Efficacy analysis of zebrafish, *Danio rerio*, as a novel demyelination model through exposure to cuprizone-infused food.

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Abstract

The central nervous system demonstrates its functional reliance on glial cells in support of neurological function primarily through its use of oligodendrocytes and the myelin they produce. Damage to myelin is caused by different pathways, one exemplified in Multiple Sclerosis (MS), where the immune system incorrectly attacks healthy tissue of the CNS. Most research focusing on these biological processes of demyelination have relied on rodent models. However, zebrafish, *Danio rerio*, are an ideal model for these goals because they are transparent and therefore are easy to monitor for demyelination and remyelination progression. In this study, the viability of zebrafish as a model for demyelination and remyelination research studies was evaluated. The initial steps were the development of cuprizone-infused food and the design of a cuprizone drug feeding protocol. Following treatment, myelin staining techniques were used to assess the level of myelin damage. The goal of this study is to develop a reliable new demyelination research model that will improve imaging of myelin damage and repair during demyelinating diseases.

Introduction

- For communication, the body uses neuronal axons to send electrical signals. The speed at which these signals travel throughout the body is related to the amount of myelin wrapped around the axon, increasing in speed with increased myelin density.
- Loss of myelin, or demyelination, occurs when damage is inflicted on the nervous system, killing the cells producing myelin; oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). [1]
- In order to study the process of demyelination, and subsequent remyelination, a functioning acute demyelination model in adult zebrafish is proposed in this study as a more effective model compared to the rodent models previously used.
- The efficacy of this model will likely be observable after inducing a cuprizone feeding technique at a 400mg/kg/day treatment concentration, once a day for 3 weeks and analyzed after whole-fish histology and staining with Luxel Fast Blue to visualize myelin density in the CNS.
- This project was carried out to develop a viable cuprizone-laden feed mixture and an effective and consistent feeding technique to introduce to zebrafish, allowing this model to be used to assess demyelination and subsequent spontaneous OPC proliferation to ultimately study the effects of antagonists of the muscarinic and histamine pathways on remyelination.

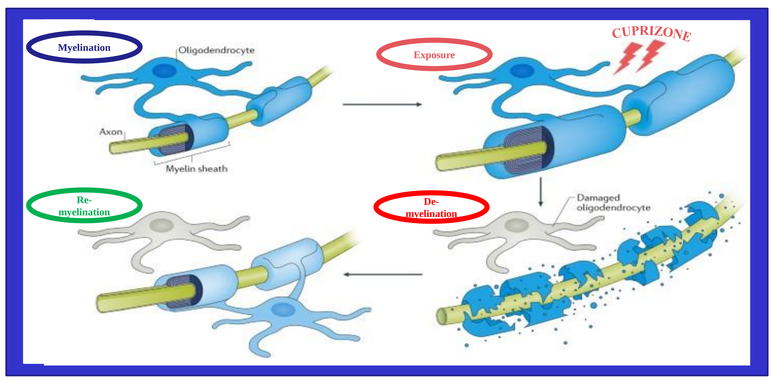

Figure 1: Flowchart of demyelination due to cuprizone exposure and remyelination following cuprizone withdraw. [2]

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References

[1]Michalski, John-Paul, and Rashmi Kothary. 2015. "Oligodendrocytes in a Nutshell." *Frontiers in Cellular Neuroscience* 9 (September). https://doi.org/10.3389/fncel.2015.00340.

[2]Franklin, R., ffrench-Constant, C. Regenerating CNS myelin — from mechanisms to experimental medicines. *Nat Rev Neurosci* **18,** 753–769 (2017). https://doi.org/10.1038/nrn.2017.136.

Methods

Housing and Water Quality– double-tanked system equipped with an external tank (15L clear storage bin and lid equipped with heater, thermometer, and filled with DI water until covering heater) holding 2 internal tanks (Aquaneering ZT180 1.8L zebrafish maintained at 700 µS/cm, pH 7-7.5, 28 C, 50ppm nitrate, 0ppm ammonia and nitrite, ½ tank exchanges daily) each holding 1 adult male zebrafish.

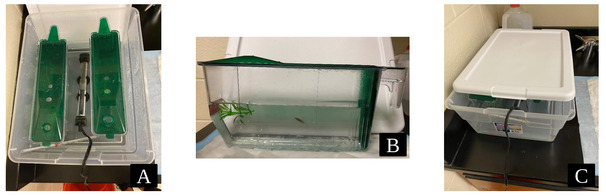

Figure 2: a) Double-tanked housing system, b) Single internal tank with male zebrafish, c) Enclosed system.

Cuprizone Food Prep. – around 0.5 µL Gemma Micro 300 ZF Feed was weighted in PCR tube for exact weight to which proprotionat cuprizone could be weighted into separate PCR tube to yield 400mg/kg/day concentration when mixed. To cuprizone PCR tube, 1 drop 1% methyl cellulose (MC), 4 drops GDW was added and vortexed. Dry food added and vortexed.

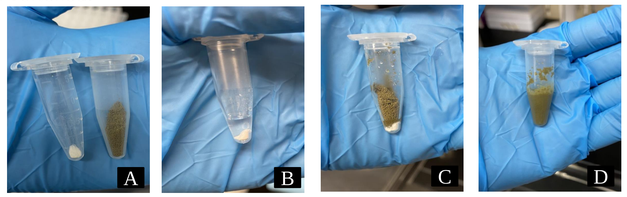

Figure 3: a) cuprizone and ZF Feed, respectively, in dosage proportions, b) cuprizone with 1 drop 1% MC and 4 drops GDW, c) combined cuprizone and feed, d) homogenous and completed cuprizone-infused food.

