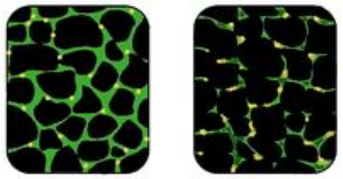
Attenuation of muscular dystrophy phenotype in drosophila by estrogen-mimics

Amandip S. Cheema, David H. Lee, Rajendram V. Rajnarayanan
Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo, NY 14214

Abstract

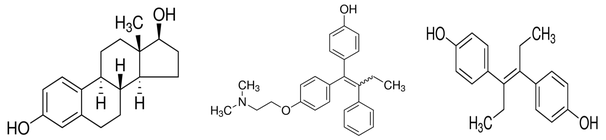
Muscular dystrophy is a disease characterized by progressive muscle wasting, respiratory and cardiac complications, and ultimately death. The disease is caused by a mutation in the protein dystrophin, which normally plays a role in the protection of muscle. Recent studies have suggested that the use of tamoxifen, an anti-cancer drug, may have therapeutic effects on the muscular dystrophy phenotype in mice. Based on our preliminary computational analyses we hypothesize that compounds targeting nuclear transcription factors estrogen receptor and\or estrogen receptor related receptor may have some beneficial effect on the muscular dystrophy phenotype in drosophila by increasing dystrophin expression. Our project aims to further investigate the role of estrogenic compounds, as a therapeutic, to the muscular dystrophy phenotype in drosophila. The compounds were administered to the flies orally by mixing them with the normal fly feed. After ingestion, we used a cluster of locomotion markers to assess the compounds' activity. Tamoxifen appears to ameliorate locomotion deficit in dystrophic flies. We believe, non-steroidal estrogenic compounds hold the key to the development of next generation therapeutics for the treatment of muscular dystrophy.



The left shows a healthy muscle with the protein dystrophin . The right shows a diseased muscle with low dystrophin, leading to damage and disorganization of the tissue.

Objective

- By using a dystrophin mutant drosophila fly model and administering estrogenic like compounds we hope to see a change in the muscular dystrophy phenotype.
- Find the role of ERR in the amelioration of the muscular dystrophy phenotype.



β-Estradiol 4-Hydroxytamoxifen Diethylstilbestrol

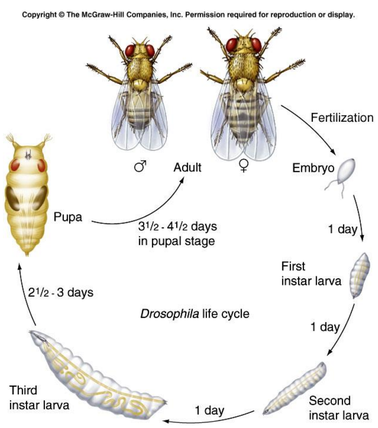
Methods

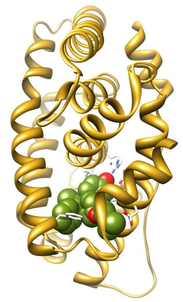
Testing of Locomotor Activity in Adult Flies

- Flies were administered various estrogenic compounds through the fly food.
- Flies were allowed to ingest the food, and lay eggs in the food.
- Larvae were allowed to grow, and parent flies were removed and tested for changes in locomotion.
- Repeated for several generations

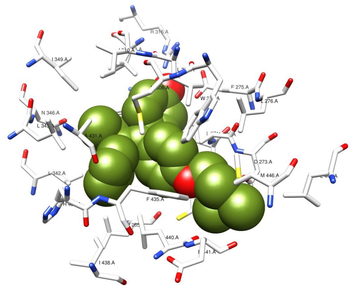
Testing of Locomotor Activity in Larvae

- Third instar larvae were removed from fly feed and placed in various solutions containing sucrose and various estrogenic compounds.
- Larvae were then placed onto agar solution in a petri dish and observed for changes in behavior and movement.
- This observation was recorded and tracked using a fly tracking program.





Tamoxifen bound to Estrogen Receptor Related Receptor (ERR)



The binding pocket of Estrogen Receptor Related Receptor (ERR), with Tamoxifen bound.

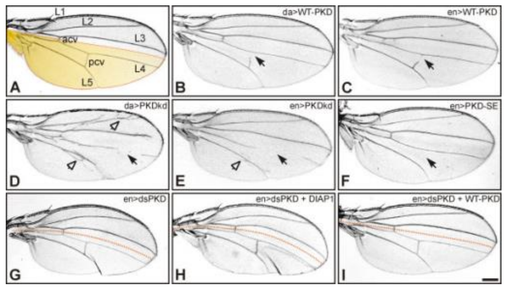
Drosophila Mutants

477-Dys$^{detached-1}$

- Flies show a flight muscle myofibril defect. Severity increases with age.
- Show posterior cross-vein (PCV) defect. Type of defect characterized by if the fly is a homozygote or a heterozygote.
- This phenotype has incomplete penetrance and selective expressivity.
- Reduced activity (crawling, flying walking)

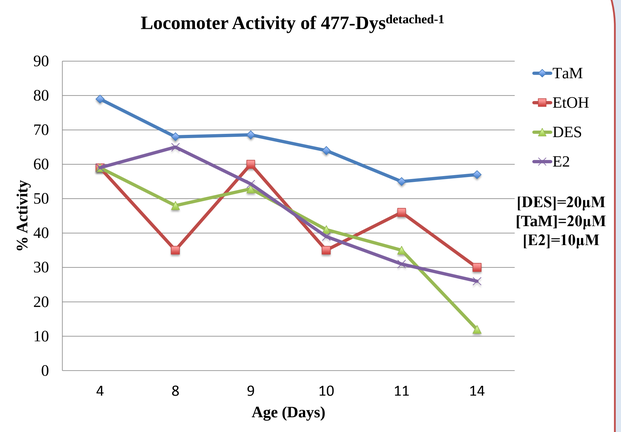
Dmel\Dys^{E6}

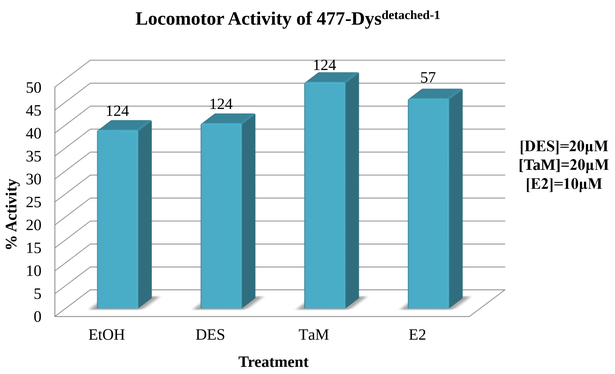
- Characterized by cross-vein defect. Most of the posterior cross-vein missing in homozygotes.
- Reduced activity (crawling, flying, walking)



This figure shows the selective expressivity of the PCV. There is incomplete penetrance leading to a range of severity in the abnormality of the PCV.

Results





Conclusions

- Tamoxifen appears to ameliorate locomotion deficit in dystrophic flies. We believe, non-steroidal estrogenic compounds hold the key to the development of next generation therapeutics for the treatment of muscular dystrophy.
- Next steps include up-scaling the study to see if results follow the pattern shown and testing novel compounds created in our lab.

Acknowledgments

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