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Ibuprofen: Effects on Developing Zebrafish Embryos

Abstract

This experiment tested the effects of the equivalent amount of two doses of human ibuprofen on zebrafish embryos for varying amounts of time. The four groups tested were a control group with no ibuprofen, a group exposed for the first 48 hours of the experiment, a group exposed from 48 to 72 hours, and a group exposed for all 72 hours of the experiment. The groups were put into wells of 5 embryos with a few drops of methyl blue and either 1 mL of instant ocean or 1 mL of two doses of ibuprofen. The zebrafish spent the majority of their time in a 28.5°C incubator. The solutions were changed and data was recorded at 48 and 72 hours of the experiment. Survival rate, deformities and hatched vs unhatched data was recorded.

The purpose of this experiment was to see the effects ibuprofen had on zebrafish embryos of varying exposure time. This specific experiment was performed to see the effects of the equivalent amount of two doses of human ibuprofen on zebrafish embryos over varying amounts of time. The results of this experiment showed that ibuprofen had an effect on how zebrafish embryos develop and their survival and hatching rates. The data for survival rates showed that if zebrafish were exposed to ibuprofen for more than one day their survival rate decreased. Hatching rates were affected if the zebrafish embryos were exposed to ibuprofen all days or the last day of the experiment. More deformities and were shown in all groups of exposed embryos.

The results from this experiment give conjecture about what happens when pregnant and breastfeeding mothers consume ibuprofen. Humans and zebrafish have similar body construction and genes, which allows the effects seen on zebrafish to be inferred to humans. Using the effects from this experiment, as a representation, it is estimated that exposure to ibuprofen during pregnancy can result in a higher rate of birth deformities and lower survival rates in human fetuses.

Introduction

Zebrafish are used as a model for humans due to their physiological similarities and ease of evaluating. Zebrafish are more convenient and cheap to store and transport than other test animals, allowing a lot of test subjects in a small area. The zebrafish embryos are transparent which allows researchers to see the development of them (Kumar, etc al, 2012). Quick and plentiful breeding, along with fast growth, allows researchers to study a multitude of eggs per study (Burke, 2016). In terms of physiological similarities, humans, and zebrafish share a lot of the same features; including two eyes, a mouth, a pancreas, liver, bile ducts, kidney, esophagus, blood, cartilage, bone, muscles, nose, ears, heart, spinal cord, and intestines (Burke, 2016). In addition to this, they also share 70% of the same genes. Testing zebrafish allow us to see the effects of toxicants on zebrafish and their DNA, which in turn, gives insight on how it affects humans (Burke, 2016).

Ibuprofen is a common, non-steroidal anti-inflammatory drug that is widely used due to its non addictive properties (Nordqvist, 2017) Ibuprofen is popular for pain, inflammation, and fever relief due to its over the counter accessibility. It can be consumed in tablets, gels, mousses, and sprays (Nordqvist, 2017). Ibuprofen lessens pain and swelling by inhibiting the production prostaglandins, which are found in the brain and work in response to bodily pain signals (Nordqvist, 2017). Ibuprofen is not classified as an addictive drug, but should not be taken in a high dosage(over 3,200 mg). Serious side effects of taking ibuprofen can include strokes or heart attacks. Along, with these effects there are a multitude of adverse side effects (Nordqvist, 2017).

Ibuprofen has been commonly used by pregnant women, it has been reported to have been used by an average of 10% (Ben Maamar, etc al, 2017). Pregnant mothers taking ibuprofen are at increased risk of complications. If taken during the third trimester ibuprofen can cause heart damage, lung damage, or even death due to its ability to close the heart of the infant. Other third trimester complications include high blood pressure in the fetus' lungs and low amniotic fluid (Briggs, 2017). Taking ibuprofen in the first trimester, or early on in general, may increase the likelihood of miscarriages (Briggs, 2017).

How will ibuprofen affect zebrafish embryos? The hypothesis of this experiment is that the sections of zebrafish embryos exposed for all three days will have the lowest survival rate, and most birth deformities. It is also hypothesized that all sections of zebrafish embryos exposed will have a lower survival rate and more birth deformities than a control section.

Materials and Methods

Amount	*Item*
1	Well Plate
85	Zebrafish Embryos
1 bottle	Instant Ocean
1 bottle	Methyl Blue
1 bottle	Ibuprofen Solution
9	Pipettes
1	Dissection and Compound Microscope
1	Phone Camera
1	Embryo and Liquid Disposal Beaker
1	Incubator
1	Permanent Marker
3 pieces	Tape

Procedure

Day 1:

1. Obtain new, clean well plate package from instructor.
2. Open and label well plate with appropriate concentration labels.
3. Put supplies away.

