2nd Student Conference *zebrafish* as an animal model

ABSTRACT BOOK

13.04.2023

Organizing Committee:
Dr Katerina Makarova (Medical University of Warsaw)
Dr hab. Krzysztof Rakus (Jagiellonian University in Krakow)

Polish zebrafish society

2nd Student Conference "Zebrafish as an animal model"

17:00	**Start of the meeting**	
17:05-17:40	**Dr Małgorzata Korzeniowska** (Mossakowski Medical Research Institute, Polish Academy of Sciences)	**Zebrafish: facts and myths**
17:40-17:50	Adrianna Gabryś (Medical University of Warsaw)	Application of chemometric methods to predict the toxicity of xenoestrogens and microplastic towards embryos of the Danio rerio species
17:50-18:00	Karolina Żółtowska (Medical University of Warsaw)	Screening of spent coffee grounds antioxidant activity using the Danio rerio embryos
18:00-18:10	Justyna Grymuza (Medical University of Lublin)	Evaluation of memory processes in adult Danio rerio
18:10-18:20	Jakub Semeniuk (Medical University of Lublin)	Effect of imperatorin on color preference in zebrafish
18:20-18:30	Lidia Krzelowska (Medical University of Lublin)	The effects of music environmental enrichment on zebrafish behavior exposed to chronic unpredictable mild stress
18:30-18:40	Piotr Mamełko (Medical University of Lublin)	Development of a zebrafish-based model of cognitive dysfunction
18:40-18:50	**BREAK**	
18:50-19:00	Emilia Seta (Jagiellonian University in Krakow)	The effect of tyrosine kinase inhibitors on peripheral and cerebral blood vessels in larval zebrafish
19:00-19:10	Aleksandra Domagalska (Jagiellonian University in Krakow)	Neuroinflammation caused by bacterial infection - research on a zebrafish model
19:10-19:20	Justyna Starzyk (Jagiellonian University in Krakow)	The study of the effect of doxorubicin treatment on human melanoma cells using zebrafish (*Danio rerio*) model
19:20-19:30	Karolina Kot (Jagiellonian University in Krakow)	Is zebrafish glycosylation altered during TiLV infection? The expression of genes involved in glycan synthesis in *Danio rerio* larvae
19:30-19:40	Aleksandra Szczepańska (University of Wrocław)	The effect of selected compounds on an animal model characterized by a reduced level of muscle glycogen phosphorylase
19:40-19:50	Kacper Kawalski (Warsaw University of Life Sciences)	The effect of long-term exposure of silver nanoparticles on the liver and germinal tissue of the butterfly splitfin (*Ameca splendens*)
19:50-20:00	Dominika Kramm (University of Warmia and Mazury in Olsztyn)	Study on cumarin antiepileptic properties using zebrafish (Danio rerio) as a model organism
20:00-20:15	Concluding remarks **Price for the best presentation**	

Application of chemometric methods to predict the toxicity of xenoestrogens and microplastic towards embryos of the Danio rerio species

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Abstract:

Zebrafish is cheap, robust in vivo model, widely used to study the toxicity of various compounds. The results obtained with zebrafish are often highly correlated with higher vertebrates. The aim of this study was to find a model that will predict the mortality of zebrafish embryos after exposure to xenoestrogen and microplastic compounds. Xenoestrogens are considered toxic for living organisms and can disrupt the functioning of the reproductive system, while microplastic can cause oxidative stress, changes in metabolic parameters and even compromise growth of organisms in long-term exposure. The Danio rerio species is commonly used for studying the properties and toxicity of these compounds in vivo. In this study, we will test the predictions of zebrafish embryo mortality rates after exposure for 24, 48, 72, 96, and 120 hours, using various types of Artificial Neural Networks. The research involved the collection and analysis of data on zebrafish embryo mortality rates after exposure to xenoestrogens and microplastic compounds. I performed molecular docking for microplastic compounds including parameters such as docking to estrogen receptors, logP, NMR structure of the compound and presence of ethanol or DMSO in the sample. Additionally, we used PCA methods to analyze the relationship between the collected data. The next stage of the project involves the creation of a model using Artificial Neural Networks. Given the growing use of artificial intelligence in pharmacy, this model may prove to be useful for researchers planning in vitro toxicity testing of xenoestrogen and microplastic compounds on zebrafish embryos.

