The Negative Effects of the Exposure of Nicotine to Zebrafish Embryos

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Abstract

Nicotine has many adverse effects on humans. Research shows humans have an increased heart rate and a higher mortality when exposed to nicotine. This paper discusses the effects nicotine has on the heart rate and mortality of zebrafish. It was also found that there may be a correlation between nicotine exposure and time of birth. These results from the experiment showed the negative, and sometimes threatening effects of nicotine exposure. The results can be compared to those of humans as well. For example, zebrafish and humans both have an increased heart rate when exposed to nicotine. The purpose of this experiment was to highlight some negative effects of nicotine. Nicotine was chosen because it is a dangerous substance that can cause much harm to humans. This experiment lasted one week. The zebrafish were placed in a well tray, each with different amounts of nicotine (0.0 mg/mL nicotine, 0.05 mg/mL nicotine, 0.1mg/mL nicotine, and 0.2 mg/mL nicotine). The results from this experiment show that zebrafish had a lower heart rate (measured in bpm (beats per minute)) when exposed to a low amount of nicotine (0.05 mg/mL nicotine and 0.1 mg/mL nicotine), but a very high heart rate when exposed to the greatest amount of nicotine. Mortality was very high in two of the wells with nicotine; in fact all the zebrafish in these two wells were dead (and all were decomposing) by the end of the experiment (96 hpf (hours post fertilization)). These results are similar to those showing the effects of nicotine exposure to humans. Research shows an increased heart rate and mortality of humans as a result of smoking. These results of zebrafish embryos exposed to nicotine can be used to educate people on the negative effects of nicotine exposure to humans.

Introduction

Nicotine is a dangerous substance present in many smoking devices. Nicotine is highly addictive and can lead to many severe health threats (e.g. lung cancer, chronic bronchitis, and emphysema), according to the National Institute on Drug Abuse (2018). These diseases can be life threatening; in fact, according to the Centers for Disease Control (2014), “Lung cancer... is now the nation’s most common cancer killer among both men and women”. It only takes eight seconds for this dangerous chemical to reach the brain (National Institute on Drug Abuse, 2007). According to the National Institute on Drug Abuse (2007), “Nicotine is highly addictive; in fact, it is as addictive as heroin and cocaine”. There are also negative side effects to nicotine consumption such as increased heart rate, more alertness, lessened appetite, and higher blood pressure (National Institute on Drug Abuse, 2007). In addition, many studies have proven that nicotine has an effect on heart rate. For example, Haass and Kübler (1997) said, “Nicotine increases heart rate, myocardial contractility, and blood pressure”. The National Institute on Drug Abuse (2007) found “Nicotine affects the neurotransmitter acetylcholine and its receptor. This receptor is located in many brain structures and body organs. It carries messages related to respiration, heart rate, memory, alertness, and muscle movement”. Acetylcholine is a neurotransmitter which transmits signals about certain things (respiration, heart rate, memory,

alertness, and muscle movement), according to the National Institute on Drug Abuse (2007). If acetylcholine and its receptors are not working correctly, these signals may be incorrect. Therefore respiration, heart rate, memory, alertness, and muscle movement could be affected. This would suggest that the heat rate, for example, could increase abnormally. This proves that the heart rate when something is exposed to nicotine may be different than when it is not exposed to nicotine. In addition, an increased heart rate could increase mortality. "Smokers lose at least one decade of life expectancy, as compared with those who have never smoked" (Jha et al., 2013). This suggests that smoking has lead to an increased mortality in humans. According to Lushniak, a former Surgeon General (2014), "Cigarette smoking remains the single leading cause of preventable mortality in the United States" (p. 678). Smoking (a preventable disease) caused the most deaths in the United States in 2014. This evidence proves nicotine has an effect on humans, and increases mortality.

