3rd Student Conference

“Zebrafish as an animal model”

18th of April 2024

ABSTRACT BOOK

Organizer: The Polish Zebrafish Society

Organizing committee:

Dr. Katerina Makarova (Medical University of Warsaw)

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Dr. Savani Anbalagan (Adam Mickiewicz University in Poznan)

AWARD FOR THE BEST PRESENTATION

Student with the best presentation will be awarded a fellowship to attend and give a presentation at the upcoming meeting of the Polish Zebrafish Society

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3rd Student Conference "Zebrafish as an animal model" [18/04/2024]

17:00	**Start of the meeting**	
17:05-17:30	**Dr Savani Anbalagan** (Adam Mickiewicz University in Poznan)	**Blind men and an elephant: The need for zebrafish-based animal model in research**
17:30-17:40	Julia Dzik (Medical University of Warsaw)	Pro-oxidant and antioxidant activity of sugar beet by-products
17:40-17:50	Agata Rogalska (Medical University of Warsaw)	Toxic Effects of Quinoline yellow : Zebrafish Embryotoxicity test (ZET) and ADMET analysis
17:50-18:00	Gabriela Żyłka (Jagiellonian University in Krakow)	Investigation of the efficacy of antibacterial-potential compounds in larval zebrafish
18:00-18:10	Natalia Pypa (Jagiellonian University in Krakow)	Generation of tools to study the role of galectin 8a and 8b in zebrafish
18:10-18:20	Jakub Michałkiewicz (Medical University of Lublin)	Evaluation of the toxicological properties of the Crinum moorei extract in the Zebrafish model
18:20-18:30	Lidia Krzelowska (Medical University of Lublin)	Exploring the effects of New Psychoactive Substances through the zebrafish Light-Dark Test: a case with selected designer benzodiazepines
18:30-18:40	Bogumił Łosiewicz (Warsaw University of Life Sciences)	Impact of silver nanoparticles and ions on morphology, growth and sex development of guppy (*Poecilia reticulata*)
18:40-18:50	**BREAK**	
18:50-19:00	Zuzanna Żelażewska (Medical University of Warsaw)	Preliminary assessment of potential hepatotoxicity of morphine and disulfiram combination
19:00-19:10	Dawid Kozłowski (Jagiellonian University in Krakow)	The comparative study of host-pathogen interactions and neuroinflammation during infection with ATCC33277 and W83 strain of *Porphyromonas gingivalis*
19:10-19:20	Ewa Bucoń (Jagiellonian University in Krakow)	Modelling infections with oral pathogens using zebrafish larvae
19:20-19:30	Adam Chromiec (Jagiellonian University in Krakow)	Effect of knock-out of *ddx1* gene on survival rate and antiviral response in zebrafish (*Danio rerio*)
19:30-19:40	Paulina Misiak (Jagiellonian University in Krakow)	Intranasal injection of tilapia lake virus (TiLV) induces both inflammatory and antiviral response in the brain of zebrafish (*Danio rerio*)
19:40-19:50	Martyna Kacprzak (Medical University of Lublin)	From pain relief to potential risk: determining ibuprofen toxicity in zebrafish model
19:50-20:00	Jagoda Szponar (Medical University of Lublin)	Swimming through metabolic disorders: zebrafish model of type 2 diabetes
20:00-20:10	Julia Bonder (Medical University of Warsaw)	Toxicity of acetaminophen and caffeine combination. Effect on Zebrafish embryos in the early stage of organogenesis
20:10-20:30	Concluding remarks	**Price for the best presentation**

Pro-oxidant and antioxidant activity of sugar beet by-products

Julia Dzik
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Presenting author: Julia Dzik (e-mail: dzikjuli@gmail.com)
Second author: dr Katerina Makarova

Sugar beet is a common plant. As for now, around twenty percent of the world's supply of sugar is derived from the root of the sugar beet. Three main by-products of sugar production are beet pulp, lime sludge and molasses. Sugar beet pulp has a varied composition consisting mainly of cellulose, hemicellulose and pectin.[1] It's often used as a high-energy, low-protein supplement for ruminants to promote optimal rumen health and increase milk production.[2]

