THE EFFECT OF THIOSTREPTON ON THE REGENERATION OF *S. MEDITERRENIA* PLANARIA WORMS

A Report of a Senior Study

by

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ABSTRACT

Planaria are widely used in regeneration studies due to their ability to regrow complete tissues after injury. Thiostrepton, a natural thiopeptide antibiotic, inhibits protein synthesis by binding to ribosomal RNA and blocking elongation factors, which suppresses cell growth. This study investigated the effects of time and thiostrepton concentration on planarian regeneration. Planaria were cut into head and tail sections and exposed to different concentrations of thiostrepton (0, 0.01 M, 0.015 M, 0.018 M) over 25 days, with body measurements recorded to assess regeneration. Statistical analyses, including ANOVA and MANOVA, were used to compare growth across time points and concentrations. Both head and tail lengths increased significantly over time ($p = 0.027$ and $p = 0.006$, respectively), indicating proper regeneration, while concentration had no significant effect ($p = 0.94$ and $p = 0.14$, respectively). No significant interaction between time and concentration was observed ($p = 0.70$ and $p = 0.75$, respectively). Width measurements remained consistent across heads and tails with no significant differences between concentrations, and histological analysis revealed no abnormalities in blastema growth. Overall, time had a stronger influence on regeneration than concentration, rejecting the hypothesis that thiostrepton concentration would have a greater effect. These findings suggest that planarian regeneration is primarily time-dependent, offering insight into the mechanisms influencing tissue growth and recovery.

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CHAPTER I

INTRODUCTION

Regeneration is a process observed in many organisms and has great potential in human medicine (see Table 1). Some of the most promising applications are regeneration of neuron function, particularly in Parkinson's disease (PD) and Alzheimer's. Current PD treatments don't stop the progression of the disease, so scientists are looking toward regenerative possibilities such as stem cell and gene therapy. Stem cell therapy involves the transplantation of mesenchymal stem cells that help preserve dopaminergic neurons (Yasuhara 2015). There is other research using different types of stem cells, such as neural or pluripotent stem cells (Yasuhara 2015).

Gene therapy focuses on transplanting specific genes to increase local dopamine concentration. Overall, these treatments are more promising for younger PD patients due to the more powerful benefits, but they are expected to still provide therapeutic effects for advanced-stage patients (Yasuhara 2015). Alzheimer's research is also looking at the possibility of stem cell transplantation as a treatment. New research shows that microglial dysfunction may be causing neurodegeneration, so scientists are now looking at restoring microglial function as a possibility of fighting the neurodegeneration (Liu 2020). Embryonic stem cells show improved special learning and memory in rats with Alzheimer's, but the

Table 1. Uses of Regeneration in Human Medicine.

Target	Therapy Type	Mechanism	Source
Cardiac	1. Stimulating Cardiac Myocyte Proliferation 2. Cardiac Cell Therapy	1. Modulating Meis1, activating YAP in Hippo-YAP, increasing Cyclin A2, lowering CDK inhibitors, and using microRNAs 2. c-kit cardiac stem cells enhance LV function and reduce infarct size and scarring. Embryonic stems cells regenerate tissue	Zhang 2015
Liver	Natural Regenerative Properties	Injury triggers cytoprotection, cell deletion, proliferation, matrix deposition, and tissue remodeling to restore hepatic structure and function	Diehl 2002
Musculoskeletal	Looking at Zebrafish mechanisms to find direction for therapy research	Muscle injury: inflammation clears damaged tissue. Muscles stem cells proliferate and differentiate Bone injury: blastema forms with progenitor cells expressing osteoblast markers to regenerate bone	Kaliya-Perumal 2022
Central Nervous System (CNS) Disorders	Cell-based Therapy with stem cells	Neural stem/progenitor cells are injected as a stem cell transplantation and differentiate to repair or replace deficient aspects of the CNS disorder	Ghosh 2016

differentiation of embryonic stem cells can cause tumors or teratomas (Liu 2020) Mesenchymal stem cells can be regulated to overexpress cytokines and vascular endothelial growth factors and show regenerative effects (Liu 2020).

Mesenchymal cells are the most common source of stem cells used in Alzheimer's research and because they are easy to select and handle after harvesting. Mesenchymal stem cells have been successfully injected into the brains of nine human patients with no serious adverse effects in the 24-month follow-up period (Kim 2015).

The extent of regeneration varies among organisms, ranging from whole-body regeneration to the regrowth of a single limb or organ. Whole-body regeneration is most associated with asexual reproduction by fission. In whole-body regeneration, there is a continuous flow of undifferentiated stem cells to body parts, following an underlying polarity control that ensures proper orientation, such as distinguishing the head from the tail. Bidirectional regeneration is not observed in vertebrates or insects; however, monodirectional regeneration can occur in appendages. Among insects, only species with a gradual larval progression can regenerate external appendages. When an appendage is lost, it regrows and becomes visible after the next proximal molt. In vertebrates, regeneration is primarily found in amphibians, specifically in urodeles and anurans, in the form of limb regeneration. Many urodele species can regenerate limbs throughout both larval stages and adulthood, whereas anurans can only regenerate limbs before metamorphosis (Goss 1969, Scadding 1977).

The regeneration process is generally consistent across different types of limb regeneration. In the past, the two distinct types of limb regeneration were classified: epimorphic or morphallactic (see Agata 2007). Epimorphic regeneration included the

formation of a blastema from which the new limb would grow. Morphallaxis, seen in hydras, doesn't form a blastema, and instead, the remaining part is remodeled to regenerate different parts of the body. A blastema occurs when a cluster of loosely packed mesenchymal cells forms and expands at the cut site. It is often recognized as a white region that forms over the cut site. Immediately after amputation in epimorphic regeneration, epidermal cells migrate to the cut surface to form a wound epithelium. The internal tissues, up to 1 mm from the cut, dedifferentiate to generate stem-like cells that can later serve various roles. If a blastema is present, limb structures are redifferentiated using the stem-like cells in a proximal-to-distal sequence. This process creates a miniature version of the regenerating limb, which continues to grow and develop over time. Fibroblast growth factors (FGFs) are expressed in the blastema cap and play a crucial role in promoting growth. Additionally, the nerve supply to the injury site is essential for successful regeneration. The blastema may form without proper innervation, but it will not grow or regenerate the missing limb (Iten 1973).