Day 2:

1. Retrieve well plate.
2. Obtain zebrafish embryos, instant ocean, methyl blue, ibuprofen solution, pipettes, and dissection and compound microscope.
3. Use a pipette to retrieve 5 fertilized zebrafish embryos for well C1. Add a few drops of methyl blue and 1 mL of instant ocean to C1. Repeat the procedure for wells C2, C3, E1, E2, and E3.
4. Use a pipette to retrieve 5 fertilized zebrafish embryos for well K1. Add a few drops of methyl blue and 1 mL of ibuprofen solution to K1. Repeat procedure for wells K2, K3, A1, A2, and A3.
5. Use microscope to make sure all wells have 5 fertilized embryos.
6. Cover well plate and place in 28.5 °C incubator. Put remaining supplies away.

Day 3:

1. Retrieve well plate from incubator and remove cover. Obtain instant ocean, methyl blue, ibuprofen solution, pipettes, disposable beaker and dissection and compound microscope.
2. Use microscope to record data regarding hatched and unhatched embryos in well plate. Remove any dead embryos from well plate, put in disposal beaker.
3. Use pipette to clean out liquid from well K1 without taking any hatched or unhatched embryos out. Add a few drops of methyl blue and 1 mL of instant ocean to K1. Repeat procedure for wells K2, K3, C1, C2, C3, and C4.
4. Use pipette to clean out liquid from well E1 without taking any hatched or unhatched embryos out. Add a few drops of methyl blue and ibuprofen solution. Repeat procedure for wells E2, E3, A1, A2, and A3.
5. Observe wells through microscope and take pictures.
6. Cover and return well plate to 28.5 °C incubator. Put remaining supplies away.

Day 4:

1. Retrieve well plate from incubator and remove cover. Obtain pipettes and dissection and compound microscope.
2. Use microscope to record data regarding hatched and unhatched embryos in well plate.
3. Dispose of embryos and clean up lab station.

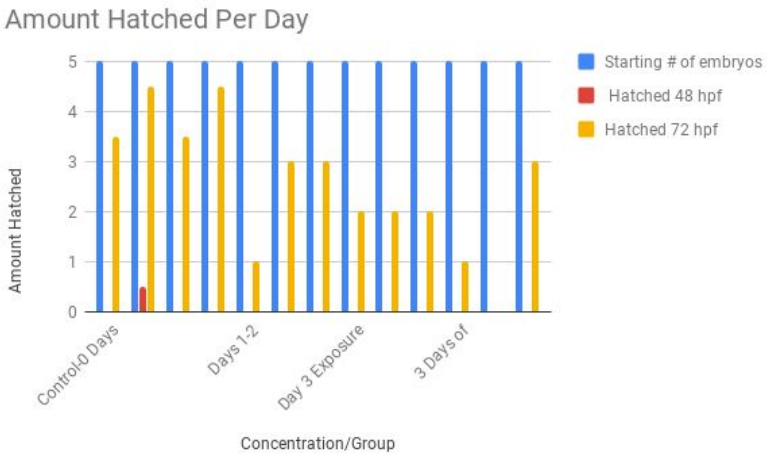
The procedure above was taken from UW-Milwaukee SEPA Program.

Data

Data Table 1: The overall data recorded for this experiment

Days of Exposure	*Well*	*Starting # of embryos*	*# alive after 48 hr*		*# alive after 72 hr*	
			Unhatched	Hatched	Unhatched	Hatched
Control-0 Days of Exposure	C1	10	10	0	5	5
	C2	10	9	1	1	9
	C3	10	10	0	1	7
	C4	10	10	0	1	9
Days 1-2 Exposure	K1	5	4	0	2	1
	K2	5	5	0	1	3
	K3	5	5	0	1	3
Day 3 Exposure	E1	5	5	0	3	2
	E2	5	4	0	2	2
	E3	5	5	0	3	2
3 Days of Exposure	A1	5	4	0	2	1
	A2	5	5	0	4	0
	A3	5	4	0	1	3

