Ciliary Inhibition in *Caenorhabditis elegans* Using Colchicine and Lithium Chloride

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Regular cell function depends on cilia, and their impairment can cause serious physiological problems. Using chemotactic behavior as a means of measuring cilia damage, this study examines the effects of colchicine and lithium chloride (LiCl) on ciliary function in *Caenorhabditis elegans*. LiCl and colchicine were given different amounts both separately and together to *C. elegans*. Colchicine at 25 µM greatly reduced movement, while colchicine at 10 µM significantly disturbed directional chemotaxis, according to chemotaxis experiments. Between 25 and 50 µM, LiCl showed the largest inhibition of movement; at 25 µM, exposure to both chemicals together produced the most total inhibition. Contrary to original expectations, lesser dosages proved to be more beneficial than doses of greater concentrations. These findings suggest that even low doses of colchicine and LiCl impair ciliary function, providing important new information about the mechanisms behind ciliary dysfunction and confirming *C. elegans* as a valuable model organism for ciliopathies research, including polycystic kidney disease (PKD). However, these results have limited practical implications and further research using improved techniques is advised.

Introduction

Due to its simplicity, easy cultivation, and well-established cell lineage, the nematode *Caenorhabditis elegans*, or *C. elegans*, is a popular model organism in biological research. An adult hermaphrodite *C. elegans* is comprised of exactly 959 somatic cells, making it an ideal system for studying fundamental biological processes. Between 60 to 80% of the *C. elegans* genome contains homologs comparable to human genes, demonstrating the evolutionary conservation of numerous critical biological pathways (Ganner and Neumann-Haefelin, 2017).

Cilia are microscopic, hair-like organelles that protrude from the surface of many eukaryotic cells (Park et al., 2015). They are necessary for vital functions such as movement, signal transduction, and sensory perception. There are two fundamental categories of cilia: motile and primary (non-motile). The primary cilia, also known as cellular antennas, are essential for detecting external stimuli and communicating them to the inside of the cell (Bae and Barr, 2008). While not directly influencing cell movement, primary cilia are essential for signaling pathways that control how cells react to changes in their environment. Primary cilia are the only cilia present in *C. elegans* and are located at the ends of sensory neurons. They are essential for the ability to detect chemical signals.

The ciliated nervous system of *C. elegans* enables the nematode to interpret environmental cues and make sensible behavioral, physiological, and developmental choices (Bae and Barr, 2008). There are exactly 60 ciliated neurons in each *C. elegans*. These neurons are not motile but they help the worm move via chemoattraction, which enables the worm to "smell" its surroundings and move accordingly. Living at the water-soil interface, *C. elegans* must navigate a challenging environment to find food while avoiding harmful compounds (Bae and Barr, 2008). As a result, chemoattraction is highly evolutionarily conserved and extensively researched.

The ciliated neurons in *C. elegans* are dispersed throughout the body, with the bulk being found in the head region as seen in Figure 1. These neurons create sensory cilia that are exposed to the environment at the tip of the nose by extending their dendrites there. Sheath and socket cells, glia-like structural cells that support and maintain appropriate dendritic pathfinding, envelop these cilia (Bae and Barr, 2008). *C. elegans* can detect and react to a variety of environmental

stimuli, including chemicals and mechanical pressures, due to the structural arrangement of these cells around cilia.