The traditional concepts of epimorphic regeneration and morphallaxis often categorize regenerative processes in rigid ways that do not account for all organisms. A more recent perspective introduces the principles of distalization and intercalation, which emphasize the regulation of positional information during healing rather than strict classifications (Agata 2007). Distalization occurs when the distal portion of a structure forms immediately after wound healing, while intercalation involves the interaction between the newly formed distal portion and the remaining proximal portion to reorganize positional information, ultimately regenerating the original structure. These principles shift the focus from the presence of a blastema, whose appearance can be difficult to define, to the way tissues interact during regeneration. Although there may be some overlap in how positional

information is reorganized, the mechanisms governing distalization and intercalation vary significantly across different organisms (Agata 2007).

Planaria Regeneration

In planaria specifically, there is cell division for both wounds and limb loss. There are two different types of responses: a generic wound response for an injury and a targeted regenerative response for a missing tissue. During injury, neoblasts rapidly proliferate at the wound site. Neoblasts are the adult stem cells in planaria that are differentiated to form other cells. They are the only actively dividing somatic cells and are located all over the body except in the pharynx and in front of the photoreceptors. The signals that cause the mass proliferation are from a signal on the G2/M progression during mitosis which results in the shortening of the G2 phase. The neoblasts cover the wound site and replace lost cells (Wenemoser 2010). If tissue is missing, a second wave of neoblast activity occurs, directing cells to differentiate into the appropriate cell types for reconstruction. This process relies on positional control genes that define the anterior-posterior and dorsal-ventral axes, ensuring that the regenerated tissue forms correctly (Petersen & Reddien, 2009). A thin layer of epidermal cells rapidly moves to cover the wound site and a blastema begins to form. The neoblasts in the blastema work to recover and rebuild the missing limb through differentiation in morphogenesis and angiogenesis.

Planaria regeneration was originally believed to be an epimorphic regeneration due to the lost tissues being formed from the blastema. Upon further study, it was found that the neoblasts are committed in a position-dependent manner before migrating to their final target position (Agata 2003). Thus, planaria regeneration is now categorized as intercalary regeneration (regeneration between existing tissues). The anterior and posterior blastemas are

formed as signaling centers to direct intercalary reorganization of body position. This allows proper integration of the new tissue with pre-existing tissues.

Thiostrepton Background

Thiostrepton is a natural macrocyclic thiopeptide antibiotic derived from bacteria of the *Streptomyces* genus. This genus of bacteria is gram-positive, which makes it highly effective in combating gram-negative bacteria. Thiostrepton is widely used in veterinary medicine, particularly as a topical ointment for treating mastitis in animals. In humans, thiostrepton has shown promising potential as a cancer treatment, inducing apoptosis specifically in different carcinoma cases (Donovick 1955, Kim 2019). However, thiostrepton has poor solubility and bioavailability, making it difficult to use as a primary treatment option. As a result, cheaper or more widely available medications are often preferred due to their better absorption and ease of administration.

Thiostrepton exerts its effects by targeting the process of translation to inhibit cell growth. It achieves this by binding to functionally critical regions of ribosomal RNA (rRNA) within the GTPase-associated center, as seen in figure 1. This binding results in the potent inhibition of elongation factor G (EF-G) and elongation factor 4 (EF4), essential for proper protein synthesis. Notably, experiments involving the GTPase activity of EF-G and EF4 in the presence of 70S ribosomes and 10 μM thiostrepton revealed a significant reduction in the release of hydrolyzed $^{32}P_i$. This observation strongly suggests that thiostrepton effectively inhibits ribosomal activity. Moreover, the presence of thiostrepton prevents EF-G•GDPNP and EF4•GDPNP from stably binding to the 70S ribosomal subunit (Walter 2012). The inability of elongation factors and nucleotides to bind to the ribosome disrupts the protein

synthesis essential for translation. This disruption leads to extensive cell death, effectively preventing the uncontrolled growth associated with mastitis or cancerous tumors.

Overall, thiostrepton primarily targets GTPase activity through different mechanisms, depending on the ribosomal subunits involved. The two main modes of inhibition include preventing the proper binding of factors on the ribosomal surface and impairing the coupling of ribosomal subunits (Naaktgeboren 1976). Additionally, thiostrepton lowers the affinity of the 50S ribosomal subunit for the 30S initiation complex, which further contributes to its inhibitory effects. When reacting with the 50S subunit, thiostrepton blocks the active site, completely halting its activity. Similarly, it disrupts the coupling between the 30S initiation complex and the 50S ribosomal subunit. This blockage inhibits GTP hydrolysis on the ribosome. Without GTP, ribosomal complexes are unable to continue protein synthesis, which ultimately compromises cell survival (Naaktgeboren 1976).

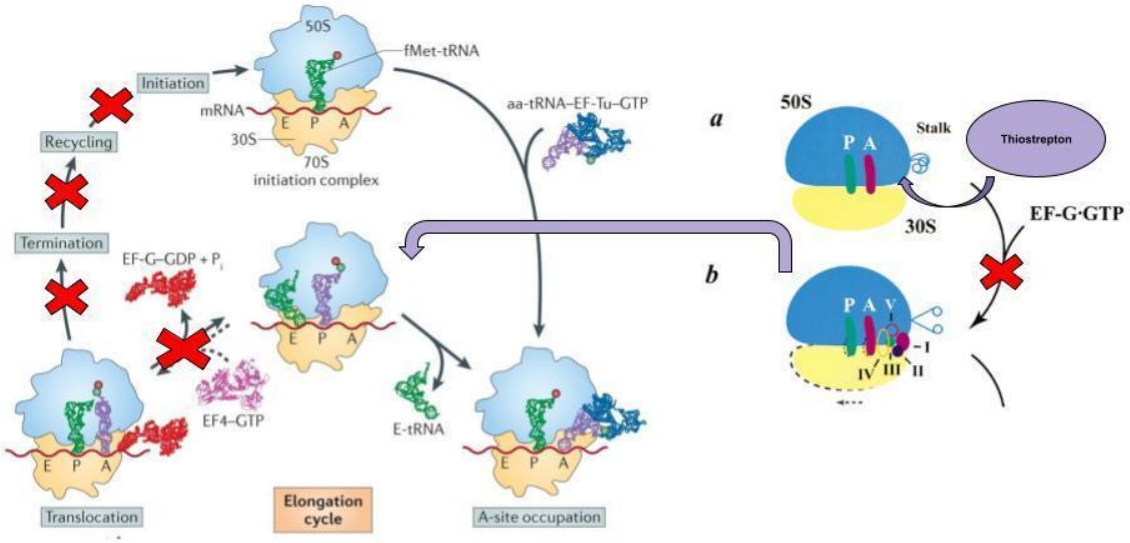

Figure 1. Model of the thiostrepton mechanism while binding to the GTPase. Adapted from Yamamoto 2014 and Agrawal 2014.