Screening of spent coffee grounds antioxidant activity using the Danio rerio embryos

Karolina Żółtowska

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Abstract

Coffee is the second most consumed drink in the world. Demand for this beverage is incredibly high due to people's fondness for taste and energizing effects. Coffee's production for the year 2020/21 was estimated at 169.60 million bags according to the International Coffee Organization. During coffee beans extraction, several by-products are generated. Spent coffee grounds (SCG) are the final by-product and the biggest contributor to coffee's biowaste [1]. Approximately 120,000 tons of SCG are created every year in Poland [2], which is only a small part of the 6 million ton figure produced around the globe [3]. The major way for used coffee utilization is by creating landfills. Those landfills are associated with the emission of greenhouse gases (methane, CO_2) due to the anaerobic degradation of coffee wastes. Apart from the atmosphere, coffee biowaste also affects the soil. SCG contain a high content of bioactive compounds: tannins and caffeine, which may contribute to soil degradation resulting in its poor quality [4]. The active chemical component of used coffee also consists of phenolic compounds, diterpenes and melanoidins [5,6], which are responsible for coffee's antioxidative properties. The protection from oxidative stress is reflected in our health as we don't age prematurely and struggle with diseases less often. To see how SCG extracts influence live organisms, the method of microscopic observations using Danio rerio embryos was applied. The outcome might present a wider perspective on coffee biowaste's use or mobilize us to find an environment-friendly way of its utilization.

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Evaluation of memory processes in adult Danio rerio

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Abstract:

Nowadays globally, there is an ongoing rise of neurological disorders such as Alzheimer disease, which is the leading cause of dementia. Currently, no prevention, cure or effective treatment for dementia is known. Therefore, there is a need to develop new animal models for evaluation of the procognitive effects of new molecules.
Neuroanatomically, the zebrafish brain is aligned similarly to the mammalian brain and posseses high homogeneity hippocampus-like and amygdala-like structure, which are main areas responsible for memory in human brain. The major neurotransmitter systems in zebrafish, including the noradrenergic, serotonergic, dopaminergic and histaminergic signaling, share many similarities to the mammalian system, thus it makes zebrafish an appropriate neurobiological model for designated research.
The aim of the present study was to establish a novel model of memory impairments in adult Danio rerio induced by scopolamine (50, 100 150 uM), which acts as a cholinolytic agent. Spatial memory and the response to novelty in zebrafish were assessed using the Y maze. Study results showed that scopolamine impairs memory processes in zebrafish. These effects were sex-dependent as scopolamine within all used concentrations decreased the time spent in novel arm in male zebrafish, whereas the concentration of 50uM did not induce statistically significant memory impairment in female zebrafish.
In conclusion, this study provides evidence that proposed zebrafish behavioral model may be considered as an effective research platform for the development of therapies for neurological disorders.

Effect of imperatorin on color preference in zebrafish

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Abstract

Imperatorin is furanocoumarin and a significant constituent of many plants from the *Apiaceae* family. Like other coumarins, it is widely recognized as a part of traditional Chinese medicine preparations. Imperatorin exhibits potent anti-cancer, anti-inflammatory, and anti-hypertensive properties, but recently has attracted increasing interest for its activity towards the central nervous system. To this day a series of *in vivo* experiments confirmed its nootropic, anxiolytic-like and antidepressant-like effects. To further examination of imperatorin neuro-properties we established and used the zebrafish model of color preference. The zebrafish has a tetrachromatic vision and can distinguish different visible wavelengths. Recently, zebrafish color preferences have gained much attention because of the easy setup of the instrument and its usefulness for high-throughput neuropharmacological applications.

For evaluation of color preference in zebrafish, 6-well plates with the bottom of the well covered and divided into two sets of different colors – blue, green, yellow, and red were prepared. The experimental procedure consisted of 3 steps. Pre-test, to assess primary color preference; conditioning, to associate tested substance with the less preferred color area; final test, to evaluate whether tested substance influence color preference. At each stage, the larvae were tested for 5 minutes. Time spent in each area and locomotor activity were gathered using Noldus DanioVision and analyzed with EthoVision 17 software.

The model of color preference in 5-dpf zebrafish was established and imperatorin effects on color preference were investigated. We believe that the presented research is a valuable tool for the evaluation of nervous system functions in zebrafish.

The effects of music environmental enrichment on zebrafish behavior exposed to chronic unpredictable mild stress

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Abstract

Introduction:
Chronic stress is a significant factor affecting well-being in both humans and animals. The data indicate that music environmental enrichment has the beneficial effects on different animal specimens including zebrafish. Little is known whether or not music can also alleviate stress-induced anxiety-like response in zebrafish.Therefore, the aim of this study was to evaluate if rock music differing in bpm can reverse stress-induced changes in animal behavior.