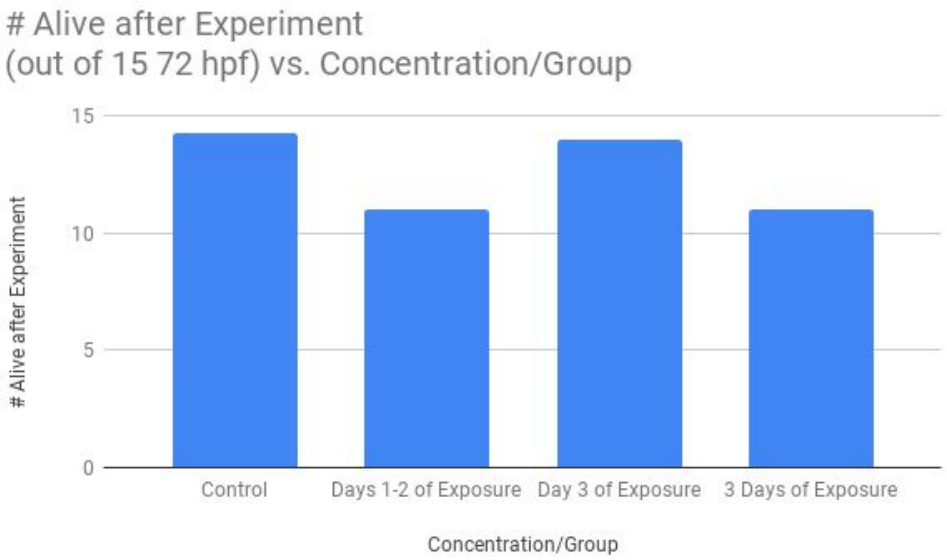
Figure 1: Shows the total amount of embryos hatched per well of each group each day



Data Table 2: Amount of zebrafish alive at 72 hpf

Concentration/Group	Control	Days 1-2 of Exposure	Day 3 of Exposure	3 Days of Exposure
# Alive after Experiment (out of 40)	14.25	11	14	11

Figure 2: Shows the amount of zebrafish alive at 72 hpf(out of 15)



Survival Rate

Data Table 3: Compares survival rate of all groups vs all other groups using a T-test 72 hpf

Test	P-Value	Significance
Control vs Days 1-2 of Exposure	.0446	Significant
Control vs Day 3 of Exposure	.8457	Not Significant
Control vs 3 Days of Exposure	.0446	Significant
Days 1-2 of Exposure vs Day 3 of Exposure	.1012	Not Significant
Days 1-2 of Exposure vs 3 Days of Exposure	.1012	Not Significant
Day 3 of Exposure vs 3 Days of Exposure	.1012	Not Significant

Hatching Rate

Data Table 4: Compares hatching rate of all groups vs all other groups using a T-test 72 hpf

Test	P-Value	Significance
Control vs Days 1-2 of Exposure	.1348	Not Significant
Control vs Day 3 of Exposure	.0272	Significant
Control vs 3 Days of Exposure	.0484	Significant
Days 1-2 of Exposure vs Day 3 of Exposure	.6433	Not Significant
Days 1-2 of Exposure vs 3 Days of Exposure	.4169	Not Significant
Day 3 of Exposure vs 3 Days of Exposure	.4918	Not Significant

Control Group

Figure 3: Model zebrafish of Control group 96 hpf

These zebrafish are an example of a healthy, average zebrafish.

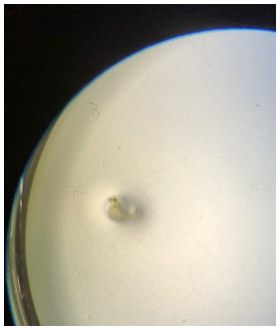
Days 1-2 of Exposure

Figure 4: Model zebrafish of Days 1-2 of Exposure 72 hpf

Compared to the Control group, Days 1-2 of Exposure were not moving very much and compared to other exposed groups had a lot hatched.

Day 3 of Exposure

Figure 5: Model zebrafish of Day 3 of Exposure 72 hpf



Day 3 of Exposure revealed numerous embryos with pericardial edema and a lower hatching rate compared to the Control groups and the other exposed groups.

3 Days of Exposure

Figure 6: Model zebrafish of 3 Days of Exposure 72 hpf

In comparison to the Control group, 3 Days of Exposure had the largest change. They were small, and if they had hatched, they had twitches. If not hatched they were excessively curled up. They also exhibited swollen stomachs and pericardial edema.

Data Analysis

The data collected from this experiment was put into a T-test using the P value of 0.05 to determine significance. The T-test was used to show how much of a statistical difference it made on the zebrafish. For this experiment the data from every category was tested against every other category for both survival and hatching rate 72 hpf. If the value from the T-test was lower than the P value of 0.05 the data was significant and the independent variable(ibuprofen) was the cause of the difference in survival and hatching rates. Significance was shown for survival rate when comparing Control vs Days 1-2 of Exposure and Control vs 3 Days of Exposure. Hatching rate difference was shown when the Control vs Day 3 of Exposure and Control vs 3 Days of Exposure were compared. When comparing all of the groups 48 hpf there was no significance. The T-test suggests that ibuprofen exposure to zebrafish embryos at certain periods of development can affect their hatching and survival rates.