Still, large amount of sugar beet by products end on landfills. Here they can penetrate to variety of water bodies, including, but not limited to underground water, lakes and rivers. In this study we'd like to examine the effect of aqueous sugar beet extracts on *Danio rerio* embryos, as well as analyze anti- and prooxidative activity using DPPH test and Fenton reaction. We believe that it can help to create and improve already existing waste management and water treatment processes in the agri-food industry and also help reduce the negative impact on the natural environment.

[1] Marzo Gago, Cristina & Díaz, Ana & Blandino, Ana. (2023). Sugar Beet Pulp as Raw Material for the Production of Bioplastics. Fermentation. 9. 655. 10.3390/fermentation9070655.
[2] MAKAMBAİ KYZY A, MAZHİTOVA A (July 1, 2023) Biotechnological valorization of sugar beet wastes into value-added products. MANAS Journal of Engineering 11 1 136–144.

Toxic Effects of Quinoline yellow : Zebrafish Embryotoxicity test (ZET) and ADMET analysis

Agata Rogalska[1], Magdalena Majdan[2], Anna Małkowska[2]

Presenting author: Agata Rogalska

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Abstract:

There has been a growing focus on the potential harm caused by additives commonly found in food products. Quinoline yellow, also known as E104 (ADI 10 mg/kg bw) is a quinophthalone synthetic dye. The aim of this work is to show possible teratogenic effect of quinoline yellow based on *Danio rerio* embryos. The findings of these experiment have underscored the need for thorough scrutiny of quinoline yellow's impact on human health. ADME-TOX in silico studies showed that quinoline yellow possessed some undesirable toxicological properties. The toxicity and genotoxicity of the compound under examination were evaluated by estimating various toxic and genotoxic endpoints using animal models. These endpoints included acute toxicity (such as the rat acute toxicity rat LD50) and chronic toxicity (specifically carcinogenicity) observed in rodent models (rat TD50 and mouse TD50). Additionally, genotoxicity was assessed through computational studies utilizing the AMES assay and clastogenicity studies, which evaluate chromosomal aberrations. The re-evaluation of quinoline yellow is essential to ensure that its use in food products aligns with established safety standards and does not pose unacceptable risks to consumers.

Investigation of the efficacy of antibacterial-potential compounds in larval zebrafish

Gabriela Żyłka[1], Ataur Rahman[2], Klara Stensvåg[2], Tomasz Prajsnar[1]

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Presenting author: Gabriela Żyłka (e-mail: gabriela.zylka@student.uj.edu.pl)

Abstract:

Given the escalating global challenge of bacterial antibiotic resistance, there is a critical imperative to seek alternatives to traditional antibiotics. Short cationic antimicrobial peptides (AMPs) serve as an example of such alternatives, offering a potential solution to this urgent concern. Indeed, it is widely believed that due to the absence of a specific target, AMPs are less prone to induce the antibiotic resistance.

This presentation focuses on the investigation of synthetic derivatives of short cationic AMPs which naturally occur in marine organisms found on the bottom of the Arctic Sea. We used zebrafish larvae to determine the toxicity of 14 tested compounds, comparing intra-yolk sac and intravenous delivery. Currently, we are determining their antibacterial activity during *Staphylococcus aureus* and *Porphyromonas gingivalis* infection in larval zebrafish. The results showed minimal toxicity to zebrafish larvae after bloodstream administration, while the activity results suggest potential antibacterial effectiveness for selected compounds. These findings suggest further research to confirm the efficacy of these AMPs as potential antibiotics.

