylthiourea, a type of tyrosinase inhibitor, was linked to the reduction of zebrafish development. Phenylthiourea contains a thiocarbamide group that suppresses thyroid hormone production, resulting in reduced eye size (Li et al., 2012). Based on these instances, it may indicate that Compound P may have abilities to reduce larval length and yolk sac size.

To understand how these complex mechanisms work, this study aims to explore the effects of Compound P, a tyrosinase inhibitor, in development and synthesis of melanocytes in zebrafish larvae. Effects of tyrosinase inhibitors on larval length and yolk sac have not been previously studied. However, based on the background information, it can be hypothesized that

Compound P will decrease melanocyte count, percent melanin concentration, larval length, and yolk sac size in zebrafish larvae, compared to zebrafish that are not treated with Compound P.

Results

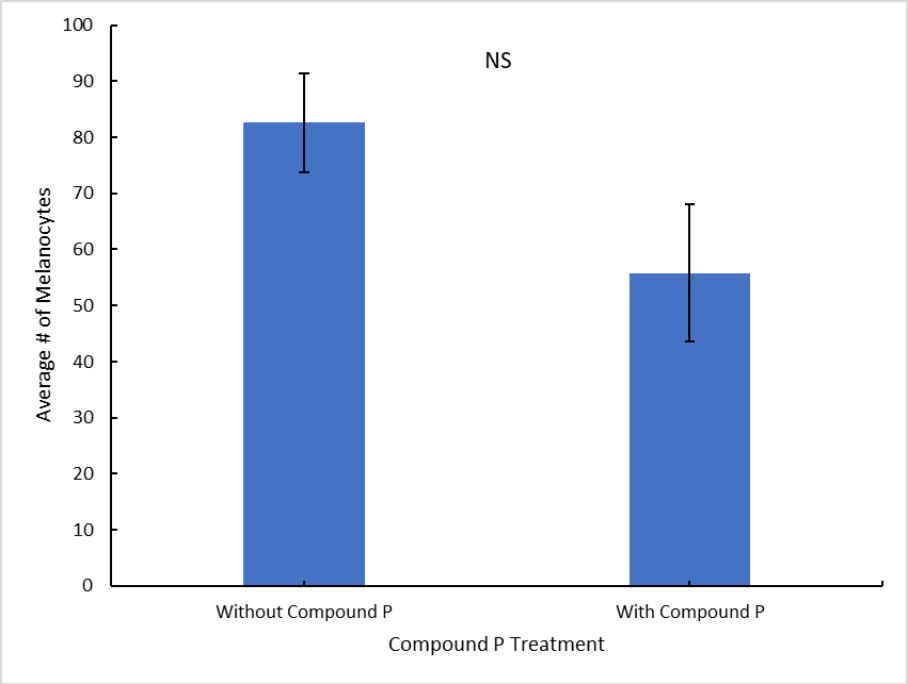

Figure 1. Average count of melanocytes for zebrafish larvae after treatment with or without Compound P: Zebrafish larvae (n= 5 per treatment, per week) were used to count the average number of melanocytes for zebrafish larvae with and without Compound P treatment (82.6 $\pm$ 8.76, 55.8 $\pm$ 12.2) using a compound microscope. The p value was > 0.05, represented with NS (not significant) between the average number of melanocytes of zebrafish larvae that were treated with and without Compound P, and error bars represent standard error of the data set.

The average count of melanocytes for zebrafish larvae treated with Compound P was 82.6 $\pm$ 8.76, while 55.8 $\pm$ 12.2 for the control zebrafish larvae group (Figure 1). The average number of melanocytes of zebrafish larvae treated with and without Compound P was statistically insignificant (p= 0.0919, unpaired t-test). Based on the p value, the hypothesis that Compound P will decrease melanocyte count was not supported.

