Molecular Behavioral Effects of Atorvastatin Exposure in Zebrafish (Danio rerio): Insights into Statin Toxicity at Environmentally Relevant Levels

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INTRODUCTION

- Atorvastatin is a cholesterol-lowering statin medication for humans that works by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase
- While this pharmaceutical is bioavailable to marine organisms, its potential toxicity to them is not well characterized
- Adverse effects in fish associated with exposure to statin drugs have included kinked notochords in Danio rerio, oxidative stress in *Oncorhynchus mykiss*, and altered lipid metabolic transcripts in *Oncorhynchus mykiss*
- **The objectives are to evaluate the potential toxicity of atorvastatin on sub-lethal developmental and locomotor-related endpoints and conduct RNA-seq analysis to identify the toxicity mechanisms of atorvastatin related to environmentally relevant exposures**

METHODS

- Zebrafish embryos at 6-hours post-fertilization (hpf) were randomly exposed either to embryo rearing medium (ERM) or one dose of atorvastatin (0.1 µg/ml – 1000 µg/ml)
- Each experimental group was conducted with 6 glass beakers containing 20 embryos and 10ml of ERM. Data related to mortality, deformities, hatch rate, as well as images using EVOS™ FL Auto Imaging System were collected daily
- On day 7 post fertilization, samples were frozen for RNA-seq, ROS, AO, and VMR analysis

ACKNOWLEDGEMENTS

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RESULTS

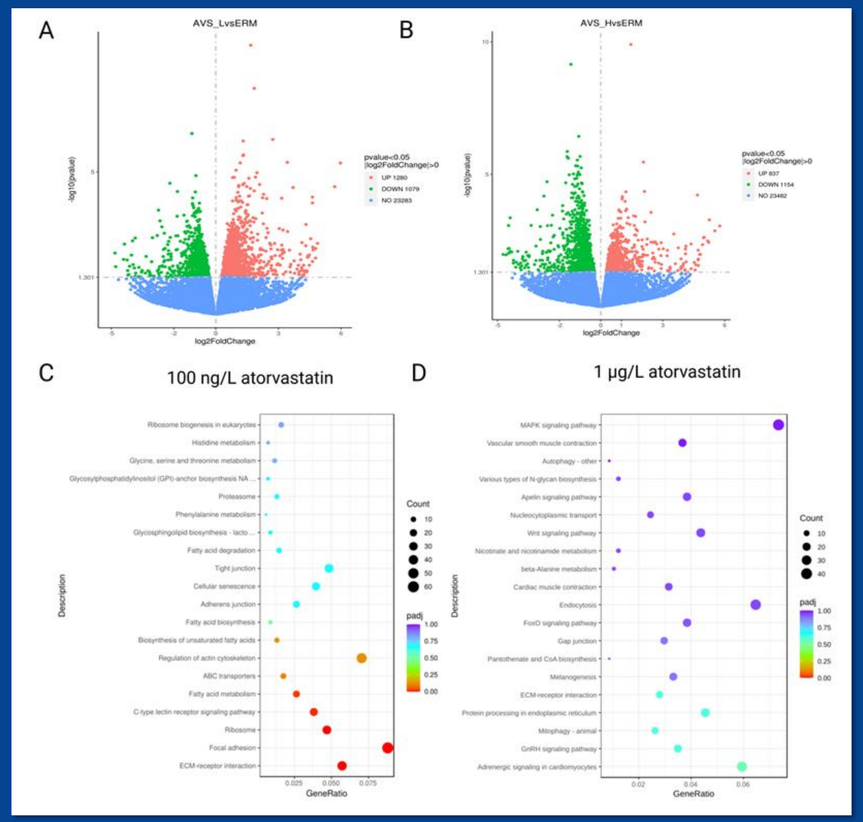

Figure 1. (A and B) Volcano plots is a graphical representation between significance and fold change for expressed genes in both the low and high concentration groups. Red indicates up regulation and green indicates down regulation. (C and D) Bubble plots of enriched KEGG pathways affected by atorvastatin. Lower dose are more significant and affect lipid related processes and ATP production.