Generation of tools to study the role of galectin 8a an 8b in zebrafish

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Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow

Presenting author: Natalia Pypa (e-mail: nata.pypa@student.uj.edu.pl)

Abstract

Gene manipulation is widely used to understand and analyse the biological role of a gene product. Zebrafish (lat. *Danio rerio*), due to their significant advantages as a genetic model organism, including a fully mapped genome, rapid and transparent embryonic development, a high reproductive rate, and accessibility for genetic manipulation are used for years as a tool to study the role of many genes. Molecular tools used until now for their gene manipulation, such as Morpholino oligonucleotides (MOs), zinc finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs) are now being substituted with new, more specific technique known as CRISPR/Cas9, an endonuclease responsible for generating a double-strand breaks (DBD) in the genome, which is supposed to lead to insertions or deletions of curtain nucleotides found in a targeted loci with the help of small guide RNAs (sgRNA). The most important thing that differentiate these techniques is the effectiveness in generating mutants, in which CRISPR/Cas9 is the pioneer. Galectin 8a and Galectin 8b, products of *lgals8a* and *lgals8b* genes respectively, seems to be contributing to a process called selective autophagy, during several bacterial infections. Here we concentrate on generating mutants with *lgals-8a* and *-8b* gene knockout via CRISPR/Cas9 as a loss of function (LoF) model of gene manipulation and on creating zebrafish models with overexpression of the same genes via mRNA in vitro transcription as a gain of function (GoF) model of gene manipulation to study the role of these genes during bacterial infection and their contribution in the selective autophagy process.

Evaluation of the toxicological properties of the Crinum moorei extract in the Zebrafish model

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[5]dr Sylwia Wośko, [6]prof. dr hab. Ewa Poleszak

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Presenting author: Jakub Michałkiewicz *(e-mail: jakubmichalkiewicz20@gmail.com)*

Introduction:

Currently, despite the widespread use of synthetic drugs, herbal medicine is often used, which in many cases can perfectly complement conventional treatment. Active compounds isolated from plants are increasingly being used as a source of medicines. However, there is evidence that indicates that herbal extracts can cause side effects. Therefore, an important aspect of research on plant raw materials, that could potentially be used in humans is their toxicity assessment.

Objective:

The aim of this research is to evaluate the toxicological properties of the plant extract of *Crinum moorei*, a plant belonging to the *Amaryllidaceae* family in the Zebrafish model *(Danio rerio)*.

Materials and methods*:*

Eggs of the *Danio rerio* species were used for the tests. Toxicological evaluation of *Crinum moore*i vegetable extract solutions at concentrations of 200 μg/ml, 100 μg / ml, 50 μg (mg/ml), 25 μg(ml) and 12.5 μg/-ml was carried out. Developmental changes were monitored every 24 hours, based on teratogenic, lethal and sublethal endpoints. The experiment was conducted for 5 days after fertilization.

Results and conclusion:

The results obtained during the experiments indicate that the extract of *Crinum moorei* at concentrations of 200 μg/ml, 100 μ g/ml and 50 μg / ml caused the swelling of heart, gallbladder and in some isolated cases bending of the spinal cord of *Danio rerio*. The results obtained may indicate developmental toxicity, but further, more detailed studies are needed to confirm the toxicities profile.

Keywords: *Crinum moorei, Amaryllidaceae, Zebrafish, Toxicity Testing.*

Reference:

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2. *Elgorashi, E.; Drewes, S.E.; van Staden: Alkaloids from Crinum moorei J. Phytochemistry 2001, 56, 637-640.*
3. *Chahardehi, A. M., Arsad, H., Lim, V.: Zebrafish as a successful animal model for screening toxicity of medicinal plants. Plants, 2020, 9, 1345.*
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Exploring the effects of New Psychoactive Substances through the zebrafish Light-Dark Test: a case with selected designer benzodiazepines

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New Psychoactive Substances are drugs that are used for their euphoric or relaxing effects. These substances are not yet controlled by conventions on psychotropic substances and narcotic drugs. The use of these drugs can result in side effects such as agitation, acute psychosis, and seizures. Mostly, the side effects of newly appearing drugs on the black market are unknown. Therefore, it is necessary to experimentally examine their pharmacological activity. This study focuses on selected designer benzodiazepines such as adinazolam, flunitrazolam, 3-hydroxyphenazepam, and nitrazolam. Benzodiazepines are a class of drugs that are known to have sedative, hypnotic, anxiolytic, and anticonvulsant properties. This study aims to examine the properties of these selected benzodiazepines and their impact on zebrafish larvae.

