Research Paper

Abstract

According to the World Health Organization, over 275 million people in the world suffer from deafness and other hearing impairments. Hearing impairments affects people of all ages, ranging from newborns to the elderly. Hearing loss is often caused by the loss of hair cells, which are mechanosensory cells that receive sound stimuli and transfer it to the brain via the auditory nerve. The human body produces about 15,000 auditory hair cells and once lost, hair cells cannot be replaced. 90% of all cases of sensorineural hearing loss are caused by damage to hair cells. The loss of hair cells can be caused by certain types of aminoglycoside antibiotics, environmental toxins, aging, as well as prolonged noise exposure. Many researchers use zebrafish as a model to visualize the viability of hair cells and study the toxicity of certain chemicals in vivo. The lateral line along the body of this fish contains hair cell bearing organs called neuromasts that allow accurate identification of the affected hair cells in an experiment. In my research, I have tested for the ototoxic (hearing toxic) effects of Bisphenol A, a common monomer used in the production of polycarbonate plastics and epoxy resins, and have compared these results with a common aminoglycoside antibiotic, neomycin, on 10 neuromasts along the head of the fish. I have concluded that acute exposure to Bisphenol A has some effect on hair cell death; however, 24 hours of chronic exposure along with slightly higher concentrations of Bisphenol A do have measurable ototoxic effects that may lead to hearing loss in humans.

Using Zebrafish to Screen for Hair Cell Damage

Zebrafish serve as an exemplary model for identifying hair cells in toxicity studies, along with lateral line protection studies. There are several reasons why Zebrafish are a great model to use, one of these reasons being that Zebrafish hair cells are anatomically similar in structure and function to human hair cells. Along with this, Zebrafish have what is known as the lateral line, with contain several hair cell bearing organs called neuromasts that are aligned throughout the body of the fish. These neuromasts are the hair cell bearing organs of the fish, and can hold anywhere between 1-30 hair cells. Neuromasts and hair cells play an important role on the livelihood of the fish, as it helps the fish identify wave currents and predation patterns. These neuromasts are equally spread apart from one another, making it easy to accurately identify where hair cells are damaged and missing via fluorescent labeling. Using zebrafish as a model to study BPA toxicity, the research was conducted in an accurate and standardized manner.

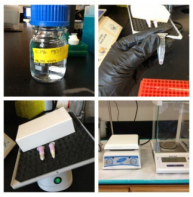
Hypothesis

I have hypothesized that as the zebrafish exposure times to BPA increase, along with the concentrations of BPA that more hair cell damage will begin to take place due to the fact that BPA might by a severe cause of neural cell death. If this is the case, a significant loss of hair cells may be visualized, having some application to hearing damage in humans.

Materials and Methods

The first step in identifying hair cell toxicity of BPA was to place embryo medium, a NaCl solution used to mimic the tropical freshwater habitat of the fish, into one well of a six plate well. A small netted fish basket was then placed into the first well and 10, 5 day post fertilization zebrafish larvae were pipetted into the tube. 10 zebrafish serve as a great sample group that helps keep the experiment accurate and also allows for error that can be caused my abnormal fish that have birth defects. After this, 6mL of embryo medium was placed in the second well of the six well plate and neomycin, a known ototoxic drug, was added at a concentration of 50, 100, 200, and 400µM so that it can serve as a standard for

comparison. The stock concentrations of the neomycin had to be diluted into the 6 mL of embryo medium and this was done by taking the molecular weight of the stock concentration (which was 16.27 mM) and calculating the molarity. 18.5 µl had to be added to 6mL of embryo medium to gain a 50 µl concentration of neomycin, 36.9 µl for a 100 µl concentration, 74 µl for a 200 µl concentration, and 148 µl for a 400 µl concentration. The larvae were then treated in the neomycin for 30 minutes and had 60 minutes of recovery time in embryo medium to allow the fish to recover from the neomycin exposure. 45 minutes into the 60 minute recovery time, 700µl of 0.05% DASPEI (2-(4-(dimethylamino)styryl)-N-ethylpyridinium iodide) dye was added to label the mitochondria within the hair cells of the neuromasts. This same procedure was repeated for the control of no chemical, and for 100 nM, 250nM, 500 nM, 1 mM, 10 mM, 20 mM, and 40 mM of BPA, however, the BPA had six hours and 24 hours of treatment and no recovery time. After the fish were incubated during the DASPEI treatment, 350 µl of an anesthetic called MS-222 was added to the fish so that the neuromasts and the lateral line could be easier to observe under the fluorescent microscope. Proper placement of the fish under the microscope was a key integral aspect to this research, as the fish needed to be placed on their side so that neuromasts were in a proper position to be scored. Many times, the swim bladder of the fish inhibits the fish from staying on its side, causing difficulty in properly positioning the fish and scoring it. Hair cell death was assessed using the Leica Fluorescent microscope and 10 neuromasts around the head were looked at. The neuromasts that were looked at included IO1, IO2, IO3, M2, IO4, O2, MI1, MI2, SO2, and SO1. On a scale of 0-2, each neuromast obtained a rating, 0 meaning that the neuromast was completely absent and dead, and 2 meaning that the neuromast was brightly labeled and viable. By doing this for all ten neuromasts, a score out of 20 was given to each fish. The results were then graphed with the averages for each concentration of the toxin, along with error bars of the standard deviation from my data, using a dose response curve to see how the effects of certain doses of BPA compare to the ototoxic effects of Neomycin. A 2 way ANOVA statistical analysis was run on both BPA concentration and exposure time to see if there was a significant correlation between the two, and if there was a p value< 0.05. The next step in the process was to then determine the accuracy of DASPEI scoring through a hair cell count. This hair cell count was needed because DASPEI is sensitive to the mitochondria in the cell, which means that they cell may not readily absorb the dye if the mitochondria is not functioning correctly. By running a hair cell count, actual individual hair cells were able to be counted, verifying the DASPEI scores. There are three parts to the hair cell count; euthanizing the fish and fixing them in paraformaldehyde overnight, treating the fish with a primary antibody called mouse anti-parvalbumin, and then treating the fish in a secondary antibody called Alexa Fluor 568 goat anti-mouse. The fish must be euthanized for this process to make sure that they are still 5 dpf for the cell counts. By first treating the fish in PFA after euthanization, the tissues of the fish can be preserved so that the proteins in the cells can still readily absorb the antibodies. The primary antibody was used to label all of the hair cells within the neuromasts so that the secondary, fluorescent antibody could tag the first antibody, giving off the fluorescence needed to accurately count the cells. Before the fish were ready to be observed under a high magnification, they were treated in glycerol, to help prevent the fish from bleaching out so that its fluorescence could be maintained.



