The Effect of Ibuprofen on the Mortality Rate of Zebrafish Embryos

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I. Title

Effect of ibuprofen on the mortality rate of zebrafish embryos

II. Background Information

Ibuprofen is a non-steroidal anti-inflammatory drug used to reduce everyday pain such as headaches, minor injuries, common colds, etc. Ibuprofen is not recommended to take during pregnancy because during the third trimester the ibuprofen may lead to one of the vessels in a fetus's heart closing. Therefore increasing blood pressure and possibly decreasing heart rate. An experiment we found (Dao-jie Xu 2011) showed that celecoxib, a prescription anti-inflammatory drug, has a negative effect on the heart rate of zebrafish. Because advil and celecoxib are both anti-inflammatory drugs we infer they will have similar effects. We chose the variable of mortality rate instead of heart rate in this situation because with the materials we had available to us, it is a much easier quantitative variable to measure.

Dao-jie Xu. "Celecoxib Impairs Heart Development Via Inhibiting Cyclooxygenase-2 Activity in Zebrafish Embryos." *Home*. N.p., Feb. 2011. Web. 30 Oct. 2011.

Hypothesis

If the concentration of ibuprofen is increased, then the mortality rate of zebrafish embryos will increase because Ibuprofen is a blood thinner and will make the fish die.

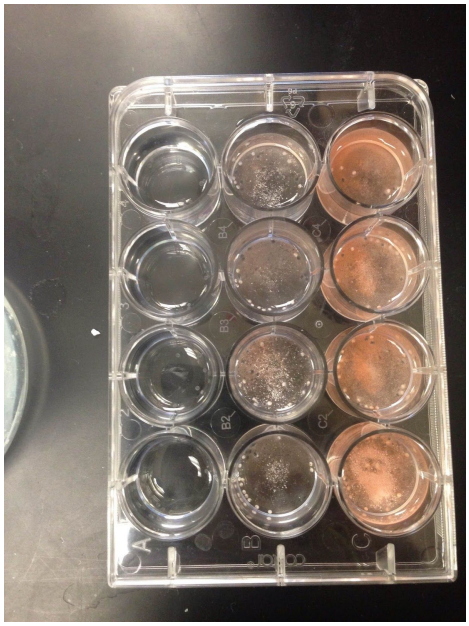
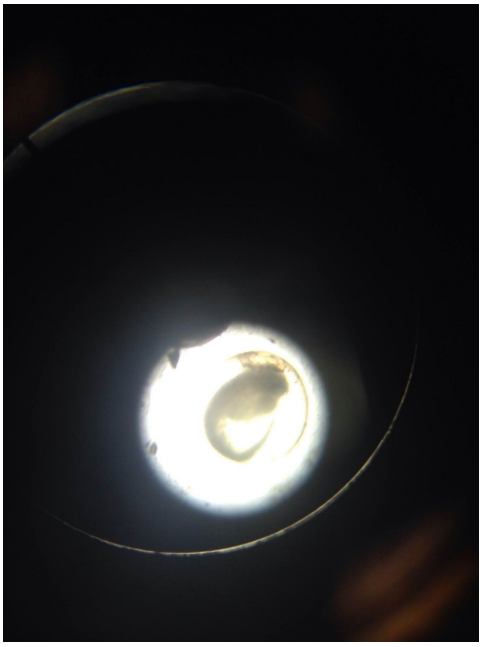
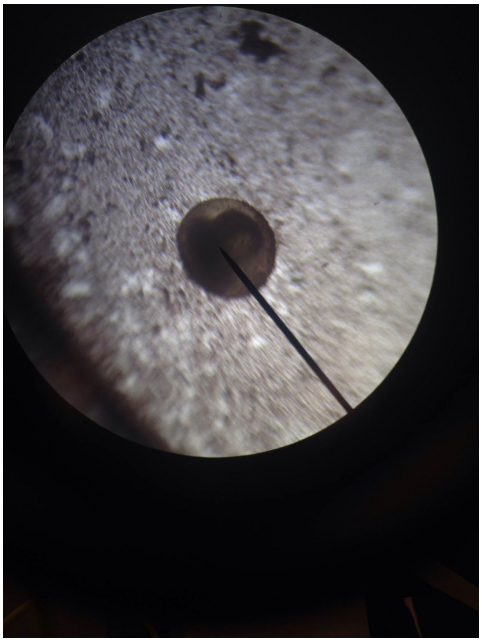
Materials

12 circular Wells
1 Microscope
.03 grams of Ibuprofen
10-15 Zebrafish embryos per well
Measurement tool for advil-scale
Camera
Incubator
Pipettes

Procedure

1. Prepare 10-15 embryos in each well
2. Crush the advil until it is a powder
3. Create two different solutions of the advil mixed with instant ocean (.03 g. Ibuprofen/34 ml Instant ocean, .03 g. Ibuprofen/68 ml Instant ocean), and one solution with only Instant ocean.
4. Put about 5 ml of solution into each well using a pipette
5. Label which wells are the control group and which wells have what amounts of advil

6. Take pictures
7. Place embryos into incubator overnight
8. Record Number of living embryos each day, and remove dead or infected embryos.