Materials and Methods:
3-month old zebrafish (AB line) were subjected to chronic unpredictable mild stress for 7 consecutive days (twice a day). Applied stressors (randomly assigned) included: a) acute net chasing, b) exposure to predator, c) heating tank water up to 33°C, d) cooling tank water down to 23°C, e)transferring the animals to another tank with low water level, f) tank change, three consecutive times, g) crowding in beaker (25 mL/fish). Two groups were additionally exposed to rock music differing in bpm (80 bpm and 130 bpm). The result were compared to control groups (non-stressed non-music and stressed non-music). 24 hrs after the last stressor application all fish were introduced to the novel tank diving test. Time and total distance traveled at the half bottom were measured as the indicators of anxiety-like response.

Conclusions:
Chronic mild stress affects animal well-being, which manifests in changes in swimming pattern of fish. Music could serve as a useful tool improving fish welfare and should be taken into consideration as environmental enrichment for zebrafish.

Development of a zebrafish-based model of cognitive dysfunction

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Dementia is a worldwide problem affecting cognitive abilities. related to imparement of logical thinking as well as memory. According to the World Health Organization (WHO), 55 million people worldwide suffer from dementia in 2022.

Therefore, the aim of this study is to develop a new high-throughput procedure to assess memory and cognition using *Danio rerio* larvae as an animal system. Scopolamine being a model compound.

Scopolamine is a commonly used alkaloid with the ability to block cholinergic receptors in humans. One of the adverse effects observed after scopolamine exposure is amnesia and impairment of cognitive skills. Because of this, scopolamine is used in animal models as a substance that may help us recreate changes in animals mimicking those observed in patients with dementia.

In this experiment, larvae are transferred into a Y-shaped maze after being held in a scopolamine solution for 1 hour. The protocol is based on observing a number of variations and repetitions when it comes to fish's swimming patterns among the arms of the Y-maze. Healthy fish will be leaning towards varyingtheir swimming routes due to their ability to remember previously taken turns. Whereas, fishes with impaired ability to memorize have a tendency to continue taking the same turn. The experiments are performed under various lighting options.

Results obtained in this research will help to establish a new model of cognitive dysfunctions in zebrafish and therefore might enhance screening of new procognitive medications forpatients suffering from dementia.

The effect of tyrosine kinase inhibitors on peripheral and cerebral blood vessels in larval zebrafish

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Abstract

Despite growing rate of success in cancer treatment, the increasing number of severe side effects on cardiovascular system have been observed among patients. The mechanisms of such vascular toxicity of many chemotherapeutic compounds are largely unknown.

A chemotherapeutic agent called ponatinib is a BCR-ABL tyrosine kinase inhibitor and a third generation drug used in therapy against chronic myeloid leukaemia and Philadelphia chromosome- positive acute lymphoblastic leukaemia. It has been shown in the phase I and II trials that treatment with ponatinib contributed to adverse vascular events in 48 % or 24 % of patients, respectively.

In this study, we use the zebrafish model that is currently gaining popularity in biomedical research as it combines the low cost, ease of use or amenability to high throughput screening of in vitro systems with complexity or genetic and functional similarity of mammalian systems. Our experiments involved microinjections of fluorescent (FITC) dextran to the bloodstream of transgenic zebrafish larvae with fluorescently tagged (mTurquoise) endothelium at 2 or 3 days post fertilization. Subsequently, the injected larvae were treated with ponatinib by immersion for 12 hours and their peripheral or cerebral vessels were finally imaged by confocal microscopy. In order to quantify the extravasation of dextran, the fluorescence intensity within 6 areas inside and outside a blood vessel was measured in each individual.

We observed reduced vessel diameter and the enhanced vascular leakage in ponatinib-treated larvae in comparison to vehicle controls. Therefore, we believe zebrafish is a valuable model for studying endothelial toxicity in vivo.

Neuroinflammation caused by bacterial infection - research on a zebrafish model.

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Growing number of evidence emphasize the infection-driven theory of neuroinflammation as a prelude to neurodegenerative disorders. Recently, oral pathogens such as *Porphyromonas gingivalis* (*Pg*), the key-stone pathogen of periodontal disease, have been associated with development of neurodegenerative disorders like Alzheimer's disease. However, the mechanism of *Pg* spreading and the impact of bacterial virulence factors (e.g. gingipains) on the brain pathology is not clear. We employed zebrafish to decipher the ability of *Pg* to affect blood-brain barrier, locate to the brain, activate microglia and cause neuroinflammation. Zebrafish is increasingly used to study various infectious and neurodegenerative diseases. In these studies, we infected systemically zebrafish larvae at 60 hours post-fertilization with wild-type (WT) *Pg* or the gingipain-null mutant (*ΔK/R-ab*) and investigated the ability of these pathogens to induce neuroinflammation. Gingipains are crucial virulence factors of *Pg*. We used RT-qPCR to study pattern of gene expression pro-inflammatory mediators and microglia activation markers at 24 and 48 h post-infection WT and (*ΔK/R-ab*). Moreover, we investigated the interaction between bacteria and microglia using real-time confocal microscopy. We found that the WT *Pg* increases expression of pro-inflammatory mediators such as *il-1β, il-8, cox2b* in the heads of zebrafish larvae. Additionally, our microscopic observation showed that WT *Pg* affect brain vasculature and activate microglia in a gingipain-dependent way. To conclude, WT Pg but not *ΔK/R-ab*, can induce neuroinflammation in the zebrafish larvae upon systemic infection, and zebrafish serves as a great model to study neurodegenerative processes upon infection with oral pathogens.