The following experiment tests the effects of zebrafish and zebrafish embryos when they are exposed to nicotine by measuring the heart rate and mortality. Is there a higher heart rate in zebrafish exposed to nicotine? Is the mortality higher in zebrafish exposed to nicotine? These are questions that were answered in the experiment. This experiment was performed to assess the effects of nicotine on zebrafish such as mortality and heart rate. The results compared to those of humans as well. If zebrafish embryos are exposed to nicotine, then they will have a higher mortality and after they hatch their heart rates will be higher than if they were not exposed to nicotine, because nicotine affects the acetylcholine neurotransmitter which sends signals affecting the heart rate.

Materials and Methods

Materials

The materials used in this experiment were one 12-hole well tray with cover (see Figure 1), fine bore pipettes (amount varied by use and by day), large bore pipettes (amount varied by use and by day), four 100 mL beakers for the nicotine (each was diluted so that 1 mL of the substance was 0.0 mg/mL of nicotine, 0.05mg/mL of nicotine, 0.1 mg/mL of nicotine, and 0.2 mg/mL of nicotine), 50 mL beaker/s (amount varied by use and by day), one microscope, 40 zebrafish embryos (10 in each well), one Sharpie, one incubator at 28.5°C (provided by teacher), three strips of blue tape for labeling, and one 100mL beaker of the clean solution (0.0 mM ethanol).

Figure 1: Part of the labeled well tray.

Methods

First, the well tray was labeled (with blue tape and a Sharpie) with the amounts of nicotine (in the order of 0.0 mg/mL. 0.05 mg/mL, 0.2 mg/mL, and 0.1 mg/mL), the substance (nicotine), and the observer's name and hour (Anna Cerroni, hour 7). Next, ten zebrafish embryos were selected for each of the four wells being used in the tray. Only the top four wells on the tray were used. The embryos chosen for the experiment were translucent to the naked eye. White embryos were avoided as this would mean they were dead. Once selected, the embryos were examined under a microscope to make sure there were exactly ten healthy

zebrafish in each section. Unhealthy or dead zebrafish had a dark spot on them. These were removed and replaced with healthy ones. Using a fine bore pipette, the liquid was extracted from each well and placed into a 50 mL beaker. Then, the nicotine was added to the corresponding wells. The substances in each of the four 100 mL beakers were already diluted, so that 1 mg/mL of nicotine would be the correct amount of nicotine for each well. Each beaker had a different dilution ratio which corresponded with the amount of nicotine needed for each well. The large bore pipette was filled with 2 mg/mL of nicotine, and the substance was released into the well until there was 1 mg/mL left in the large bore pipette. This would lessen the risk of air bubbles. This was repeated for all four of the wells. Photos and/or videos were also taken of each well with zebrafish. The well tray was placed in a 28.5 °C incubator overnight.

On day two, three, and four, the embryos and hatched zebrafish were examined. Observations were written down. Qualitative observations included the heart rate in bpm (this was measured by counting the amount of heartbeats of a zebrafish for ten seconds, and multiplying that by six), responsiveness, hatched/unhatched, turned upright, color, location in well, shape, size, and other important information. This information was found by looking at the zebrafish and zebrafish embryos, usually through a microscope. In some cases, a drawing was used to show the shape or location of the zebrafish. Quantitative data included how many had hatched and how many were alive after a certain number of hours (0 hpf, 24 hpf, 48 hpf, 72 hpf, and 96 hpf) in each well. The nicotine was again extracted, and replaced with a fresh solution. Next, if there were living hatched zebrafish, the heart rate was measured, and the zebrafish were tested for response to stimuli. This was done by gently tapping their side and/or back with a fine bore pipette. A fresh pipette was used each time to not contaminate other wells (if the pipette was used in a higher amount of nicotine prior). Gloves were provided for the nicotine to avoid coming in contact with skin. Dead and/or decomposing zebrafish were extracted by a fine bore pipette. In addition, photos and/or videos were taken, as well as observations (qualitative and quantitative data). Each night, the well trays were covered up and placed in a 28.5 °C incubator. The previous steps were repeated on day five, but instead, the wells containing living zebrafish were filled with a clean solution (see Figure 2). These zebrafish were placed in a nursery tank to further develop. A Chi Square analysis of the data was completed to ensure statistical significance.

