Aldicarb Exposure, and the Effects of the GBB-1 Gene in Caenorhabditis elegans

Haley Parks, Hope Brown, Kylie Curtis, Alex Romer, Rebecca Thiemann-Siepelt
Middle Tennessee State University

Introduction

- In all living creatures that reside on the planet earth, gene expression plays a critical part in keeping that species alive and fully functional to survive within its ecosystem. Gene expression plays its part by expressing certain proteins that allow full functionality of multiple life essential internal systems in a living body.

- A neurotransmitter labeled GABA serves as a vital role in the development and the overall function within the nervous system. GABA uses synaptic transmission through both ionotropic receptors and G-protein-coupled receptors. Ionotropic receptors are made using seven channels gated through ligand to create small, incredibly fast signals between the neurons, while the GPCRs are gated by neurotransmitters and they prefer to make longer, yet much slower, signals between neurons (Dittman and Kaplan 2008).

- Acetylcholine is used in the process of contracting muscular tissue. The acetylcholine that is released from cholinergic moto-neurons, the neurons that help send signals to the muscle fibers in the muscular system, is used to stimulate postsynaptic receptors located in the muscles making the wall, thus allowing these muscles to contract and the organs can perform locomotion (Mahoney et al. 2006). The gene GBB is one of the subunits that makes up a GABA receptor, and it aids in the expression of the cholinergic moto-neurons (Schultheis et al. 2011).

- Aldicarb is an inhibitor to acetylcholine, and it is commonly used as a pesticide against unwanted nematodes. If a nematode is exposed to aldicarb, it prevents the acetylcholine from breaking down the acetylcholine, making a large buildup of acetylcholine within the synapse. This leads to hyper-contractions within the muscles before gradually leading to paralysis.

- When exposed to both 2.628 millimolar and 5.256 millimolar of aldicarb, the RNA from the GBB-1 gene will be alternatively spliced to form different isoforms in regions of the C. elegans nervous system, compared to those not associated with the exposure of aldicarb.

Methodology

- Designed and designed the reference sequence, predicted alternative isoforms due to treatment, designed PCR primers, and calculated the expected PCR size for the gene GBB-1.

- Cultiivated and harvested C. elegans on plates of bacteria, assuring that they were C. elegans. The first group would be the control, and the second and third would be the two experimental groups.

- Treated the control group with 900 microliters of saline followed by 100 microliters of 5.256 mM aldicarb, all three groups of nematodes were kept in saline.

- Isolated and extracted RNA from nematode pellets and performed reverse transcription using the thermocycler, producing cDNA. Gradient PCR was run with 900 microliters of saline, then followed by 50 microliters of deionized water and 100 microliters of 5.256 mM aldicarb. All three groups of nematodes were kept in saline.

- Designed the PCR primers, and calculated the annealing temperature for our RNA samples will be 51.2 degrees Celsius.

- Extending exposure time of aldicarb among the motoneurons (Schultheis et al. 2011).

- After running PCR on our samples, the image shows that alternative splicing did not occur in both the 5.256 mM and the 2.628 mM experimental samples, however it did show that the expression of GBB-1 is inversely correlated with the concentration of aldicarb. It showed that as the concentration of aldicarb that was added to the nematode pellets increased, the expression of GBB-1 in the nematode pellets decreased. No difference in band placements were shown between the control and the two experimental samples.

- Since the image showed the inverse correlation between the concentration of aldicarb and the expression of the GBB-1 gene, it concludes that aldicarb reduces the expression of the cholinergic moto-neurons (Schultheis et al. 2011), inhibiting the release of acetylcholine and preventing neural signals from reaching muscle fibers located in the muscular system that are vital to perform locomotion in C. elegans (Mahoney et al. 2006).

Future Directions

- Repeated testing is needed in order to confirm no other possible factors affected the two experimental samples.
- Tests on electrophoresis would be wise to reassure assumption that there was no possible contamination among the three samples.
- Further testing on the GBB-1 gene involving aldicarb would be recommended to see if other possible dependent variables are affected by the exposure of aldicarb.
- Experiment using different primers to look for possible alternative splicing in other exons (such as exon 17, 9, or even 7).
- Extending exposure time of aldicarb among the nematodes longer than 25 minutes.

References


Huskey MA, Kunkel TA, Bowden WC, et al. 2005. Alternative splicing did not occur in both the 5.256 mM and the 2.628 mM experimental samples, however it did show that the expression of GBB-1 is inversely correlated with the concentration of aldicarb. It showed that as the concentration of aldicarb that was added to the nematode pellets increased, the expression of GBB-1 in the nematode pellets decreased. No difference in band placements were shown between the control and the two experimental samples.

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Conclusions

- The best annealing temperature for the primers for GBB-1 was 51.2°C.

- After running PCR on our samples, the image shows that alternative splicing did not occur in both the 5.256 mM and the 2.628 mM experimental samples, however it did show that the expression of GBB-1 is inversely correlated with the concentration of aldicarb. It showed that as the concentration of aldicarb that was added to the nematode pellets increased, the expression of GBB-1 in the nematode pellets decreased. No difference in band placements were shown between the control and the two experimental samples.