The effects of the investigated substances were evaluated using a light-dark test to determine their impact on the central nervous system. This test involved assessing locomotor activity and tigmotaxis in different light phases. For the behavioral experiment, 24-well plates were used, with one 5 dpf zebrafish larvae per well. After the incubation with the drug and habituation period, the test started with 5 minutes of light followed by 5 minutes of dark. The video was registered using the DanioVision chamber and activity parameters were calculated by the EthoVision program (Noldus). Additionally, a Maximum Tolerated Concentration test was conducted to establish the drugs' toxicological profile.

The investigated substances have shown dose-dependent behavioral responses. The animal model used in the study is highly effective in rapidly assessing the activity of novel psychoactive substances.

Impact of silver nanoparticles and ions on morphology, growth and sex development of guppy (*Poecilia reticulata*)

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Presenting author: Bogumił Łosiewicz (e-mail: s213028@sggw.edu.pl)

Abstract:

The aim of the study was to examine the impact of silver nanoparticles and ions on morphology, growth and sex traits development of guppy (*Poecilia reticulata*) from the day of hatching for the next 60 days. Fish younglings were exposed to AgNPs concentrations of: 0 (control group), 0.01, 0.05, 0.1, 1 ppm and 1ppm of silver ions. On the last day of the experiment fish were euthanized, measured and weighed, then subjected for histological processing (whole fish). The highest mortality rate was observed in 1ppm silver nanoparticles and ions groups and oscillated around 40% compared to 10% in control. Fish in AgNPs 0.05, 0.1, 1 ppm groups showed the least number of individuals with developed secondary sexual characteristics with over 70% of undefined sex specimens in whole group. Similar dependency showed fish weight and body length in which individuals of those 3 groups were the lightest with the lowest value of 0,029 (± 0,023) g in AgNPs 0.05 group, however they were also the largest ones in context of body length with 11,68 (± 3,31) mm even exceeding in this parameter control group. Despite that AgNPs 0.05 group were the smallest ones in total length with reaching 12,59 (± 2,00) mm. All exposed groups showed significantly smaller hepatocytes and lower intestinal fold height than individuals in control group. Obtained results indicate that exposure to AgNPs may delay sexual maturation and disturbs morphology and growth of fish.

Preliminary assessment of potential hepatotoxicity of morphine and disulfiram combination

Zuzanna Żelażewska[1], Marzena Łazarczyk[2], Marta Marszałek-Grabska[3], Kinga Gaweł[3], Anna deCorde-Skurska[1], Joanna Giebułtowicz[4], Mariusz Sacharczuk[2], Justyna Paszkiewicz[5], Anna Leśniak[1]

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Presenting author: Zuzanna Żelażewska (e-mail: zuzanna.zelazewska@gmail.com)

Introduction:
Morphine-induced tolerance and hyperalgesia are burdensome side effects that hinder chronic pain management. Recent data shows that disulfiram – a drug registered for alcohol use disorder, was shown to fully suppress those side effects.

Both clinical and preclinical studies show that disulfiram treatment may pose an increased risk of acute liver injury. However, the potential toxicity of the disulfiram-morphine combination has not been thoroughly scrutinized so far.

The aim of the study:
This study aims to determine the dose-dependent impact of the disulfiram and morphine combination on: i) HepG2 cell line viability; ii) *Danio rerio* larvae development and behavior; iii) serum liver toxicity markers in rats.

Material and methods:
HepG2 cell viability following disulfiram, morphine alone or their combination was assayed in the MTT test after 24h and 7 days. Hatching rate and locomotor activity of *Danio rerio* larvae were determined following 24h and 5-day exposure. The levels of liver toxicity markers were measured in rat serum with the ELISA assay after 21 days of morphine and disulfiram co-treatment.

Results:
A synergistic antiproliferative effect of morphine and disulfiram co-treatment was observed in HepG2 cells after 7 days of exposure. Also, a decreased hatching rate of *Danio rerio* larvae and swim bladder underdevelopment were observed. Locomotor activity was however unaffected. Chronic morphine and disulfiram co-treatment in rats did not affect serum liver toxicity markers.

Conclusion:
Disulfiram could serve as a promising future therapeutic option for counteracting morphine-induced tolerance and hyperalgesia. However, caution is advised in individuals with impaired liver function.

The comparative study of host-pathogen interactions and neuroinflammation during infection with ATCC33277 and W83 strains of *Porphyromonas gingivalis*

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Presenting author: Dawid Kozłowski (e-mail: dawid.kozlowski@student.uj.edu.pl)

Numerous evidence supports the link between microbial infections of the oral cavity and neurodegenerative diseases. However, the exact mechanisms of bacteria-driven central nervous system pathologies are yet to be fully understood.

Pophyromonas gingivalis (*Pg*) is a key-stone pathogen in periodontitis. Many studies suggest that differences in infection severity and systemic pathologies correspond to differences in *Pg* strains and their virulence factors, e.g. fimbriae or capsules.

In this study, we compared the pathogenic potential of two strains of *Pg*; fimbriated ATCC 33277 and non-fimbriated W83, using a zebrafish systemic infection larvae model. We studied zebrafish mortality and bacterial ability to induce neuroinflammation upon systematic infection with both *Pg* strains. Neuroinflammation was examined by gene expression of pro-inflammatory cytokines in the heads of infected larvae. We also compared the ability of these *Pg* strains to cross the vascular barrier, disseminate into the brain parenchyma and activate microglia. In addition, we studied bacteria interactions with phagocytes (macrophages and neutrophils) and endothelium in zebrafish larvae.

We found that, in comparison to W83 strain, ATCC 33277 was: (i) less pathogenic, (ii) aggregated upon injection, and (iii) adhered more to the endothelium. We also observed that W83 *Pg* was able to cross the vascular barriers of the brain, degrade brain vasculature and activate microglia.

Zebrafish larvae provides invaluable insight into the complex mechanisms of *Pg* infection and neuroinflammation, being a promising model in neuroimmunological research of microbial-driven neuropathologies.

Modelling infections with oral pathogens using zebrafish larvae

Ewa Bucoń[1], Magdalena Widziołek-Pooranachandran[1], Magdalena Chadzińska[1]

[1]*Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow*

Presenting author: Ewa Bucoń (e-mail: ewa.bucon@student.uj.edu.pl)

Dental plaque is a complex biofilm composed of commensal and pathogenic bacteria surrounded by an extracellular matrix. This specialised niche protects bacteria from the host's immune system and limits the penetration of therapeutics. Reports suggest that oral pathogens can cause periodontal diseases and systemic ones such as cardiovascular diseases and neurodegenerative disorders; however, the mechanism remains unknown. One possibility is that microcolonies detach from the growing biofilm and enter the bloodstream.

Zebrafish larvae are a widely used infection model to study human pathogens, including a key-stone pathogen in periodontitis – *Porphyromonas gingivalis.*

For decades, infections with single bacterial species were studied, which do not consider the complex bacterial interaction in multi-species biofilms. Therefore, the **aim of this study is to establish a new zebrafish infection model to study infections with biofilm-forming bacteria.** We investigate: (i) new oral pathogens (single species infections) and (ii) microbiofilms (multi-species infections).

We examined the pathogenic potential of commensal *Streptococcus mitis* and pathobiont *Fusobacterium nucleatum*, both frequently present in oral biofilms, in zebrafish larvae infected systemically. We studied dose-dependent mortality of zebrafish and bacterial survival. We observed that *S. mitis* was not pathogenic for zebrafish larvae in contrast to the pathogenic *F. nucelatum.*

Currently, we are developing multi-species biofilm ranging from non- to very pathogenic bacteria, which are a part of the core oral microbiome in human. Moreover, we are developing methods to study bacterial composition within the biofilms.