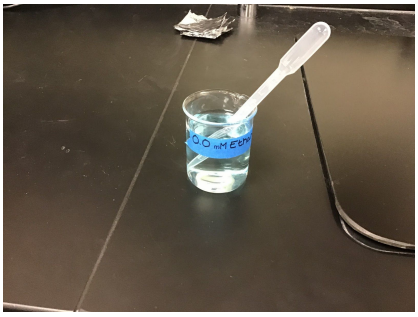

Figure 2: Clean solution (0.0 mM ethanol) and a large bore pipette.

Results

This study was designed to test the effects of nicotine on zebrafish. The experiment was set up to create an easy way to measure mortality and heart rate in the zebrafish and zebrafish embryos exposed to increasing concentrations of nicotine. The dependent variables in this experiment were the heart rate and mortality. The independent variables in this experiment were 0.0 mg/mL of

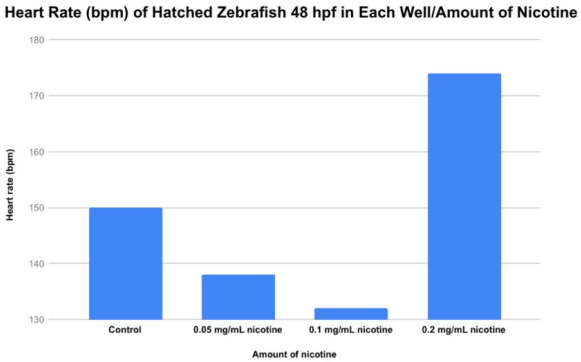

Figure 3: Figure 3 shows the heart rate (in bpm) of a zebrafish that is hatched in each well 48 hpf.

nicotine, 0.05 mg/mL of nicotine, 0.1 mg/mL of nicotine, and 0.2 mg/L of nicotine. The control group was the well with 0.0 mg/mL of nicotine. The controlled variables in this experiment were the temperature in the incubator (28.5 °C), amount of zebrafish in each well, time in incubator, and size of the wells.

The chi square value was 22.42, and the null hypothesis was rejected using a degree of freedom of three and a critical value of 7.82. This means the chi square value (22.42) was higher than the critical value, and the results were not by chance alone, but due to the application of nicotine.

It was found that as the zebrafish that were in the well with 0.2 mg/mL of nicotine hatched, the heart rate of one of the zebrafish in this well was dramatically higher than that of the zebrafish in other wells (48 hpf). However, as the concentration of nicotine increased (from control to 0.1 mg/mL nicotine), the heart rate of the zebrafish (48 hpf) lowered (see Figure 3). The heart rate of a hatched zebrafish in the control well was 150 bpm 48 hpf and 130 bpm 72 hpf. The heart rate of a hatched zebrafish in the well with 0.05 mg/mL nicotine was 138 bpm 48 hpf. The heart rate of a hatched zebrafish in the well containing 0.1 mg/mL was 132 bpm 48 hpf, 144 bpm 72 hpf, and 222 bpm 96 hpf. The heart rate of a zebrafish in the well with 0.2 mg/mL nicotine was 174 bpm 48 hpf.

Table 1 illustrates that as the concentration of nicotine increased, the zebrafish started to hatch sooner. For example: in the control well and the well with 0.05 mg/mL nicotine, one zebrafish hatched in the first 48 hpf, but in the other wells with higher amounts of nicotine (0.1 mg/mL nicotine and 0.2mg/mL nicotine) no zebrafish hatched until 72 hpf.