By looking at the fish under 400 x magnification, each individual hair cell within the neuromast of the fish was observed and counted. This was done for three groups; a control group, 20 µM BPA, and 40 µM BPA, and 10 fish were scored per group. A total of four neuromasts were looked at; IO1, IO2, IO3, and M2.

Results

In my experiment, the data I have collected is directly tied in with my hypothesis. The results of my experiment show that the 24 hours of exposure to high concentrations of BPA do in fact cause a significant amount of hair cell damage (see figure 1 in appendix for graph). After running statistical analysis, time is shown to have an effect on hair cell survival, with a p value of 0.002. BPA concentrations themselves have a p value of 0.007 which is also a significant value, which has an effect on hair cell death. Neomycin was my standard of comparison for all BPA tests (see figure 2 in appendix) and is a definite ototoxic antibiotic. When compared to 6 hours of BPA with low concentrations, there isn't a significant amount of ototoxicity shown (see figure 3 in appendix). When combining neomycin and BPA together to determine whether or not BPA sensitizes hair cells and makes it more susceptible to the damaging effects of known ototoxins, there is a slight correlation (see figure 4 in appendix). My final, time course experiment shows that that as time progresses, BPA does show higher otoxicity (shown in figure 5 in appendix). My results through the DASPEI analysis was verified through the hair cell counts, as the number of total hair cells of 4 neuromasts decreases has BPA concentrations increase (see figure 6 in appendix).

The Future

With this research, one simple change can be the implementation of my research. One family switching to stainless steel bottles is reducing their risk of diabetes, cancer, infertility, and even hearing loss. Though motivating the consumer to make smart choices about what products they buy, I believe that as a whole, our world can significantly change the way companies manufacture and create their products. By saying "no" to BPA, companies will be forced to create alternative products and together we can create a BPA free, healthy environment that reduces the risk of obtaining many health ailments. I hope that my research can be that push for changing the perspective of the common American household, and that with my research, we can become one step closer to a BPA- free planet.

Appendix

Figure 1:

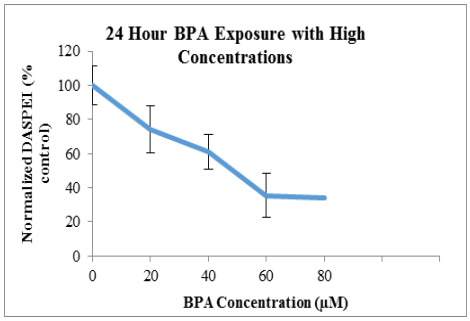


Figure 2:

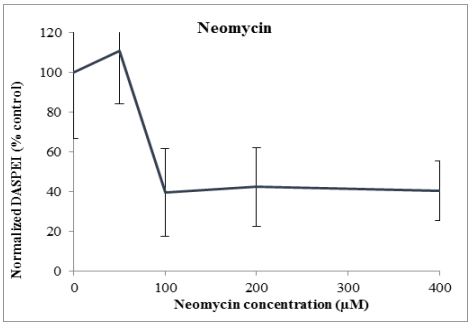


Figure 3:

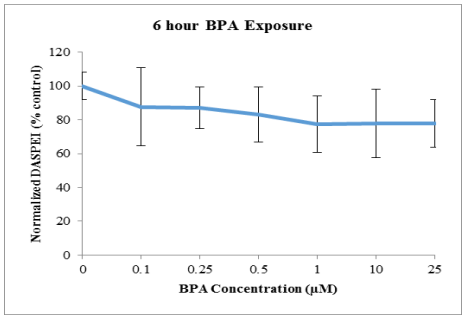
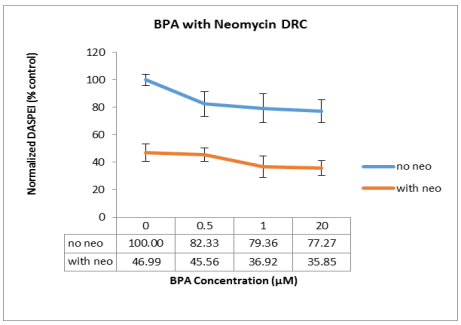


Figure 4:



	0	0.5	1	20
no neo	100.00	82.33	79.36	77.27
with neo	46.99	45.56	36.92	35.85

Figure 5:

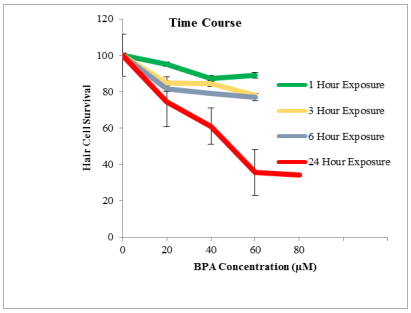


Figure 6: