Impact of fluoxetine exposure on microRNA expression on primary cultured hepatocytes from rainbow trout (*Oncorhynchus mykiss*)

Wasim Karkache, Paul M. Craig, Marilyn Vera Chang, Kelly Levesque, Thomas W. Moon
University of Ottawa

Introduction

- The increasing prescription rate of human drugs and the inability to effectively remove them from sewage wastewater, has lead to concerns that the active ingredients excreted in human waste will accumulate in aquatic ecosystems and have detrimental effects on the resident aquatic organisms.
- MicroRNAs (miRNA) are major post-transcriptional regulators; they are essential in numerous biological processes, and are sensitive to these toxicological wastes, such as fluoxetine, the active ingredient in Prozac. Prozac is a widely used antidepressant drug; it is in the selective serotonin reuptake inhibitor (SSRI) family.
- Previous reports have demonstrated that fluoxetine impacts several important miRNAs found in zebrafish. In order to render these impacts economically important, we examined if fluoxetine had a similar negative impact on the miRNAs of rainbow trout fish. This is possible because miRNAs are heavily conserved across species.
- In this experiment, hepatocytes were cultured from female trout (Figure 1) and exposed to either control conditions, to fluoxetine, to serotonin or to fluoxetine and serotonin. These hepatocytes were then collected and miRNA was extracted in order to eventually preform qPCR on specific miRNA known to be impacted by fluoxetine exposure in zebrafish.

Figure 1: Image of a rainbow trout[1].

Methodology

Hepatocytes were extracted from female trout, and were then cultured and exposed to either control conditions (no fluoxetine), fluoxetine (100 μM), fluoxetine and serotonin or pargyline (100 μM as a positive control) for 96 hours (Figure 2).

↓

The cells were then collected and miRNA was then extracted using the miRNeasy mini kit (Qiagen).

↓

The miRNA was converted into 1st strand cDNA using the 1st strand miRNA cDNA kit (Agilent).

↓

qPCR was preformed on 5 specific miRNA (let-7d, miR-140-5p, mir-210-5p, miR-22b, mir-301a) that are known to be responsive to fluoxetine exposure in zebrafish.

↓

Finally, in order to establish a link between miRNA expression pattern and target transcript, an *in silco* approach was taken to confirm specific targets of these miRNA transcripts in rainbow trout.

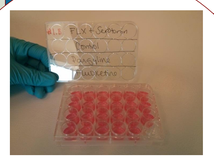
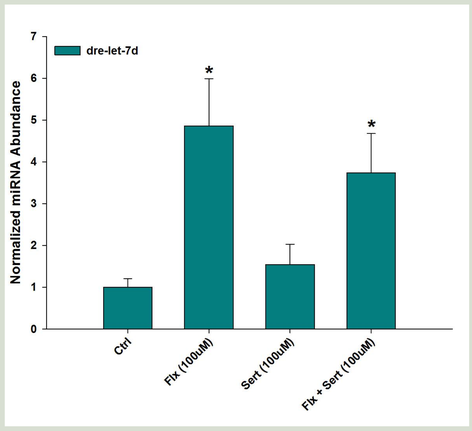


Figure 2: Cultured female hepatocytes, exposed to either fluoxetine and serotonin, control conditions, pargyline or fluoxetine.

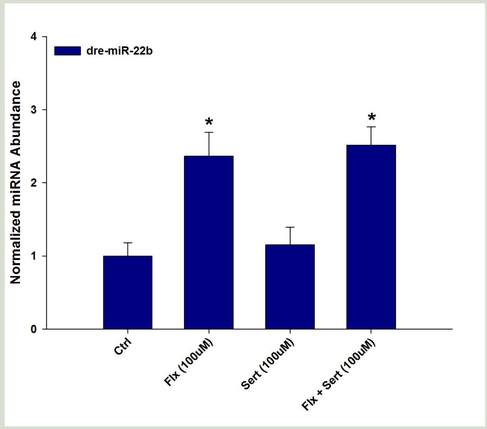
Results

After the qPCR analysis, the miRNAs that were the most severely impacted by fluoxetine exposure were the miRNAs: let-7d, miR-140 and miR-22b. It was possible to demonstrate that fluoxetine exposure severely impacts the expression of these miRNAs (Figure 3).