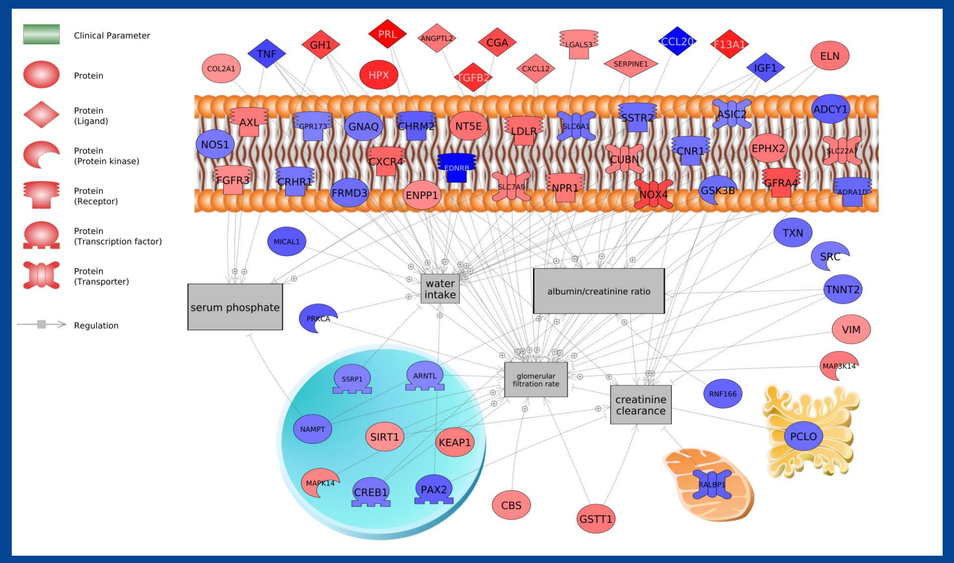

Figure 2. Gene set enrichment analysis revealed molecular gene network and pathway related to lipid regulation to kidney function with atorvastatin exposure. Red indicates up-regulation and blue indicates down-regulation.

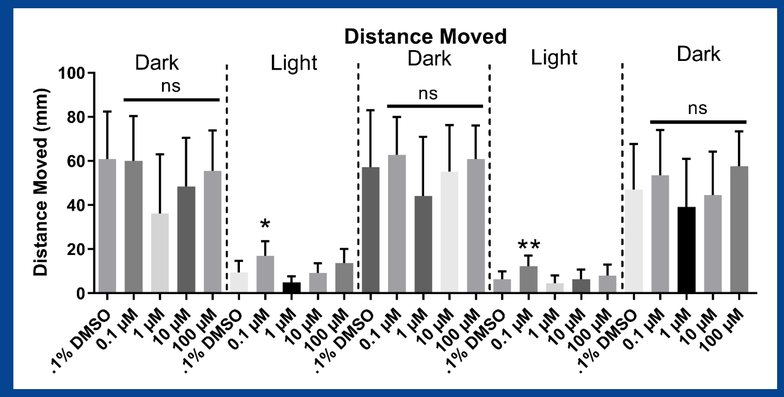

Figure 3. Locomotor behavior was affected by atorvastatin. However, these responses were only observed during exposure to light and not dark. Ns= not significant. Asterisk indicates significance at P<0.05.

DISCUSSION & CONCLUSIONS

- In the United States, statins are among the most frequently prescribed medications (Golomb et al., 2004); however, data is lacking on developing toxicity mechanisms related to statin drugs. As such, we narrowed this knowledge gap by investigating molecular and behavioral responses in larval zebrafish
- **For developmental end-points, atorvastatin lowers survival and hatch success with increasing concentration. Deformity frequency is insignificant in zebrafish larvae**
- **For locomotion, activity based on distance traveled was increased by 0.1 µg/ml atorvastatin exposure**
- **RNA-seq revealed altered gene networks associated with lipid regulation and ATP levels that are directly impacts kidney function, even in low dose**
- This study improves knowledge regarding the relative toxicity of atorvastatin to marine species to improve risk assessment strategies for statin drugs

REFERENCES

Golomb, B. A., Criqui, M. H., White, H., & Dimsdale, J. E. (2004). Conceptual foundations of the UCSD Statin Study: a randomized controlled trial assessing the impact of statins on cognition, behavior, and biochemistry. *Archives of internal medicine*, *164*(2), 153-162.