Feeding protocol – cuprizone food was extracted from tube with 1cc syringe, no needle. Air expelled and food compacted to end, and 27G needle was attached. Cell culture dish was filled with GWD into which 0.3cc of cuprizone food was expelled. Pipette used to break up food by collecting and expelling until food appeared as pellets. A final collecting of food was taken into the pipette and fed individually to fish in small bowl. This was found to be too stressful and prevented fish from eating. Technique changed to direct feeding into fish tank. Feeding was done 1x/day for 2 weeks and increased to 2x/day for the last week because no effects were seen.

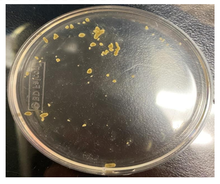

Figure 4: cuprizone-infused food in pelletized form, ready to be fed.

Euthanasia, Fixation, and Decalcification – each fish was euthanasia by Tricaine-S (MS222), tail clipped, and fixed in 4% PFA in PBS for min. 24 hrs. at 4C. for 7 days. A 7-day decalcification in 0.5M EDTA at room temp. followed.

Dissection and histology – each zebrafish was dissected by a ventral cut, from to operculum. Abdominal internal organs (swim bladder, GI, heart) were removed, leaving muscular walls of abdominal cavity to allow for better infusion of whole specimen.

Staining – standard procedure for H&E and Luxel Fast blue were used on the prepared slide.

Future Research

- **Further trials to increase sample size.**
- **Start from beginning with less stressful feeding technique to help keep ingestion consistent.**
- **Further evaluation of fixation and dehydration techniques on a whole, adult zebrafish to provide better sections of samples.**
- **Once model is established, continuing experiment through cuprizone-laden food withdraw to observe remyelination processes.**

Results

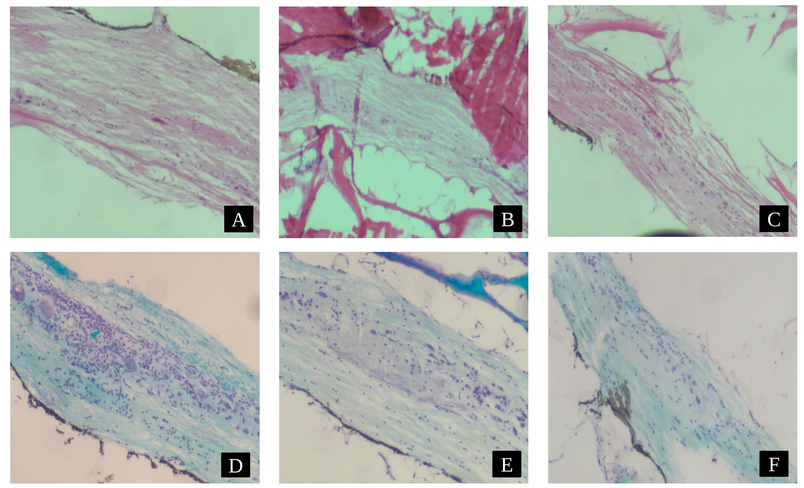

Figure 5: Sections imaged at 100x in areas of lower cell body densities and similar spinal locations. All H&E and LFB comparative slides were taken from adjacent sections in each respective sample; a) Control fish stained with H&E, b) Fish 1 stained with H&E, c) Fish 2 stained with H&E, d) Control fish stained with LFB showing qualitative darker blue saturation indicating higher myelin presence, e) Fish 1 stained with LFB showing qualitative lighter blue saturation suggesting lower myelin presence consistent with loss of myelin due to cuprizone exposure, f) Fish 2 stained with LFB qualitative lighter blue saturation suggesting lower myelin presence consistent with loss of myelin due to cuprizone exposure.

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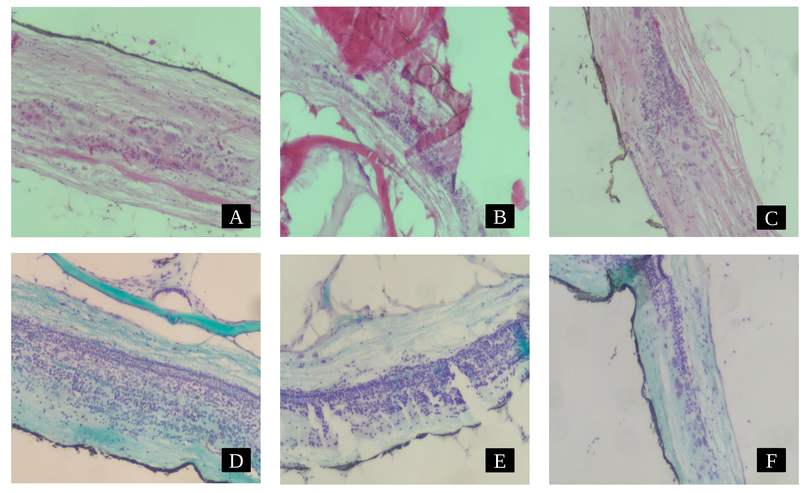

Figure 6: Sections imaged at 100x in areas of higher cell body densities and similar spinal locations. All H&E and LFB comparative slides were taken from adjacent sections in each respective sample; a) Control fish stained with H&E, b) Fish 1 stained with H&E, c) Fish 2 stained with H&E, d) Control fish stained with LFB showing qualitative darker blue saturation indicating higher myelin presence, e) Fish 1 stained with LFB showing qualitative lighter blue saturation suggesting lower myelin presence consistent with loss of myelin due to cuprizone exposure, f) Fish 2 stained with LFB qualitative lighter blue saturation suggesting lower myelin presence consistent with loss of myelin due to cuprizone exposure.