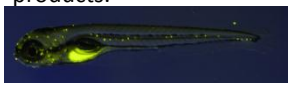
Ginkgo Biloba Flavonols cause Oxidative Damage in Zebrafish Hair Cells

Anna Roche

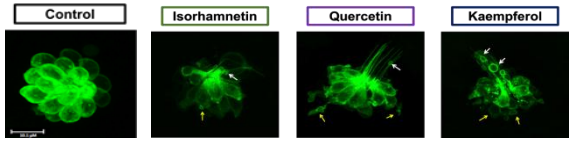
Overview

Several drugs, including aminoglycosides and chemotherapy agents, are known for their ototoxicity. The ototoxic potential of most therapeutic compounds is unknown. FDA-approved drugs are not tested for ototoxicity, so their potential to affect hearing goes unrecognized. There no FDA oversight and the manufacturer is responsible for ensuring the safety of their natural products.



5 day-old zebrafish with DASPEI labeled neuromasts (yellow dots) visible on the head and trunk.

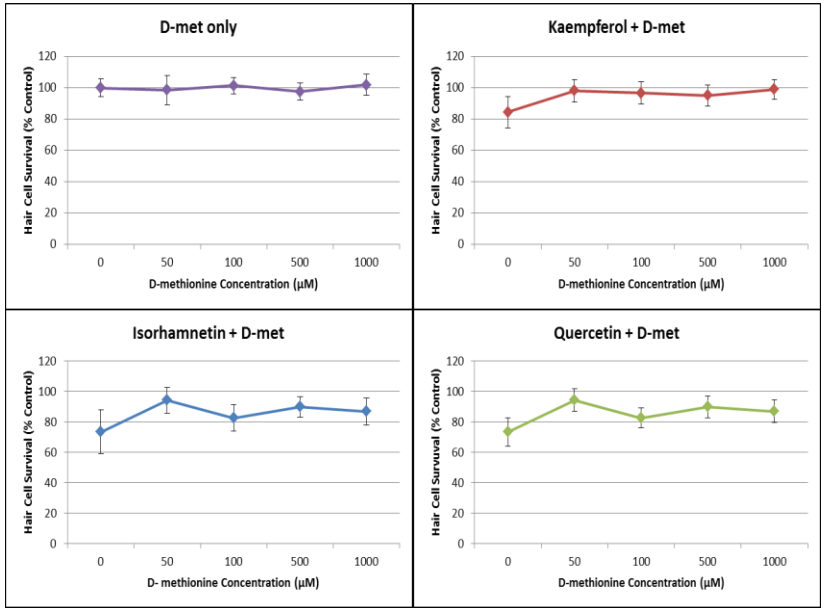
Using the zebrafish lateral line, we screened a natural products library to identify new potential ototoxins. We found three flavonols from the Gingko Biloba plant, quercetin, kaempferol, and isorhamnetin, all demonstrated significant ototoxicity.



Methods

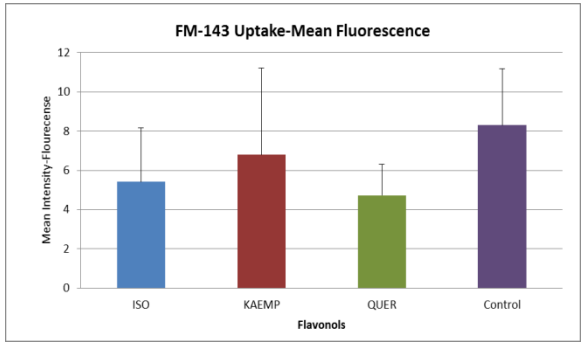
Five groups of zebrafish larvae were exposed to 5 concentrations D-methionine. Five other groups of fish were treated with the same concentrations of D-met and then exposed to 50µm of a flavonol. Fish were incubated then exposed to a mitochondrial dye, DASPEI. This causes the neuromasts in fish to glow yellow. Ten main neuromasts of zebrafish have consistent locations on each fish. Each neuromast is given a score from 0-2. 0-the neuromast is missing; 1-it is missing hair cells; 2- the neuromast is healthy. The dye FM-134 is only taken up by healthy cells. Three groups were exposed to the flavonols and then exposed to FM-143. They were viewed on a confocal microscope to assess fluorescence.

Flavonols Paired with D-methionine



Fish exposed D-met: F value of $F(4.55)=0.94$ and $p<0.45$. D-met+quercetin: $F(4,55) = 10.84$ and $p<0.00$, all concentrations were protected. Fish treated with D-met and isorhamnetin: $F(4,55) = 7.89$, $p<0.001$. There was protection from all concentrations except 100µM. Fish treated with D-met and kaempferol: $F(4,55) = 7.14$, $p<0.001$. All concentrations were protected.

FM-143 Mean Fluorescence



Isorhamnetin had a fluorescence of 5.409. Kaempferol had a fluorescence of 6.800. Quercetin had a fluorescence of 4.718. The control fish had fluorescence of was 8.296.

Discussion

The lower average fluorescence from FM-143 dye uptake in fish exposed to the three flavonols, shows that the remaining hair cells that are still alive, are not as healthy as they would be. By looking at the graphs of the flavonol and antioxidant data it supports that antioxidants are protecting hair cells. This is shown by looking at the first point on each graph, at 0µM D-met where the hair cell survival is lower than all the other points. The antioxidant experiments ran error free until the last few trials with quercetin and D-met some fish that had been previously exposed to quercetin did not uptake the dye and were not able to be scored.

Conclusion

This experiment has served to expand our understanding of how flavonols from Gingko Biloba are affecting hair cells in larval zebrafish. The knowledge that antioxidants may prevent cell death is a large step in understanding and hopefully preventing such damage. In the future, using ROS indicators would help confirm that Ginkgo Biloba flavonols cause oxidative damage. Ultimately, herbal supplements are not going to lead to deafness or other overly serious health concerns that we know of, but having unregulated medication is still a risk. Hopefully more awareness will be centered on herbal supplements.

Acknowledgements: Thanks to my mentor Allison Coffin for all her support and the WSU-V lab space.

Coffin, A. B., Ou, H., Owens, K. N., Santos, F., Simon, J. A., Rubel, E. W., & Raible, D. W. (2010). Chemical Screening for Hair Cell Loss and Protection in the Zebrafish Lateral Line. *Zebrafish*, *7*(1), 3–11.