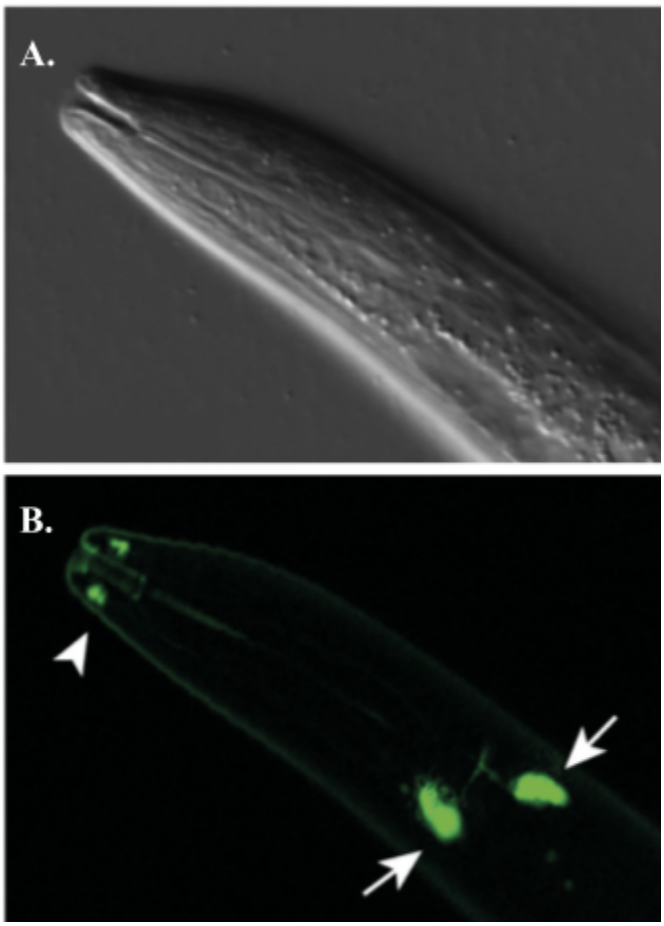

Figure 1. *Visualization of C. elegans head and ciliary structures.*
(A) Light microscopy image of the head region of Caenorhabditis elegans.
(B) Fluorescent microscopy image showing green fluorescent protein (GFP) bound to tubulin, highlighting major tubulin deposits in neurons (arrows) and sensory cilia (arrowheads).
(Adapted from Ganner and Neumann-Haefelin, 2017)

A cylindrical scaffold made of microtubules called the axoneme serves as the structural foundation for cilia. Consequently, microtubules are found in very high concentrations in cilia (see Figure 1). The axoneme is essential to the cilia's ability to move and sense things. A highly conserved mechanism known as intraflagellar transport (IFT) controls the development and maintenance of cilia. It involves the transportation of proteins along the axoneme using the motor proteins dynein and kinesin-2 (Mukhopadhyay et al., 2008). Various proteins are transported to and from the ciliary tip by the IFT machinery, which maintains the cilium's correct construction and operation. The ciliary membrane is home to a variety of ion channels and receptors that take part in the principal ciliary signaling pathways, which include mechanosensing and olfactory sensing.

Impact of Colchicine on Ciliary Function

Microtubule dynamics are disrupted when tubulin, the subunit of microtubules, is bound by colchicine and its polymerization is inhibited (Paschke et al., 2013). The disruption results in a severe impairment of the intraflagellar transport channel, which inhibits ciliary activity by depolymerizing the axoneme and stopping IFT. Colchicine's anti-inflammatory and anti-mitotic properties have led to its medicinal usage in the treatment of gout and other conditions. Unfortunately, its restricted therapeutic index limits its wider relevance and use in medical therapies, as beneficial doses are virtually always close to potentially lethal amounts. (Forkosh et al., 2020).

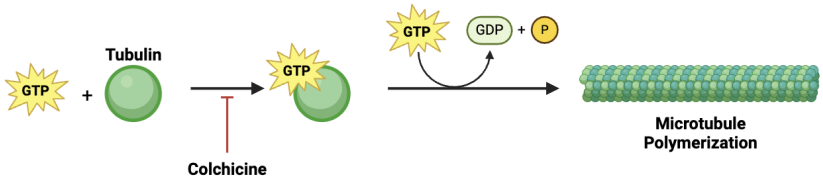

Figure 2. *Mechanism of Colchicine-Induced Ciliary Dysfunction.*
Colchicine disrupts ciliary function by inhibiting tubulin polymerization, thereby preventing the assembly of the axoneme, a key structural component of cilia.

Colchicine is helpful for studying the structural and functional characteristics of cilia in the context of ciliary function because of its capacity to inhibit microtubule polymerization. As seen in the simplified mechanism in Figure 2, colchicine effectively prevents tubulin from polymerizing, which leads to ciliary dysfunction and prevents the axoneme from further assembly. This makes it useful for researching how microtubules affect ciliary function as well as the more general effects of ciliary malfunction in human disorders.