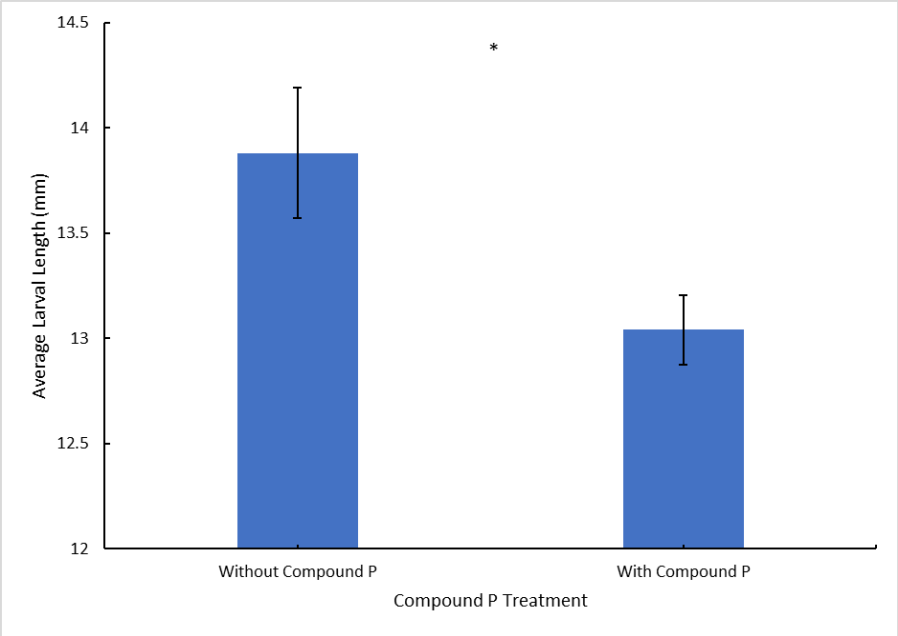

Figure 2. Average larval length (mm) of zebrafish larvae after treatment with or without Compound P: Zebrafish larval length (mm; 13.9 ± 0.310, 13.0 ± 0.165) was measured using images captured from a compound microscope onto ImageJ of zebrafish larvae (n= 5 per treatment, per week) with and without Compound P treatment. The single asterisk (*) indicates statistical significance, a p-value <0.05, between the average larval length between zebrafish larvae that were treated with and without Compound P, and error bars represent standard error of the data set.

The average larval length of zebrafish larvae after Compound P treatment was 13.9 ± 0.310 mm, and the average larval length for zebrafish that were not treated with Compound P was 13.0 ± 0.165 mm (Figure 2). The average larval length between zebrafish larvae treated with and without Compound P was statistically significant (p= 0.0281, unpaired t-test). Because the p value was statistically significant, the hypothesis that Compound P will decrease larval length compared to untreated zebrafish larvae was supported.