Fish wells after procedure (left), Living Zebrafish Embryo (Middle), Dead Zebrafish Embryo (Right)

Abstract

In this experiment, we tested how the anti-inflammatory drug Ibuprofen affects the health of Zebrafish embryos. Zebrafish have very similar genomes to humans, so it could have a correlation with taking anti-inflammatories while pregnant. Ibuprofen is a painkiller/anti-inflammatory which can be bought over the counter and can cause complications in a fetus during the third trimester of pregnancy if taken my the mother. In our experiment, we took different concentrations of an Ibuprofen/instant ocean solution to test what effect it had on the zebrafish. In both experimental groups where Ibuprofen was used, all the fish died. In conclusion, we saw that Ibuprofen has a very negative effect on zebrafish, which could make people become more worried about the effect of Ibuprofen has while they are pregnant, since it killed all of the Zebrafish it was tested on.

Data

Number Of Living Zebrafish Embryos	Concentration of Ibuprofen in Instant Ocean (g/ml)		
	Control (No Ibuprofen)	.03 g. Ibuprofen/34ml Instant Ocean	.03 g. Ibuprofen/68ml Instant Ocean
	a1: (Day 1=11, Day 2=11, Day3=11)	b1: (Day 1= 13, Day 2=0, Day 3=0)	c1:(Day 1= 15, Day 2=0, Day 3=0)
	a2:(Day 1=10, Day 2=10, Day 3=10)	b2: (Day 1=13, Day 2=0, Day 3=0)	c2:(Day 1= 16, Day 2=0, Day 3=0)
	a3:(Day 1=8, Day 2=8, Day 3=8)	b3: (Day 1=14, Day 2=0, Day 3=0)	c3:(Day 1=12, Day 2=0, Day 3=0)
	a4:(Day 1=13, Day 2=13, Day 3=13)	b4: (Day 1=10, Day 2=0, Day 3=0	c4:(Day 1=10, Day 2=0, Day 3=0)

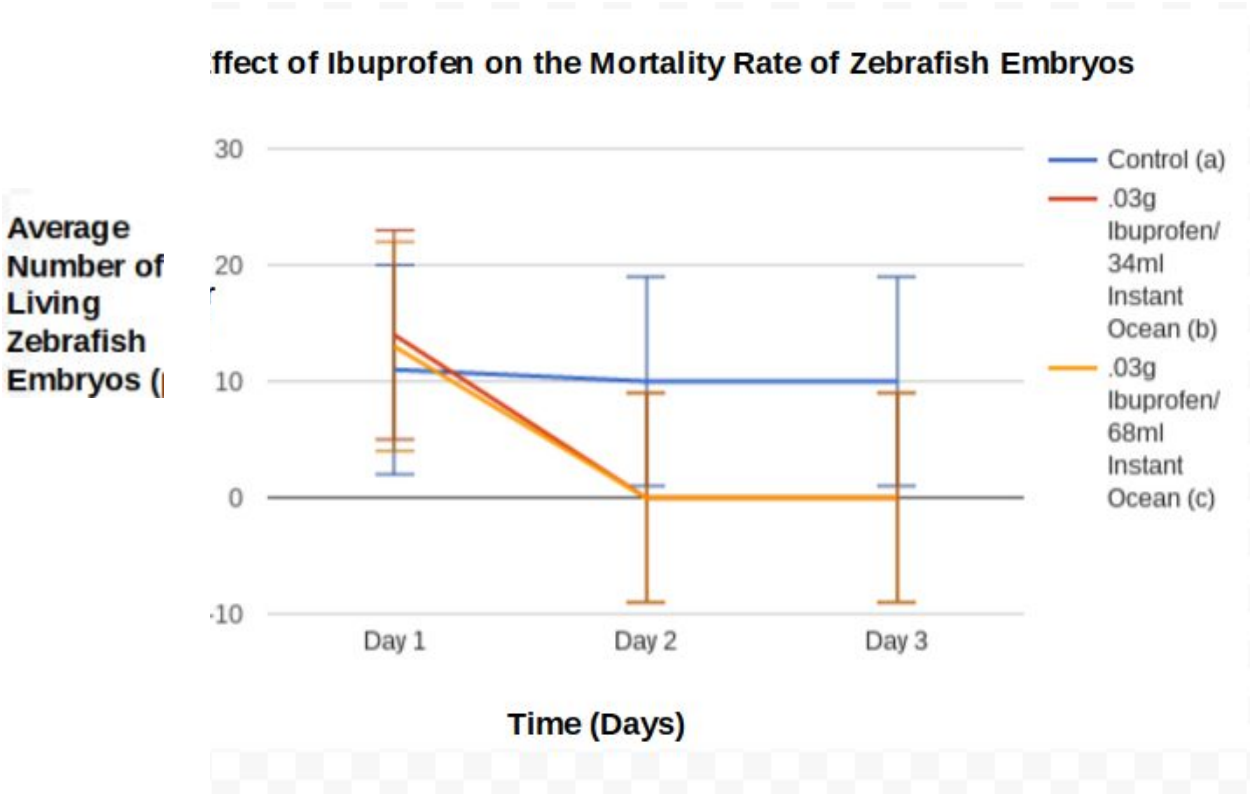
NOTE: 1st day we did NOT have solutions ready, so we only had 3 experimental days.