Lithium Chloride and Its Effects on Ciliary Dynamics

Another substance that influences ciliary dynamics is lithium chloride (LiCl), albeit distinctly from colchicine. Although LiCl is more commonly used to treat bipolar disorder, it heavily impacts signaling pathways within cells. LiCl specifically interferes with the Wnt and Hedgehog pathways, a complicated network that is important in tissue homeostasis, cell differentiation, and proliferation (Fliegauf et al., 2007, Thompson et al., 2016). The process of cilia creation and maintenance, known as ciliogenesis, is regulated by Wnt signaling as well and is most pertinent in the context of this experiment (Niehrs et al., 2024).

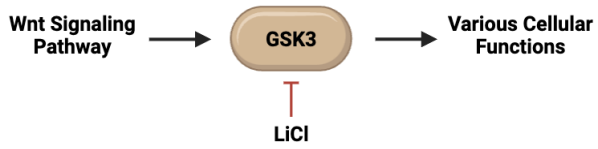

Figure 3. *Mechanism of Lithium Chloride on Ciliary Elongation.*
Lithium chloride (LiCl) promotes the elongation of primary cilia by inhibiting glycogen synthase kinase-3 (GSK-3), a critical regulator in the Wnt signaling pathway.

Primary cilia elongate as a result of LiCl's inhibition of glycogen synthase kinase-3 (GSK-3), a crucial regulator in the Wnt pathway as seen in the simplified mechanism of Figure 3. Although this could appear advantageous, aberrant ciliary lengthening can interfere with regular ciliary signaling and function. The ability of the organism to sense and react to its surroundings may be impacted if LiCl disrupts these pathways and changes ciliary dynamics.

Aims of the Study

The objective of this research is to examine the impact of colchicine and lithium chloride on the function of cilia in *C. elegans*, a model organism possessing distinct ciliated neurons that are crucial for chemosensory activities. These substances, which interfere with microtubule dynamics and modify signaling pathways essential for ciliogenesis, were given to adult nematodes. By detecting aberrant chemosensory behaviors, ciliary dysfunction was evaluated and quantified, offering insight into how certain compounds impact ciliary function. The investigation also looks at the combined effects of the two substances, which could result in stronger cilia function inhibition due to their different processes. This study will advance collective knowledge of ciliary dysfunction and how it relates to illnesses in people.

(Methods and Results sections removed for brevity)

Discussion

The primary goal of biomedical research is to understand the normal functioning of biological pathways and how defects in these pathways contribute to disease (Ganner and Neumann-Haefelin, 2017). This work examined the effects of lithium chloride and colchicine separately and in combination on *C. elegans* chemotactic behavior, aiming to provide insights into how ciliary dysfunction could lead to ciliopathies; diseases resulting from dysfunctional cilia.

Summary of Results

The experiment demonstrated that colchicine and LiCl significantly reduce worm mobility, both individually at lower concentrations and even more so when combined. The two different chemotaxis index calculations revealed that motility and directional movement followed distinct trends and were affected differently. This suggests that while movement ability and functional chemosensory capability are inextricably linked, they are fundamentally distinct processes. This distinction was particularly evident in the combined trial, where motility was the most impaired of any trial yet there was no difference in the direction of movement.

In the context of cilia function, the results indicated that exposure to lower concentrations, specifically 25 μM, was the most effective across all trials. Given that multiple pathways are involved in cilia maintenance, this finding raises an intriguing possibility: at higher concentrations, a repair pathway for cilia may be activated, mitigating the observed impairment in movement and direction as cilia are being repaired. These results emphasize the complexity of ciliary function and highlight the need for further investigation into how these chemical agents disrupt cilia and their associated pathways.