a)



b)



c)

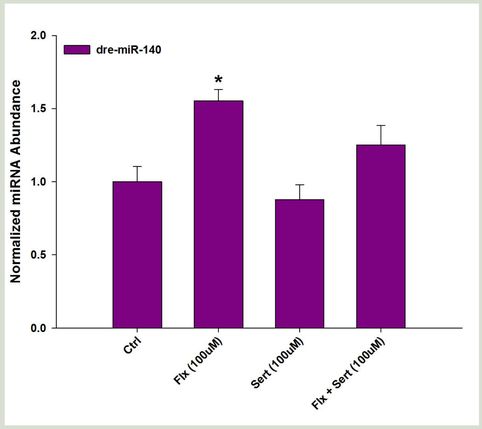


Figure 3: These graphs represent the normalized miRNA abundance of a particular miRNA in different environments that cells were exposed to. In all graphs, it is evident that fluoxetine severely impacts the miRNA's expression; this is proven by the apparent difference in abundance from the environments containing fluoxetine (Flx and Flx +Sert) to the control group (Ctrl) and the serotonin group (Sert).
(a) Normalized let-7d abundance in the 4 different conditions.
(b) Normalized miR-140 abundance in the 4 different conditions.
(c) Normalizedmir-22b abundance in the 4 different conditions.

Discussion

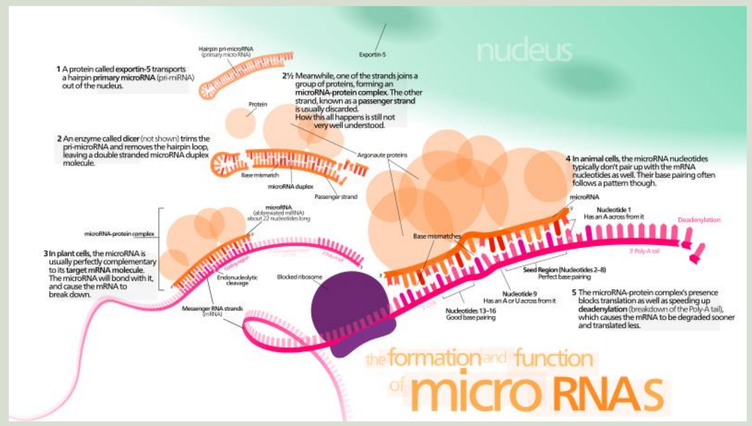


Figure 4: Brief overview of miRNA functions. miRNAs are major post-transcriptional regulators acting on the mRNA[2].

We initially predicted to see an alteration of the expression pattern of miRNA in the miRNAs treated with fluoxetine. Our research results support this hypothesis; we were able to concluded that fluoxetine exposure does in fact impact the expression of the miRNAs: let-7d, miR-140 and miR-22b. These findings are particularly significant seeing as these miRNAs that are the most conserved throughout species, such as the rainbow trout.

These results are interesting and important as it makes a link between drug consumerism in Canada, and the negative toxicological impacts they may have on aquatic species. These findings demonstrate the extent to which our actions may negatively impact other species and ourselves.

The next steps to take in this research would be to determine the exact metabolic and physiological impacts that these changes in miRNA expression may have on fish. As well as determine if these impacts are reversible with other intermediates.

References

[1] Kraft, C.E, D.M. Carlson, and M. Carlson. 2006. *Inland Fishes of New York (Online),* Version 4.0. Department of Natural Resources, Cornell University, and the New York State Department of Environmental Conservation

[2] *MiRNA*, http://commons.wikimedia.org/wiki/File:MiRNA.svg. (01, 23, 2014)

[3] Solon, Olivia. 2012. *Sheets of gold and DNA strands used in unconventional dark matter detector (Online)*. Wired.co.UK, Science.

Acknowledgements

I would like to thank Dr. Thomas W. Moon, Dr. Paul M. Craig, Marilyn Vera Chang and Kelly Levesque

Wassim Karkache, wkark064@uottawa.ca