After calculating the SEM, SD, and P difference, I can conclude that our data is very accurate.

P value and statistical significance:
The two-tailed P value is less than 0.0001
By conventional criteria, this difference is considered to be extremely statistically significant.

Group	Control	Groups b and c (same results)
Mean	0.975	0.000
SD	0.050	0.000
SEM	0.025	0.000
N	4	4

Graph



Trend Statement

Control: As the days passed, the number of living fish only decreased by one, then plateaued.
A.: As the days passed, the number of living fish decreased quickly to zero.
B.: As the days passed, the number of living fish decreased quickly to zero.

Sources of Error

1. One source of error we had was that we didn't have the same number of zebrafish embryos in each well. This could have caused our ending data to be less accurate.
2. Another source of error we had was that we only had 2 experimental groups. If we would have been able to have more time and do more math to make safely concentrated solutions, our experiment would might have had better results.
3. One more source of error we encountered was that the pills we crushed up had to be crushed up with their outer reddish coating, which could have caused the results to be different. If we had just Ibuprofen medicinal powder, then we might not have ran into that issue.

Analysis

In this experiment, we tested Ibuprofen's effect of the mortality rate of Zebrafish embryos. We hypothesized that as the concentration of Ibuprofen increased, then the mortality rate would increase as well because the blood-thinning aspect of the drug would be hard on the fish. Our hypothesis was supported in this experiment, but we were surprised with how well it was supported. Something that supports the accuracy of the data is that the SEM was only 0.025 in the control group and 0 in the 2 experimental groups. The P difference was only 0.001, which is very promising for our results. In our control group, the mean (average) percent of fish that survived was 97.5%, and in the experimental group 0% survived. This is very strong in supporting the fact that Ibuprofen kills Zebrafish embryos. However, we do not know whether or not the coating of the pill we crushed up had any effect on the fish. We also do not know if we were using absurd concentrations of Ibuprofen either. These are both very important in finalizing the legitimacy of our data, so it is hard to say, even though are statistics are sounds based on our data, that our results are as accurate as they seem. Had we done this experiment again and had more resources, I believe it would be good to conduct an experiment more similar to the one we based ours off of. This experiment used Celecoxib as the anti-inflammatory drug and measured heart rate instead of mortality rate of the embryos (Dao-Jiie Xu, 2016). Since heart rate is a lot more precise and can be measured with less time between intervals, I think we would have had more conclusive results. I also think that if we had more time, we should have tried to remove the pill coating and done more research on how much medicine to use. This experiment gives me a better understanding of what this drug is capable of. Even though the results may not be accurate, it cannot be safe for a baby if it kills Zebrafish embryos. It really shows just how important it is to make sure you know what you are putting into your body.

References

Dao-jie Xu. "Celecoxib Impairs Heart Development Via Inhibiting Cyclooxygenase-2 Activity in Zebrafish Embryos." *Home*. N.p., Feb. 2011. Web. 30 Oct. 2011. From http://anesthesiology.pubs.asahq.org/article.aspx?articleid=1933376