Broader Context and Literature Comparision

The results of this investigation are consistent with other studies on the vital role cilia play in cellular function and the pathophysiology of disease. One well-known ciliopathy is polycystic kidney disease (PKD), in which abnormalities in the cilia cause kidney cysts and eventually significant organ dysfunction (Ganner and Neumann-Haefelin, 2017). The proteins linked to PKD, called polycystins, localize to renal primary cilia and function as mechanosensors that control calcium influx, which is essential for preserving cellular homeostasis (Park et al., 2015). The significance of cilia in renal health is emphasized by the fact that the most common kidney disease, autosomal dominant polycystic kidney disease (ADPKD), can be caused solely by dysfunction in these cilia.

Clarifying the essential functions of cilia in cystic kidney disorders has been made possible through research outside of traditional kidney models, particularly with *C. elegans*. This nematode is important for investigating ciliopathies because of the conservation of many genes and molecular pathways between it and humans. The knowledge that ciliary dysfunction can result in major pathophysiological alterations is supported by the interruption of cilia function that was seen in this investigation. It is possible that even a partial disruption of microtubule assembly or ciliogenesis can significantly affect their function, as seen by the considerable movement suppression observed at 25 μM colchicine and LiCl. This discovery is especially important for creating treatment plans that target ciliary circuits in illnesses.

The findings of this research add to the collective knowledge of how cilia function and how disruptions can worsen ciliopathies. The results also highlight a crucial problem: although this study has shed light on the various ways in which cilia might be damaged, it has not yet produced results for possible treatments of ciliopathies. In fact, these findings suggest ways to worsen ciliopathies by further compromising cilia function and producing more severe symptoms of the condition.

Limitations and Impact on Conclusions

When evaluating the data, it is important to understand the many limitations that persisted over the course of this project. Despite conducting 180 successful chemotaxis assays, each compound's concentration was represented by only 9 plates. The impact of any conclusions drawn is likely hindered by this relatively small sample size. If aberrant findings are obtained

from a single plate, the CI values for that concentration may be greatly skewed. Increasing the number of replicates would likely result in more trustworthy data.

Furthermore, the controls did not achieve CI values of 1, indicating perfect chemosensory behavior. Although values of 1 fell within the margins of error for the controls and perfect behavior is unlikely in living organisms, considerable error may have occurred. Residual E. coli on the plates, despite washing the worms with S. Basal, could have influenced the worms' behavior, causing them to stick to their original deposition sites and artificially lowering the CI values. Additionally, the worms may have had defective cilia prior to the experiment. Future investigations could address this issue by employing different cleaning techniques for the plates or using genetic analysis to examine the stock worms.

Moreover, the CI values may have been affected by the center circle the worms were initially deposited in, which measured one centimeter in diameter. Variations in this size could either inflate or deflate the CI, highlighting the need to standardize this aspect of the experimental setup. Consistent comparison with controls is essential to mitigate this variability and was attempted in this experiment.

The use of *C. elegans* as a model organism for human diseases has an inherent disadvantage as well. Despite the conservation of many genes and pathways, *C. elegans* lacks some sophisticated systems. These results are relevant for understanding the general functions of cilia but do not directly apply to specific human ciliopathies, which are more pertinent to practical medical applications. In an altered version of this experiment, however, knocking out homologous genes in *C. elegans* using gene-editing methods such as CRISPR could yield more focused insights and increase the applicability of the results to human diseases.

While the findings of this experiment are valuable, caution is warranted in their interpretation; they should be viewed as trends for further investigation rather than concrete evidence. The results highlight the significant effects of LiCl and colchicine on ciliary function, but additional replications and methodological adjustments are necessary to strengthen these conclusions. Although these results are promising, they are not conclusive due to the limited sample size and potential methodological errors. Future studies with more rigorous controls and larger sample sizes are needed to validate these findings and explore their implications.

This investigation utilized the chemosensory behavior of *C. elegans* to demonstrate that even at very low concentrations, colchicine and LiCl significantly impair ciliary activity. These findings are pertinent to human ciliopathies and offer insight into how chemicals may disrupt ciliary function. The study does, however, also draw attention to the difficulties in converting these findings into feasible therapeutic strategies. Although the results provide insight into ciliary dysfunction, they do not yet point to a specific course of treatment; rather, they highlight the need for more investigation to stop ciliopathies from getting worse. Future studies must address the limitations of this work to enhance understanding of ciliary dysfunction and its broader implications for human health.