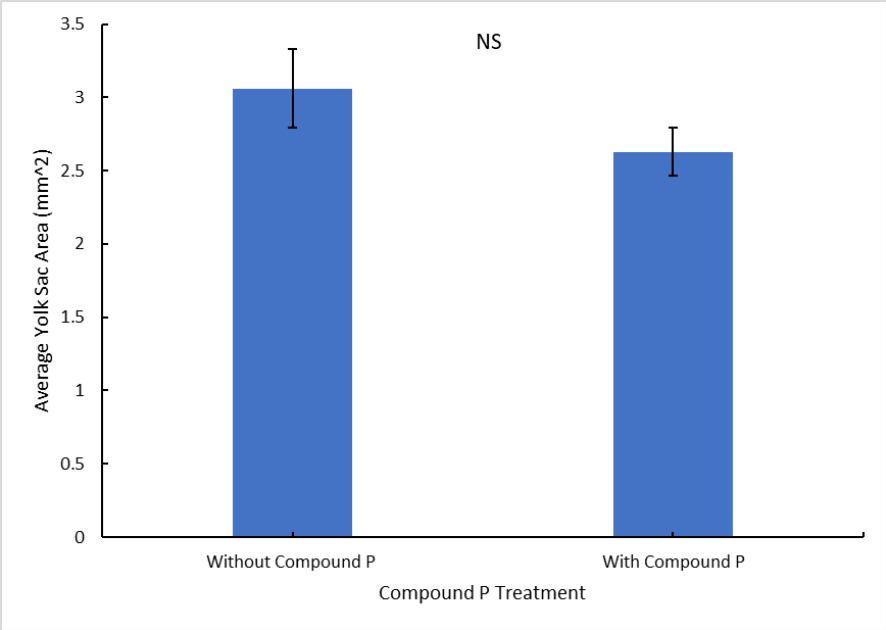

Figure 3. Average yolk sac area (mm^2) of zebrafish larvae after treatment with or without Compound P: The average yolk sac area of zebrafish larvae (mm^2; 3.06 $\pm$ 0.266, 2.63 $\pm$ 0.162) was measured using images captured from a compound microscope onto ImageJ of zebrafish larvae (n= 5 per treatment, per week) with and without Compound P treatment. The p value was > 0.05, represented with NS (not significance) between zebrafish larvae treated with and without Compound P, and error bars represent standard error of the data set.

The average yolk sac area (mm^2) for zebrafish larvae treated with Compound P was 3.06 $\pm$ 0.266 mm^2, and those that were not treated with Compound P had an average of 2.63 $\pm$ 0.162 mm^2 (Figure 3). Between these two groups, the averages were statistically insignificant (p= 0.186, unpaired t-test). Since the p value was statistically insignificant, the hypothesis that Compound P will decrease yolk sac area compared to untreated zebrafish was not supported.

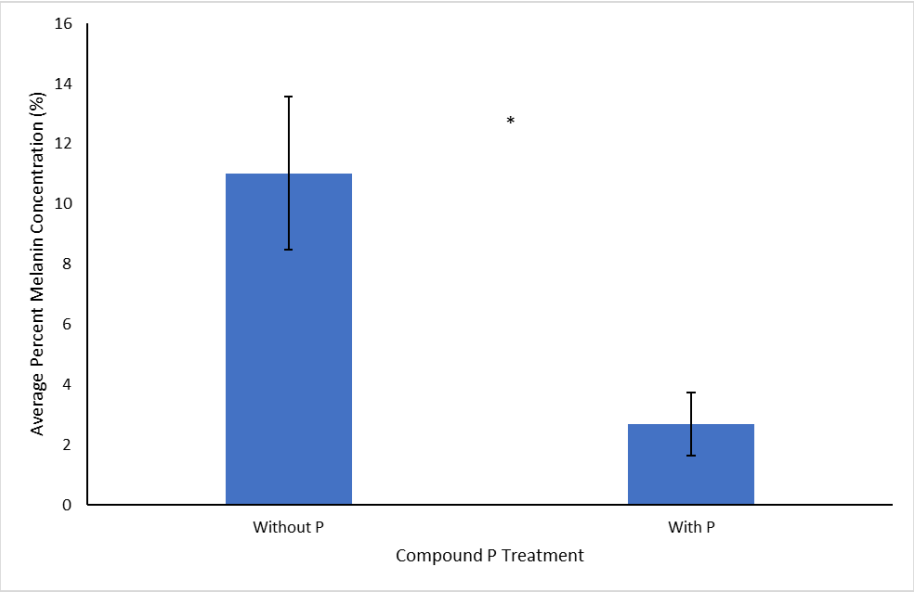

Figure 4. Average percent melanin concentration (%) in zebrafish larvae with and without Compound P treatment: Zebrafish larvae (n= 10, per treatment) were used to calculate the average percent melanin concentration with and without Compound P treatment (11.02 $\pm$ 2.55, 2.68 $\pm$ 1.06); data sets were collected by 5 different groups to calculate the average percent melanin concentration. Error bars represent standard error of the data set, and the single asterisk indicates statistical significance ($p < 0.05$) between the percent melanin concentration values of zebrafish larvae with Compound P treatment versus without P treatment.