As shown in Figure 4 and Figure 5, the zebrafish embryos died in the first 24 hpf in the wells containing less nicotine (control group and 0.05 mg/mL nicotine). Throughout the rest of the week, no more zebrafish died in the control group in the well with 0.05 mg/mL, none died 48 hpf, but by 72 hpf all of the zebrafish had died. One zebrafish embryo died in the first 24 hpf in

Amount of Zebrafish in Each Well (control, 0.05mg/mL nicotine, 0.1 mg/mL nicotine, and 0.2 mg/mL nicotine) the Hatched Each Day (24 hpf, 48 hpf, 72 hpf, and 96 hpf)

Amount of Nicotine	24 hpf	48 hpf	72 hpf	96 hpf
Control (0.0mg/mL nicotine)	0	1	5	0
0.05 mg/mL nicotine	0	1	4	0
0.1 mg/mL nicotine	0	0	5	3
0.2 mg/mL nicotine	0	0	8	0

Table1: This table shows how many zebrafish hatched each day in each well. Some days zero zebrafish embryos hatched, while other days five zebrafish embryos hatched.

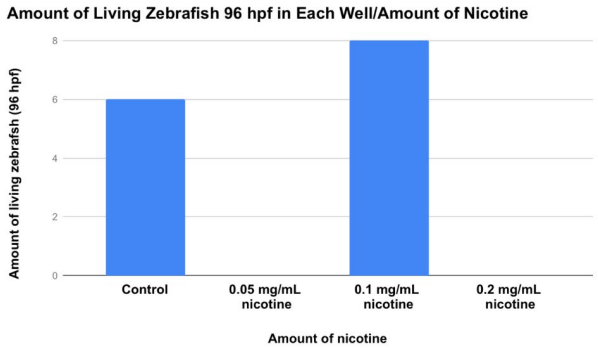

Figure 4: Figure 4 shows the total amount of living zebrafish in each well at the end of the experiment.

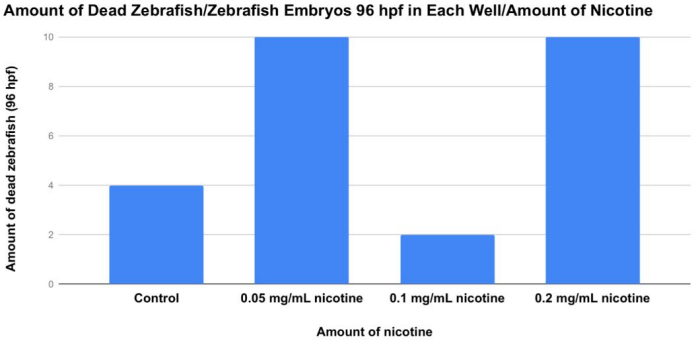

Figure 5: Figure 5 shows the amount of dead zebrafish/zebrafish embryos in each well at the end of the experiment.

the well containing 0.1 mg/mL nicotine. This remained consistent until 96 hpf when one zebrafish had died. Two zebrafish embryos died in the well containing 0.2 mg/mL nicotine in the first 24 hpf. This remained unchanged 48 hpf, but seven more had died 72 hpf and started decomposition. In the well with 0.2 mg/mL nicotine, all the zebrafish embryos had died and some started decomposing by the end of the week (see Figure 6).

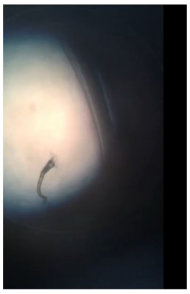

Figure 6: Figure 6 shows a picture under a microscope of a zebrafish 96 hpf that was dead and had started decomposition in the well with the highest concentration of nicotine. It was the last living zebrafish in this well.