Thiostrepton is also emerging as a potential medicine for tumor suppression. A common tumor suppressor protein, p53, typically drives cells into cell cycle arrest, DNA repair, or apoptosis in response to cellular dysregulation. However, mutations in this protein have been found to cause approximately 50% of cancers. p53 negatively regulates the expression of molecules associated with cellular proliferation, such as forkhead box protein M1 (FOXM1) (Kalathil 2018, Su 2024).

Many experiments have looked at the expression of ubiquitin (Ub)—a protein that attaches to other proteins to regulate their stability, activity, and localization—and p53 in the presence of thiostrepton in SW480 cells. The results of the thiostrepton treatment showed an accumulation of Ub molecules and a reduction in p53 proteins. The overall destabilization of p53 caused by thiostrepton treatment varies depending on the mutational status of p53 and the type of mutation present. The downregulation of p53 mRNA occurs at the transcriptional level rather than the translational level, yet proteolysis is still observed. Thiostrepton downregulates mutant p53 by facilitating the conversion of Bcl-2-associated athanogene 1 (BAG1) to Bcl-2-associated athanogene 3 (BAG3), which mediates autophagy. The BAG1-to-BAG3 switching is a hallmark of proteasome inhibition, and thiostrepton accelerates this process (Kalathil 2018, Luo 2023). This switching induces autophagy in mutated p53, which helps slow tumor growth. These findings highlight the potential of thiostrepton as a drug for tumor suppression.

Purpose of Study

The use of thiostrepton as a tumor suppressant in medicine may provide valuable insight into the mechanisms of regeneration suppression in planaria. If more concentrated thiostrepton is added to the planaria tanks, the blastema growth is predicted to lessen or halt altogether. If thiostrepton halts regeneration in planaria, the exact sites and processes involved in tissue regrowth can be pinpointed. These findings could then be compared to tumor growth mechanisms in various types of cancer. This approach could lead to the development of more precise chemotherapy treatments, targeting specific pathways involved in tumor formation rather than indiscriminately attacking rapidly dividing cells—a strategy that often results in severe side effects.

CHAPTER II

METHODOLOGY

Brown planaria were sourced from Carolina Biological Supply (Burlington, NC). Thiostrepton was purchased from MedChemExpress (Monmouth Junction, NJ). Planaria were stored in a 10°C refrigerator from delivery until use. Spring water was collected from the Maryville College Woods Springhouse. Sixteen petri dishes were filled with 20 mL of spring water, and 5 planaria were placed in each petri dish. Each petri dish got a pinch of frog food mixture for 24 hours. After 24 hours, the planaria from each dish were put into a clean dish with fresh spring water while the original dish was cleaned and the water was replaced. After refreshing the water of each dish, they were arranged in 4 rows of 4 to symbolize the 4 trials of 4 concentrations of thiostrepton as seen in Figure 2.

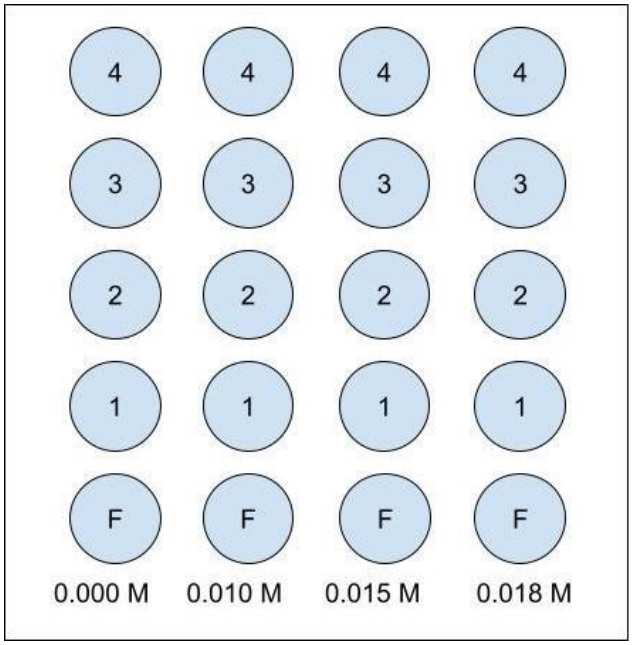

Figure 2. Organization of Petri dishes for planaria housing and feeding. 16 plates were arranged in 4 rows of 4 for 4 trials and a feeding plate. 5 planaria were placed in dishes 1-4 in 20 mL of spring water. 20 µL of the corresponding concentrations of thiostrepton solution (seen under each row) was added to each dish. For feeding, a plate was added to each row (labeled F). Planaria were added to the plate, then returned to their original plates, one trial at a time, with each feeding cycle

Thiostrepton was prepared by combining 0.0302 g of powder thiostrepton with 1 mL of n-n,dimethylformamide (DMF) to create a concentration of 0.018M. The stock solution was diluted with DMF to create the other concentrations. Two hundred microliters of stock plus 0.04 mL of DMF created a concentration of 0.015 M. 0.2 mL of stock plus 0.16 mL of DMF created a concentration of 0.010 M. Each dish was labeled "LNP Thesis [], trial #" (ex. LNP Thesis 0.018 M 3). The planaria in each dish were cut in half using a scalpel blade, and 20 µL of the corresponding concentration was added to each dish. After 84 hours, the planaria were fed again using egg yolk. To do this, 4 new petri dishes were assigned as feeding dishes to avoid concentration contamination. One plate at a time, the planaria were transferred from their original plate to the feeding plate containing fresh spring water and a pinch of cooked egg yolk using a transfer pipette. The planaria were left to

feed for 2 hours before being transferred back to their original plate. This process was repeated 4 times for each trial. After feeding, 10 μL of the corresponding concentration of thiostrepton solution was added to each plate to refresh the drug. After 72 hours, one head and tail of a planaria from each plate were removed and preserved in Bouin's Fixative (Ricca Chemical Company, Arlington, Tx) for use in histology later. The Bouin's Fixative was cleared using 70% ethanol. The preserved worms were viewed under a dissection scope (7x), and the length and width of each were recorded. A photo was taken of each planaria removed. The feeding process with egg yolk was repeated, and pictures were taken of each plate. This process was repeated following Table 2.