We believe that zebrafish larvae will serve as great tool to study infections with complex biofilms *in vivo*.

Effect of knock-out of *ddx1* gene on survival rate and antiviral response in zebrafish (*Danio rerio*)

Adam Chromiec[1], Miriam Mojżesz[1], Niedharsan Pooranachandran[1], Justyna Starzyk[1], Magdalena Chadzińska[1], Mikołaj Adamek[2], Krzysztof Rakus[1]

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[2] *Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany.*

Presenting author: Adam Chromiec (adam.chromiec@student.uj.edu.pl)

Abstract:

RNA helicases of the DExD/H-box family play a key role in all steps of RNA metabolism in the cell, from transcription and pre-mRNA processing to RNA translation and degradation. In addition, many of the DExD/H-box RNA helicases are multifunctional proteins that also play a role in cell cycle regulation, apoptosis, tumour development and the antiviral immune response. DDX1 is a DExD/H-box RNA helicase that has been previously shown to be up-regulated following viral infection in the zebrafish model. In order to verify the role of this protein in the antiviral response, the *ddx1* gene was knocked-out using the CRISPR/Cas9 method. We demonstrated that *ddx1* knock-out larvae do not display any development defects and are viable until 14 days post fertilisation, but no adult $ddx1^{-/-}$ homozygotes were observed. Next, we studied antiviral response in zebrafish larvae upon infection with tilapia lake virus (TiLV). We did not observe any significant difference in the survival, viral load and expression of antiviral genes (*rig-I, ifn1,* and *mxa*) between three groups of zebrafish: $ddx1^{-/-}$, $ddx1^{+/-}$, and $ddx1^{+/+}$. Thus, DDX1 is not crucial for anti-TILV response in zebrafish.

Intranasal injection of tilapia lake virus (TiLV) induces both inflammatory and antiviral response in the brain of zebrafish (*Danio rerio*)

Paulina Misiak[1], Maria Zawisza[1,2], Miriam Mojżesz[1,3], Magdalena Chadzińska[1], Mikołaj Adamek[4], Krzysztof Rakus[1]

[1]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Krakow, [2]Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, Poland, [3]Department of Microbiology, Immunology and Transplantation, Rega Institute, Laboratory of Virology and Chemotherapy, Leuven, Belgium, [4]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine Hannover, Hannover, Germany.

Presenting author: Paulina Misiak (e-mail: paulina.misiak@student.uj.edu.pl)

Tilapia lake virus (TiLV) is an enveloped virus that contains single-stranded RNA as its genetic material. TiLV has a very negative impact on global aquaculture, as it causes mass mortality of wild and farmed Nile tilapia (*Oreochromis niloticus*). Previously, the zebrafish - TiLV infection model was developed to study TiLV biology and fish immune response. Intraperitoneal injection of TiLV resulted in virus replication in multiple organs of zebrafish, with highest replication and persistence in the brain. To better study the virus, we developed a novel model of TiLV infection by intranasal injection. We showed that intranasal injection of adult zebrafish by TiLV resulted in virus replication in the brain and to a lesser extent in other organs. In addition, the expression of genes involved in the type I interferon (IFN) pathway, the inflammatory response and genes encoding markers of macrophage/microglia and astrocyte activation in the brain of TiLV-infected fish was examined. An up-regulation of the expression of genes encoding pattern recognition receptors binding viral RNA (*rig1*, *tlr-3*), transcription factors that induce the expression of type I interferons (*irf-3*, *irf-7*), antiviral protein (*mxa*), and genes encoding proinflammatory cytokine (*il-1β*) was observed at day 14 post-infection. In addition, an up-regulation of the expression of macrophage/microglia activation markers (*csf1r*, *cd68*) and an astrocyte marker (*gfap*) was demonstrated at the same time point. The study shows that intranasal injection of TiLV may be a suitable tool for studying the immune response of zebrafish to infection considering neurotropic nature of the virus.