Discussion

The experimental hypothesis was if zebrafish embryos are exposed to nicotine, then after they hatch their heart rates will be higher and they will have a higher mortality than if they were not exposed to nicotine because nicotine affects the acetylcholine neurotransmitter which sends signals about the heart rate. Previous research shows nicotine negatively effects mortality and heart rate of zebrafish. In humans, mortality can be increased because of smoking. Also, many studies have shown an increase in humans' heart rates when exposed to nicotine. This is because the nicotine affects a neurotransmitter which sends messages to the heart. The evidence proves the heart rate of the zebrafish was affected by the nicotine. The heart rate of a zebrafish in the well with 0.05 mg/mL nicotine 48 hpf was lower that the heart rate of a zebrafish in the control group, and the heart rate of a zebrafish 48 hpf in the well with 0.2 mg/mL nicotine was even lower. However, the heart rate of a zebrafish 48 hpf in the higher concentration of nicotine was dramatically higher than the zebrafish in other wells (see Figure 3). The heart rate of a zebrafish in the control group 48 hpf was 150 bpm (it is possible the zebrafish that were tested for heart rate throughout the experiment were newly hatched. It was speculated during the experiment that zebrafish which had recently hatched seemed to have a higher heart rate than those who were not newly hatched).

The heart rate of a zebrafish in the control well was also measured 72 hpf, and had lowered to 130 bpm. The heart rate of a zebrafish of the well with 0.1 mg/mL nicotine was also measured 72 hpf and had risen to 144 bpm. The heart rate of a zebrafish in this well was also measured 96 hpf and was 222 bpm (the zebrafish measured seemed to be newly hatched). The reason for this difference in unknown, but there may be a correlation with nicotine since the evidence suggests an irregular/different heart rate in zebrafish exposed to nicotine.

The results suggest an increased mortality for the zebrafish exposed to nicotine. This would prove the hypothesis correct because all the zebrafish in two of the wells with nicotine were dead and some were decomposing by the end of the experimental week (see Figure 6). It was interesting, however, that the zebrafish in the well with 0.1 mg/mL nicotine survived longer than the other zebrafish in the wells with 0.05 mg/mL nicotine and 0.2 mg/mL nicotine. Furthermore, the least amount if zebrafish died in the well with 0.1 mg/mL nicotine by the end of the experiment (see Figure 4 and Figure 5). Previous research has shown that nicotine affects mortality. Research shows that smoking causes the most deaths that are preventable (Lushniak, 2014, p. 678). Higher mortality may also be due to and/or correlated with an increased heart rate. This is shown in this study. The zebrafish in the well with the highest concentration of nicotine had the highest heart rate, and many were decomposing by 96 hpf (see Figure 6). Furthermore, the null hypothesis was rejected, so the nicotine had an effect on the nicotine, and it was not

chance that some zebrafish were dead. This proves part of the hypothesis true, since zebrafish had a greater mortality when exposed to nicotine.

According to Table 1, the zebrafish in the wells with a higher concentration of nicotine (0.1mg/mL nicotine and 0.2 mg/mL nicotine) hatched later than the zebrafish in the wells with a lower concentration of nicotine (control well and 0.05 mg/mL nicotine). It is speculated that this is a result of the nicotine in the wells. Does nicotine affect the time it takes for a zebrafish embryo to hatch?

This experiment could have been improved in many ways. For example, it could have been repeated, had more wells with different amounts of nicotine, and/or had more zebrafish to test. If this experiment was repeated, the results could have been more accurate, and there would be more data to compare. In Day 1 of the experiment (set-up day), there was a confusion with the amounts of nicotine in each well. When adding the nicotine to each well, the solution with 0.2 mg/mL nicotine may have been added to the well labeled for 0.1 mg/mL nicotine, and the same for the well labeled for 0.2 mg/mL nicotine. Due to this, the experiment should be repeated.

This study was conducted to test the negative impacts of nicotine. It was found that exposure to nicotine affected the mortality, heart rates, and time of birth of zebrafish. The evidence relates to previous research. Evidence from this experiment and previous research both show a decreased life span and elevated heart rate with exposure to nicotine. The results from zebrafish and zebrafish embryos are similar to those of humans. Both zebrafish and humans demonstrated an increased mortality and heart rate when exposed to nicotine. This study answers the question of how nicotine exposure affects the heart rate and mortality of humans.

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