Table 2. Schedule of planaria care throughout the experiment.

	Sun	Mon	Tues	Wednesday	Thurs	Fri	Sat
Week 1				**Starting Day:** Prepare petri dishes, cut planaria, add planaria and thiostrepton solution			
Week 2	Feed			Feed, change water, preserve 1 head and 1 tail from each trial, take pictures, add 10 μL of Thiostrepton solution			
Week 3	Feed			Feed, change water, preserve 1 head and 1 tail from each trial, take pictures			
Week 4	Preserve the remaining planaria						

After the planaria were fixed for 7 days (after the last planaria were taken for preservation), the samples were cleared using 70% ethanol. The samples were cleared 2x a day for 7 days. After clearing, the length and width of each sample were measured under a dissection microscope (7x). One sample from each preservation (head or tail, concentration, day) was set aside for histology. The samples were placed in cassettes with ChemWipes to prevent loss of the sample during the histology process. The histology process outlined in the *Animal Tissue* Techniques was followed to preserve the samples (Presnell and Schreibman 1977). After histology, the wax blocks were cut into 12 μm sections and placed on slides. The slides were stained using Hematoxylin, and they were counterstained using Eosin. Coverslips were applied using Permount. The slides were viewed under a microscope at 40x-400x.

R Studio was used to perform a two-way ANOVA, a MANOVA, and an ANCOVA for each data set (planarian heads, planarian tails, and blastema width). The two-way ANOVA tested the independent and combined effects of time (day) and thiostrepton concentration on single dependent variables, such as head length or tail length. This test was used to see if growth changed over time or across concentrations, and whether the two factors interacted. The MANOVA included two dependent variables (head length and head width) to examine the overall effects of time, concentration, and their interaction. This test was chosen because head length and width are related measures of regeneration and analyzing them together helps account for their correlation. The ANCOVA was used for the blastema data to compare growth across concentrations while controlling for time. This allowed the effect of concentration to be tested more accurately by adjusting for the influence of time on blastema development. The code for the statistical analysis was written using ChatGPT.

CHAPTER III

RESULTS

The head and tail length in the 0M concentration increased over time (slope=0.0038, slope=0.0404, respectively). The head and tail width in the 0M concentration had a negative slope (slope=-0.0068, slope=-0.0138). The head length in the 0.01M concentration had a positive slope (slope=0.0113), whereas the head width, the tail length, and the tail width had a negative slope (slope=-0.008, slope=-0.0076, slope=-0.0095, respectively). The head length, head width, and tail width in the 0.015 M concentration increased over time (slope=0.026, slope=0.0033, slope=0.0076, respectively), and the tail length had an overall negative slope (slope=-0.0252). The head length in the 0.018M concentration increased over time (slope=0.0519), and the head width, tail length, and tail width had a negative slope (slope=-0.0062, slope=-0.0202, slope=-0.0506, respectively). The linear trendlines are shown in Figures 3-6 (Raw measurements can be found in Appendix I).

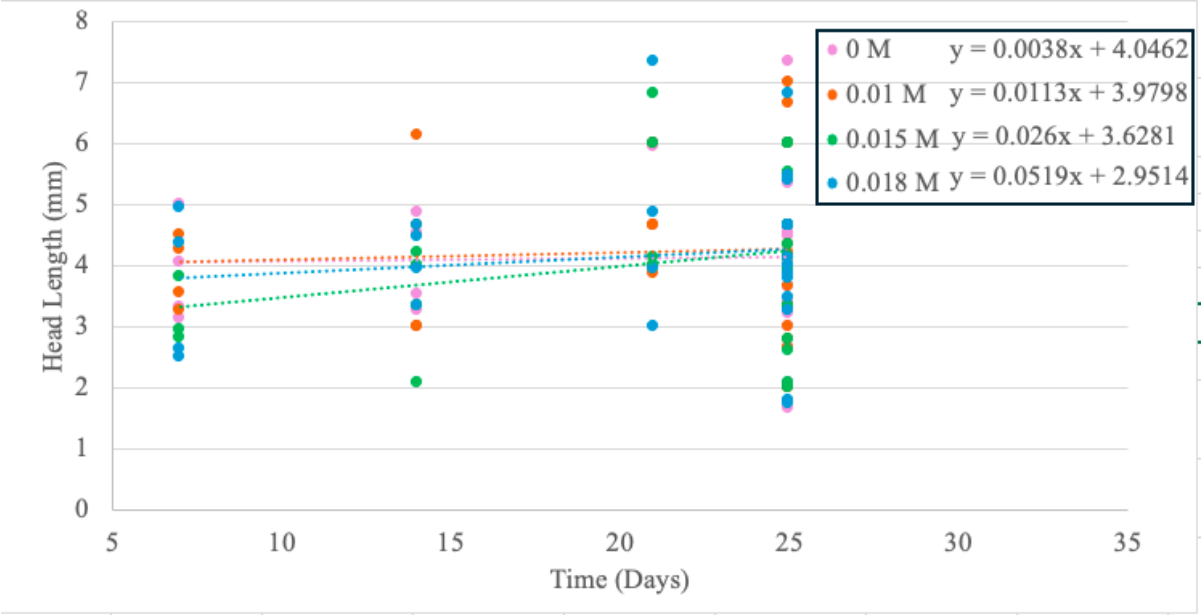

Figure 3. Effect of time on the head lengths of planaria in different concentrations of thiostrepton. Planaria worms were introduced to different concentrations (0M, 0.01M, 0.015 M, 0.018 M) of thiostrepton for 25 days. Linear trendlines are added for each concentration group, and the equations are shown.

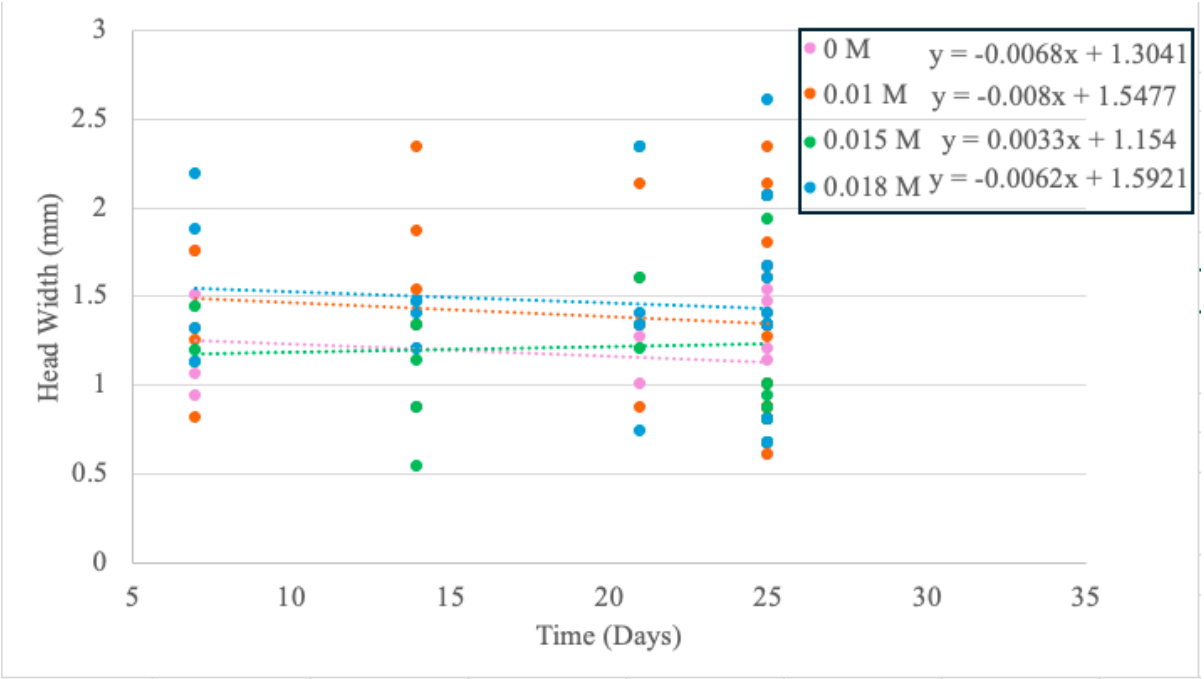

Figure 4. Effect of time on the head widths of planaria in different concentrations of thiostrepton. Planaria worms were introduced to different concentrations (0M, 0.01M, 0.015 M, 0.018 M) of thiostrepton for 25 days. Linear trendlines were added for each concentration group, and the equations were shown.

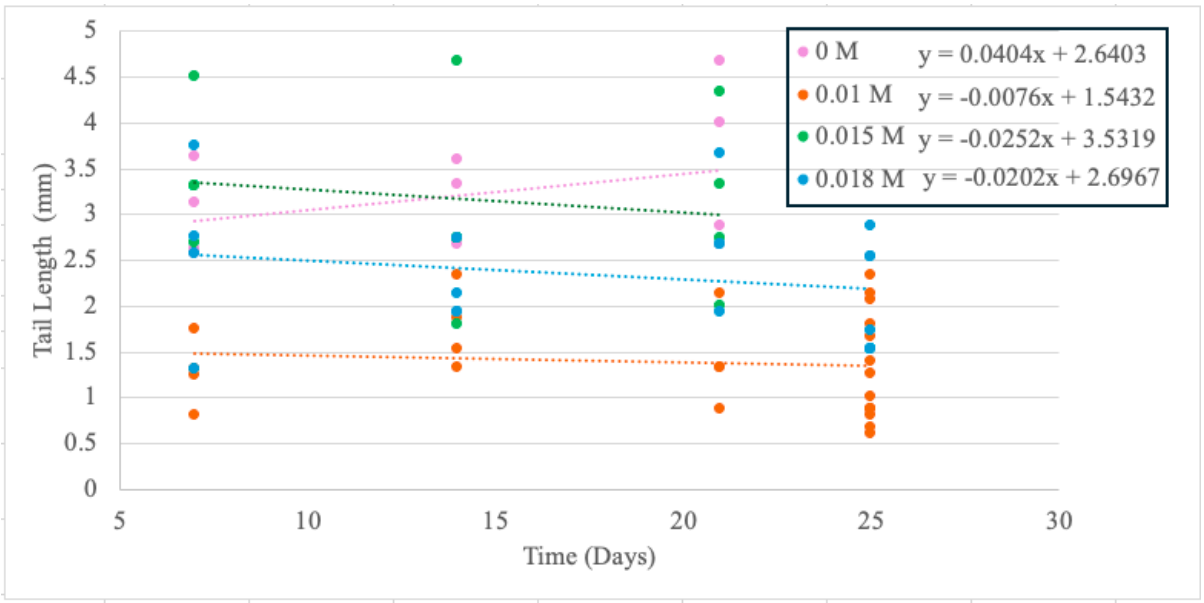

Figure 5. Effect of time on the tail lengths of planaria in different concentrations of thiostrepton. Planaria worms were introduced to different concentrations (0M, 0.01M, 0.015 M, 0.018 M) of thiostrepton for 25 days. Linear trendlines were added for each concentration group, and the equations were shown.

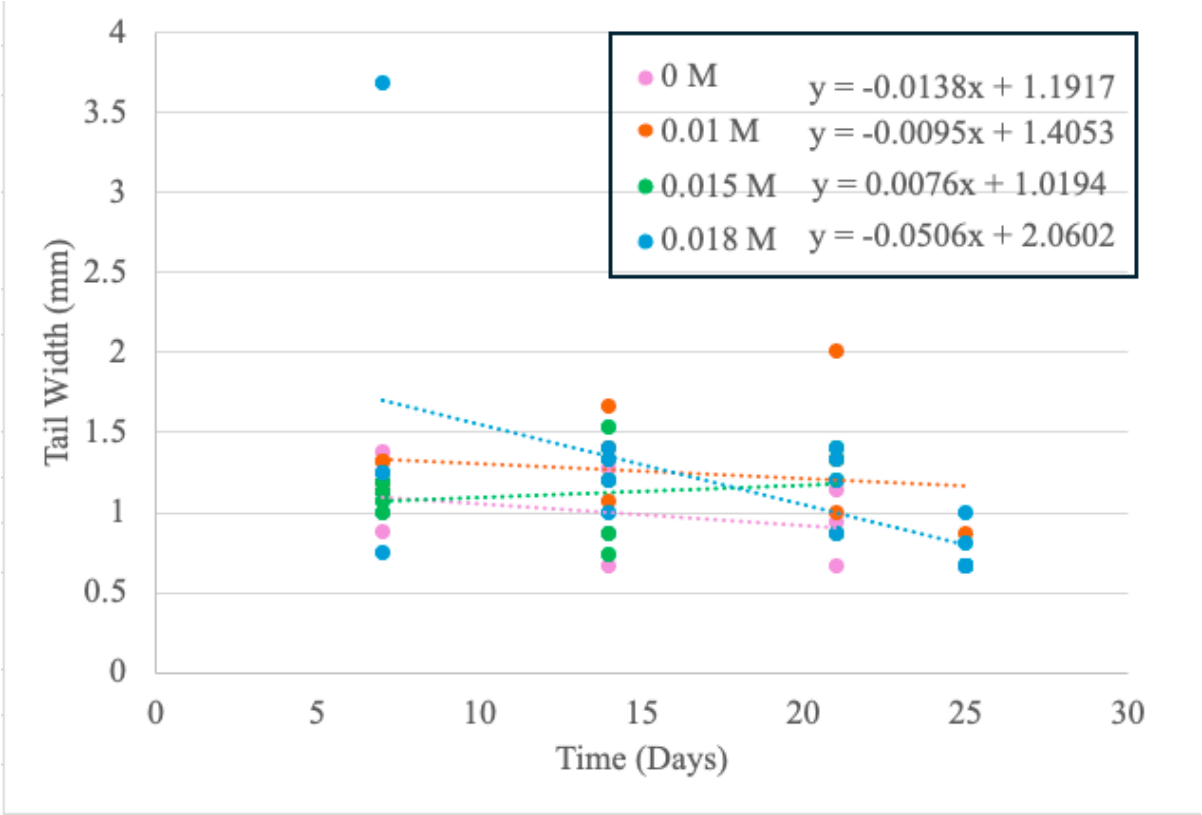

Figure 6. Effect of time on the tail widths of planaria in different concentrations of thiostrepton. Planaria worms were introduced to different concentrations (0M, 0.01M, 0.015 M, 0.018 M) of thiostrepton for 25 days. Linear trendlines were added for each concentration group, and the equations were shown.

When considering the effect of time (days), the change in head length was statistically significant (p=0.027). When considering the concentration effect on head lengths, it was not statistically significant (p=0.94). The interaction between the day and concentration of the head sizes was also not statistically significant (p=0.99). When considering the effect of time, concentration, and the interactions between the two, the change in head width was not statistically significant (p=0.54, p=0.061, p=0.61, respectively). When considering the effect of time and concentration on the length and width of the heads together, it was overall significant (p=0.017, p=0.0058), but the interaction was not significant (p=0.70).

When considering the effect of time, the change in tail length was statistically significant (p=0.006). The effect of concentration and the interaction (Day x Concentration) on the change in tail length were not significant (p=0.14, p=0.75, respectively). When considering the effect of time, the change in tail width was significant (p=0.023). The effect of concentration and the interaction were not significant (p=0.089).

The blastema measurements showed no statistical significance across day, concentration, or interaction (p=0.16, p=0.50, p=0.12, respectively). Images of the histology samples are shown in Figure 7.

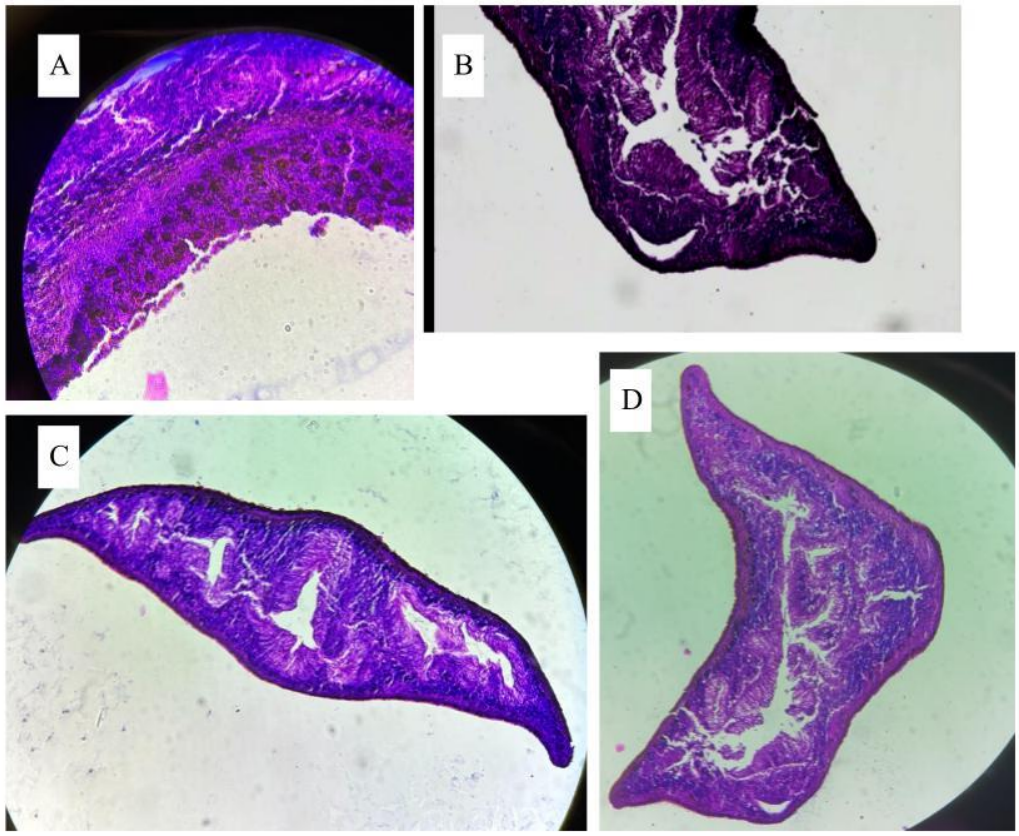

Figure 7. Planaria histology samples viewed under a microscope. A) 0.01 M tail sample from day 21 viewed at 400x. B) 0.0 M tail sample from day 7 viewed at 100x. C) 0.018 sample from day 25 viewed at 100 x. D) 0.015 M head sample from day 7.

CHAPTER IV

DISCUSSION

The results indicate that thiostrepton had no significant effect on planarian regeneration. Both head and tail growth increased over time, while concentration and interaction effects were minimal. These findings contrast with previous studies showing that thiostrepton can inhibit protein synthesis and induce apoptosis in other cell types, particularly in bacteria and cancerous tissues.

Although thiostrepton effectively inhibits protein synthesis in prokaryotic and certain eukaryotic cancer cells, the results of this study suggest that it did not inhibit regeneration in planaria. This outcome may be explained by several biochemical and experimental factors. Thiostrepton has poor solubility and limited bioavailability, which can reduce its uptake and activity in aqueous environments (Kim 2019). Due to the planaria being maintained in water, thiostrepton likely exhibited reduced stability and incomplete absorption. Additionally, thiostrepton is typically dissolved in dimethylformamide (DMF), a solvent that may separate when mixed with water. This separation could have prevented consistent delivery of the compound to planarian tissues, further reducing its efficacy.

At the molecular level, thiostrepton's inhibitory mechanism primarily targets prokaryotic ribosomes, where it binds to the GTPase-associated center of the 23S rRNA on

the 50S ribosomal subunit. This interaction blocks the binding and activity of elongation factors EF-G and EF4, thereby halting protein synthesis (Walter 2012; Naaktgeboren 1976). However, planarian cells contain eukaryotic ribosomes, which differ structurally from bacterial ribosomes. As a result, thiostrepton may not have effectively interacted with planarian ribosomal RNA or elongation factors, limiting its ability to disrupt protein translation in this system. The absence of significant effects on head and tail regeneration, as well as the consistent histological structure across concentrations, supports this explanation.

Furthermore, thiostrepton's apoptotic activity in mammalian systems is often mediated through specific molecular pathways, including the inhibition of forkhead box protein M1 (FOXM1) and modulation of p53-related mechanisms (Kalathil 2018; Su 2024; Luo 2023). These pathways are primarily associated with tumor suppression rather than regenerative processes. In contrast, planarian regeneration depends on pluripotent stem cells called **neoblasts**, which utilize signaling networks such as Wnt/β-catenin and ERK to regulate proliferation and differentiation. Because thiostrepton does not directly target these regeneration-specific pathways, it is unlikely to interfere with neoblast-driven tissue regrowth.

The significant increase in head and tail lengths over time indicates proper growth of planaria during regeneration. The effects of time were stronger than the concentration effects, indicating that the hypothesis was not supported. When considering head growth, the combined length and width measurements were significant for both day and concentration but not for their interaction. This suggests that while both time and concentration influence growth overall, the pattern of growth across days remained parallel among concentrations.

Similar trends were observed in tail growth, where small differences between concentrations were present, but time effects were consistent across all groups.

The negative slopes found in head and tail width growth were most likely due to variation in the initial sizes of the planaria. Heads and tails were selected at random for preservation each week, which may have resulted in smaller tails being chosen over time. Another possibility is natural tapering that occurs during tail regeneration. The parallel slopes across concentrations indicate that thiostrepton had no measurable effect on these changes. When considering blastema growth and formation, there were no significant differences or structural abnormalities across concentrations. Histological examination revealed consistent tissue organization among all treatments, suggesting that thiostrepton did not affect blastema structure or differentiation. This finding differs from the original hypothesis, which predicted that thiostrepton would suppress the uncontrolled cell proliferation typically observed in regenerative blastema. Instead, the blastema grew consistently across all concentrations, and increased cellular activity was evident on histology slides. This could be attributed to thiostrepton targeting specific proteins rather than inducing widespread apoptosis.

This study was limited by sample size, planaria housing, and thiostrepton variability. The limited number of samples may have reduced statistical power and accuracy. Housing multiple planaria in the same petri dish introduced variability in which heads and tails were selected for preservation each week. Isolating individuals in separate dishes would have allowed for more precise tracking of individual growth. Additionally, only preserved planaria were measured, so the absence of an initial baseline measurement limited assessment of growth magnitude. The thiostrepton compound may have degraded during shipping or separated when mixed with water, decreasing its activity. Because this experiment relied on

physical measurements of growth, it may not have captured subtle molecular or protein-level changes. Future research should address these limitations by using larger sample sizes, maintaining individual planaria, and employing protein-based assays to detect molecular differences. Testing higher or more diverse concentrations of thiostrepton may also help clarify its effects on regeneration.

Overall, the results indicate that planaria regenerated normally regardless of thiostrepton concentration, with time being the primary factor influencing growth. Both head and tail regeneration increased significantly over time, while concentration effects were minimal and largely parallel across all groups. This suggests that thiostrepton did not inhibit regeneration or alter blastema development, contrary to the original hypothesis. The consistent histological structure and absence of abnormalities further support that thiostrepton had no observable effect on tissue formation. Despite the limitations, this study provides a foundation for future research into the molecular mechanisms of regeneration and thiostrepton's potential role in protein regulation.