From pain relief to potential risk: determining ibuprofen toxicity in zebrafish model

Martyna Kacprzak[1], Łukasz Kurach[1]

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Presenting author: Martyna Kacprzak (e-mail: 61068@student.umlub.pl)

Abstract

Introduction

Ibuprofen, as one of the easily available drugs with analgetic, antipyretic and anti-inflammatory properties, is currently widely used by patients of almost all ages. However, despite this, there are not many studies focusing on its potential toxicity and impact on the development of embryos of various organisms.

Materials and Methods

0-day-old *Danio rerio* embryos were placed in 6-well plates (15 embryos per well) in the following ibuprofen concentrations: 30-180μM. They were observed for four days for coagulation, hatching and developmental changes. At 96hpf, heart rate measurements were taken, and then all alive and hatched embryos were transferred to 48-well plates (1 embryo per well) and subjected to a locomotor activity in Light-Dark Test. The results were compared to the control group, which was the E3 solution.

Conclusions

Ibuprofen can cause numerous developmental changes in *Danio rerio* embryos, such as the occurrence of pericardial edema, yolk edema, and changes in tail shape. A decrease in heart rate in higher concentrations and a decrease in motor activity were also observed. The toxicity of ibuprofen in the context of developmental changes should therefore be taken into account as an important problem and tested on other model organisms.

Swimming through metabolic disorders: zebrafish model of type 2 diabetes

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Presenting author: Jagoda Szponar (e-mail: jagodaszponar111@interia.pl)

Abstract

Studies have concluded that non-treated type 2 diabetes mellitus can lead to cardiovascular failure, retinopathy, poor wound healing, or impaired cognitive function. It is estimated that due to negative lifestyle choices and the obesity epidemic, around 39 million people have diabetes and around 150.9 million are prediabetic. This indicates an urgent need for the development of new medications, which will allow to reduce the prevalence of diabetes.

Expanding the range of diabetes therapeutics requires continuous evolution of validated animal models in parallel with the latest discoveries and advancements. Zebrafish are a unique model for studying metabolic disorders due to their pancreas structure, glucose homeostasis, and lipid metabolism, which are similar to that of mammals.

Our initial research involved establishing the toxicity profile of glucose and metformin as a first-line therapy for diabetes treatment. Both substances were tested by incubating the zebrafish in a glucose solution of 200, 150, 100, 50, and 1 mM and metformin solution of 0.5, 1, 6, 15, and 30 mM for 96h. No toxicity was registered in the used substance concentrations. The diabetic model was tested using 7 dpf larvae by immersing the zebrafish til 14 dpf in 40 mM – 160 mM glucose solutions. At 14 dpf animals were kept for 24h with solution without glucose or treated with 1-10 μM metformin. Glucose levels were determined using a Glucose Fluorometric Assay Kit.

In conclusion, we developed a diabetes type 2 zebrafish model with high body glucose levels and responsiveness to standard treatment using metformin.

Toxicity of acetaminophen and caffeine combination. Effect on Zebrafish embryos in the early stage of organogenesis

Julia Bonder

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Caffeine is the most widely consumed stymulant. In Poland a person's average coffee consumption is around 95 litres every year which is 26600 mg of caffeine per year. Paracetamol is first on the list of most popular OTC medicines in the world. It is used by more than 50% of pregnant women and it is also well tolerated in eldery people goup. 5% of caffeine and paracetamol is not metabolised and excreted in the urine, reaching the sewege system. This may have harmful influence on environment, fish and water creatures. The aim of the experiment was to find if there is an interaction beetween paracetamol and caffeine during simultaneous sumbission in three different groups: caffeine 300 mg/L; caffeine 300 mg/L with paracetamol 1 mM; caffeine 300 mg/L with paracetamol 3 mM. There was a control group as well. They were observed in 72 hour after fertilization, and the results showed sure malformations and increased spinal curvatures depending on concentration. This findings suggest that there is an interaction between these two medications and experiments should be continued.