Results

This experiment was designed to test the significance of ibuprofen on zebrafish embryos. Zebrafish embryos were tested with ibuprofen for different time constraints and monitored vs a control group to gather results. The independent variable of the experiment was the ibuprofen level and the

amount of time embryos were exposed to ibuprofen. The dependent variable was the survival and hatching rates of the zebrafish embryos. There were clear results that ibuprofen affected the zebrafish embryos. Either slower hatching rates or lower survival rates were shown at Control vs Days 1-2 of Exposure, Control vs Day 3 of Exposure, and Control vs 3 Days of Exposure. Results also showed deformities and other mannerism present in all groups other than the control group. Exposure during the first two days led to a high rate of hatching, low movement in the hatched embryos. Exposure during the third/last day showed pericardial edema and a lower hatching rate. Three days of exposure yielded results of smaller fish, fish with twitches, curled up unhatched fish, swollen stomachs, pericardial edema, and a low hatching rate.

Discussion

The results of this experiment align with what is considered scientifically acceptable. Zebrafish embryos exposed early on and zebrafish exposed for the whole time had higher death rates. Embryos exposed for the last third and the entire time had more internal deformities (Briggs, 2017). The control group of this experiment was a good model due to its high survival and hatching rates.

All groups of exposed zebrafish exposed to ibuprofen 72 hpf either had a lower survival rate or a slower hatching rate than the control group. Zebrafish embryos exposed for all three days experienced both a lower survival rate and a slower hatching the than the control. Embryos exposed for the first two days had a lower survival rate and embryos exposed for the third/last day had a slower hatching rate. All groups exposed to ibuprofen had higher birth deformities than the control group. This experiment yielded results that showed that exposure to ibuprofen does affect zebrafish embryos, particularly in early stages of fetal development.

This experiment had some limitations. Due to small amounts of zebrafish embryos we were not able to do a control group at the same time as the exposed groups, which could cause differing results. Other limitations included not being able to regulate temperatures at all times, not having all embryos from the same group experience the exact same variables, only being able to do one trial, and having amateur students perform this experiment.

The results gathered from this experiment can be used as a model in human pregnancy and fetal development when ibuprofen is consumed. According to the results of this experiment when pregnant and breastfeeding mothers ingest ibuprofen there is a higher risk of birth deformities and a lower fetus survival rate; particularly in the early stages of development that represented the first and second trimesters (Briggs, 2017;Kumar, 2012).

References

Ben Maamar, M., Lesné, L., Hennig, K., Desdoits-Lethimonier, C., Kilcoyne, K. R., Coiffec, I., Rolland, A. D., Chevrier, C., Kristensen, D. M., Lavoué, V., Antignac, J. P., Le Bizec, B., Dejucq-Rainsford, N., Mitchell, R. T., Mazaud-Guittot, S., … Jégou, B. (2017). Ibuprofen results in alterations of human fetal testis development. Scientific reports, 7, 44184. doi:10.1038/srep44184

Briggs, G. (August 2017). *Is it safe to take ibuprofen during pregnancy?* Retrieved from https://www.babycenter.com/404_is-it-safe-to-take-ibuprofen-during-pregnancy_1246873.bc

Burke, E. (2016, October 14). *Why Use Zebrafish to Study Human Diseases?* Retrieved February 27, 2018, from https://irp.nih.gov/blog/post/2016/08/why-use-zebrafish-to-study-human-diseases

Petering, D. H., Berg, D., Tomasiewicz, H., Carvan, M., Petering, L., and Hesselbach, R. (2012). *"Zebrafish as Models: Studying the Effects of Environmental Agents on Human Health."1-96.*

Kumar, R. B., Kar, B., Dolai, N., Karmakar, I., Bala, A., & Haldar, P. K. (2012). Toxicity study of deflazacort on morphological development in zebrafish embryos. *Comparative Clinical Pathology,22*(6), 1205-1210. doi:10.1007/s00580-012-1551-1

Moynan, A. B., & Welsh, C. A. (2012). The Photodegradation of Ibuprofen and Dissolved Organic Matter in Lake Superior and St. Louis River Water. *Zebrafish, Volume 9*(4), 1-6 https://www.liebertpub.com/doi/10.1089/zeb.2012.0754

Nordqvist, C. (Wednesday 21 June 2017). *What to know about ibuprofen?* Retrieved from https://www.medicalnewstoday.com/articles/161071.php