APPENDIX 1: Raw Measurements of Preserved Planaria

Concentration	Day	Head Length (mm)	Head Width (mm)	Tail Length (mm)	Tail Width (mm)
0	7	3.125	1.0625	2.5625	1
0	7	5	1.5	3.125	1.125
0	7	4.0625	1.75	2.625	1.375
0	7	3.3125	0.9375	3.625	0.875
0.01	7	3.25	0.8125	3.3125	1.125
0.01	7	4.5	1.3125	3.9375	1.3125
0.01	7	3.5625	1.25	3.1875	1.3125
0.01	7	4.25	1.75	3.9375	1.1875
0.015	7	2.9375	1.1875	2.6875	1.125
0.015	7	2.8125	1.125	3.3125	1.0625
0.015	7	3.8125	1.4375	3.3125	1
0.015	7	N/A	N/A	4.5	1.1875
0.018	7	2.5	1.125	2.5625	0.75

0.018	7	4.9375	2.1875	3.75	1.25
0.018	7	4.375	1.875	2.75	1.25
0.018	7	2.625	1.3125	1.3125	3.6875
0	14	4.533333333	1.333333333	2.733333333	1.266666667
0	14	3.533333333	0.866666667	2.666666667	0.866666667
0	14	3.266666667	0.866666667	3.6	1.2
0	14	4.866666667	1.2	3.333333333	0.666666667
0.01	14	3	1.533333333	2.8	1.666666667
0.01	14	6.133333333	2.333333333	3	1.4
0.01	14	4.666666667	1.866666667	4	1.333333333
0.01	14	3	1.333333333	3.4	1.06666667
0.015	14	2.066666667	0.533333333	2.733333333	0.866666667
0.015	14	4.2	0.866666667	2.733333333	1.533333333
0.015	14	4	1.333333333	4.666666667	1.2
0.015	14	3.333333333	1.133333333	1.8	0.733333333
0.018	14	3.333333333	1.2	2.133333333	1.4

0.018	14	3.933333333	1.466666667	2.733333333	1.333333333
0.018	14	4.466666667	1.466666667	1.933333333	1
0.018	14	4.666666667	1.4	1.933333333	1.2
0	21	6	1.6	4.666666667	1.133333333
0	21	4	1	2.666666667	0.666666667
0	21	4.666666667	1.333333333	2.866666667	0.866666667
0	21	5.933333333	1.266666667	4	0.933333333
0.01	21	3.866666667	0.866666667	2.2	1
0.01	21	4.666666667	1.333333333	2.2	1.333333333
0.01	21	4.666666667	1.333333333	6	2
0.01	21	6	2.133333333	3.333333333	1.333333333
0.015	21	4	1.333333333	2.733333333	1.2
0.015	21	4.133333333	1.2	2	0.866666667
0.015	21	6.8	1.6	4.333333333	1.4
0.015	21	6	2.333333333	3.333333333	1.333333333
0.018	21	3.933333333	1.333333333	2.666666667	1.2

0.018	21	3	0.733333333	2.666666667	1.3333333333
0.018	21	4.866666667	1.4	3.666666667	1.4
0.018	21	7.333333333	2.333333333	1.933333333	0.866666667
0	25	4.066666667	0.8	N/A	N/A
0	25	3.4	0.8	N/A	N/A
0	25	3.2	1.2	N/A	N/A
0	25	4.666666667	1.333333333	N/A	N/A
0	25	4.466666667	1.133333333	N/A	N/A
0	25	2.066666667	0.6	N/A	N/A
0	25	3.333333333	0.8	N/A	N/A
0	25	4.533333333	1.466666667	N/A	N/A
0	25	1.666666667	0.666666667	N/A	N/A
0	25	3.666666667	1.333333333	N/A	N/A
0	25	4.6	1.533333333	N/A	N/A
0	25	7.333333333	2.066666667	N/A	N/A
0	25	5.333333333	1.333333333	N/A	N/A

0	25	3.666666667	0.866666667	N/A	N/A
0	25	3	0.866666667	N/A	N/A
0.01	25	7	2.333333333	1.8	0.866666667
0.01	25	3.666666667	0.8	1.666666667	0.666666667
0.01	25	2.666666667	0.866666667	N/A	N/A
0.01	25	2	0.666666667	N/A	N/A
0.01	25	3.333333333	1	N/A	N/A
0.01	25	6	1.8	N/A	N/A
0.01	25	2	0.6	N/A	N/A
0.01	25	4	1.4	N/A	N/A
0.01	25	4.666666667	2.133333333	N/A	N/A
0.01	25	4.333333333	1.266666667	N/A	N/A
0.01	25	6	1.666666667	N/A	N/A
0.01	25	3	0.866666667	N/A	N/A
0.01	25	3.933333333	0.866666667	N/A	N/A
0.01	25	6.666666667	2.066666667	N/A	N/A

0.01	25	4.2	1.6	N/A	N/A
0.01	25	2.8	1	N/A	N/A
0.015	25	2.6	0.8	N/A	N/A
0.015	25	3.933333333	0.866666667	N/A	N/A
0.015	25	5.533333333	1.933333333	N/A	N/A
0.015	25	2	0.666666667	N/A	N/A
0.015	25	4.133333333	0.8	N/A	N/A
0.015	25	2.066666667	0.933333333	N/A	N/A
0.015	25	6	1.333333333	N/A	N/A
0.015	25	4.666666667	1.333333333	N/A	N/A
0.015	25	4.133333333	1.333333333	N/A	N/A
0.015	25	4.666666667	1.333333333	N/A	N/A
0.015	25	2.8	0.8	N/A	N/A
0.015	25	6	2.066666667	N/A	N/A
0.015	25	4.333333333	1	N/A	N/A
0.015	25	3.333333333	1.333333333	N/A	N/A

0.018	25	1.8	0.666666667	1.533333333	1
0.018	25	3.866666667	1.333333333	1.533333333	0.66666667
0.018	25	3.8	1.333333333	1.733333333	0.66666667
0.018	25	1.733333333	1.666666667	1.533333333	0.8
0.018	25	4.666666667	1.666666667	2.533333333	0.66666667
0.018	25	5.466666667	2.066666667	2.533333333	0.66666667
0.018	25	4.666666667	1.4	2.866666667	0.66666667
0.018	25	5.4	2.066666667	N/A	N/A
0.018	25	4.666666667	1.333333333	N/A	N/A
0.018	25	4.666666667	1.333333333	N/A	N/A
0.018	25	3.266666667	0.8	N/A	N/A
0.018	25	6.8	2.6	N/A	N/A
0.018	25	4.133333333	1.6	N/A	N/A
0.018	25	4	1.333333333	N/A	N/A
0.018	25	3.466666667	1.333333333	N/A	N/A
0.018	25	4	0.666666667	N/A	N/A

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