The average percent melanin concentration for larvae treated with Compound P was 11.02 $\pm$ 2.55% while the average without treatment was 2.68 $\pm$ 1.06% (Figure 4).. The average percent melanin concentration between zebrafish larvae with and without Compound P treatment was statistically significant ($p= 0.0164$, unpaired t-test). The hypothesis was that the percent melanin concentration was supported because the p value was statistically significant.

Discussion

The average melanocyte count experiment showed that untreated zebrafish larvae did not have a significant difference between treated zebrafish larvae, which did not support the hypothesis. The average larval length with Compound P treatment had significant differences compared to zebrafish larvae without the treatment, which supported the hypothesis. However, the average yolk sac area treated zebrafish larvae did not have a significant difference compared

to untreated larvae, thus not supporting the hypothesis. Finally, the average percent melanin concentration for Compound P-treated larvae was less than untreated larvae, supporting the hypothesis of Compound P decreasing melanin concentration compared to untreated zebrafish larvae.

It was expected that the development of zebrafish larvae would decrease due to tyrosinase inhibitors. 1-phenyl 2-thiourea (PTU) is a tyrosinase inhibitor that has shown to block skin pigmentation of zebrafish. 0.03% concentration of PTU resulted in the inhibition of cranial neural crest development (Bohnsack, 2011). With the decrease in cranial neural crest development, this may decrease larval length. This suggests that Compound P may potentially reduce larval length. In addition, the percent melanin concentration declined after Compound P treatment. This is supported by a study where tyrosinase inhibitor, 4-(6-hydroxy-2-naphthyl)-1,3-benzenediol (HNB), reduced melanin content from 100% to 35% at 100 μM concentrations (Ha et al., 2007). This further illustrates that tyrosinase inhibitors have the ability to decrease melanin concentration.

On the contrary, average melanocyte count and yolk sac area experiments were unexpected results. The average melanocyte count was unexpected since the melanin concentration yielded significantly different results. However, this may be supported by melanocytes arising from bipotent melanogenic progenitors from neural crest cells (Richard et al., 2015). The body structures of zebrafish larvae with Compound P treatment remained normal, with minimal structural distortions. This may indicate that the neural crest cells were developed normally under the treatment of Compound P, which produced normal melanocytes counts. However, due to the inhibition of tyrosinase, melanin production is decreased. In addition, the yolk sac area did not have significantly different results compared to the untreated controls.

Tyrosinase inhibitors may not have a direct effect on the development of the yolk sac area. In previous studies mentioned, phenylthiourea treatment reduced the eye size and cranial neural crest development (Li et al., 2012 and Bohnsack et al., 2011). However, there were no other studies that resulted in reduced bodily structures. Thus, the inhibition of tyrosinase may affect the development of the anterior region but not specifically the torso segment.

It is critical to note that this study has several limitations. First, the sample size was somewhat small (n=10 to 20 zebrafish larvae), which would have reduced its statistical ability to identify important variations in some parameters. For example, the research study conducted by Li et al., each group (treated and untreated zebrafish embryos) had 343 zebrafish embryos. Future research might benefit from expanding their sample sizes and number of trials to produce more trustworthy results. In addition, there was a lack of previous research on effects of tyrosinase inhibitors on larval length and yolk sac size. Due to this, it was difficult to conclude on whether the results yielded were reasonable.

Further research is needed to accurately determine the impact of Compound P on skin pigmentation, including melanocyte count, melanin production, larval length, and yolk sac area. This study has altered the understanding of how tyrosinase inhibitors affect zebrafish larvae development, revealing their inhibitory effect on larval length. Future investigations could involve a larger sample size of zebrafish larvae and varying concentrations of Compound P to explore its influence on melanin production and development in greater detail.

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