Key words: zebrafish, *Porphyromonas gingivalis*, microglia, neuroinflammation

The study of the effect of doxorubicin treatment on human melanoma cells using zebrafish (*Danio rerio*) model

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Malignant melanoma is an aggressive and frequently fatal skin cancer that is especially difficult to treat in case of spread beyond lymph nodes, i.e. metastasis. Responsible for cancerogenesis are excessive UV irradiation and mutations in melanocytes, the melanin-producing cells found in the skin. They can change from regular into cancerous cells in a matter of months. Zebrafish (*Danio rerio*) larvae can be used as a laboratory model organism to study the effect of different chemotherapeutics against cancer cells. Zebrafish larvae don't have an adaptive immunity, so human cells are not rejected after xenotransplantation. The larvae are transparent making possible observations of tumor development *in vivo*. Finally, the therapeutic agents can be easily administered into zebrafish larvae through immersion. The main aim of this work was to study the effect of doxorubicin, a well-known therapeutic with cytostatic activity, on human melanoma cells using zebrafish larvae model. Zebrafish larvae (AB-TL line) were injected into the yolk with 1205Lu human melanoma cells at 2 days post fertilization (dpf). Cells were stained with fluorescent dye, making them detectable using fluorescence microscopy. Doxorubicin (10 mg/L) dosage was administered via water (changed daily). This concentration was chosen based on our previous tests. Larvae were imaged at 1 hour and after 1, 2, and 3 days post drug administration. The tumor surface area was calculated using ImageJ software. Our results demonstrated that there were no statistically significant differences in the surface area of tumors between doxorubicin-treated and control larvae. Next, other melanoma-specific drug will be tested.

Is zebrafish glycosylation altered during TiLV infection?
The expression of genes involved in glycan synthesis in *Danio rerio* larvae.

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Abstract:

Due to the constantly growing threat from viruses, alternative methods of virus elimination or attenuation are being sought. During infection, viruses use the various offensive strategies, including coating their virions with host glycans. This provides them the protection against the host immune system or regulates their adhesion to host receptors. Therefore, glycosylation as a post-translational modification of proteins is an important process viral infection development. The aim of the study was to determine whether Tilapia Lake Virus (TiLV) infection affects the expression of genes encoding enzymes involved in glycosylation using the zebrafish experimental model.

Genes encoding enzymes responsible for adding fucose residues to glycans were selected: *fut8, fut9B*; sialic acid hydroxylase and synthase: *cmah, cmas1*; as well as sialidases: *neu1, neu4* and sialyltransferases: *st6GalNAc5A, st6Gal1, st3Gal4, st8Sia7*.

TiLV microinjections into duct of Cuvier were performed on zebrafish larvae (2 days post fertilization, dpf). At 24 and 48 hours post infection (hpi) larvae were collected for RNA isolation by the TRIzol method and cDNA was synthesized. Gene expression was examined using real-time qPCR (RT-qPCR). PCR products were also visualized by 2% agarose gel electrophoresis.

Our study showed that *st8Sia7* expression was significantly up-regulated in non-infected four-day larvae in relation to three-day subjects. The expression level of *st8Sia7* was lower in TiLV infected larvae as compared to control ones at 48 hpi, although the difference was not statistically significant. We also observed the statistically significant decrease of *neu4* expression in zebrafish larvae at 48 hpi in comparison to 24 hpi. The obtained results may suggest the use of altered forms of glycans is required for mimicry and tissue tropism during infection in zebrafish.

Keywords: *Danio rerio* ● glycosylation ● virus infection ● sialic acid ● TiLV virus

The effect of selected compounds on an animal model characterized by a reduced level of muscle glycogen phosphorylase

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Keywords: glycogen phosphorylase, zebrafish, McArdle disease

The muscle glycogen phosphorylase (PYGM) is a key enzyme in glycogen metabolism in muscles. During physical exercise, the energy supplied to skeletal muscles comes from glycogen, broken down in the process of glycogenolysis. Mutations within *Pygm* gene cause disorder of carbohydrate metabolism in skeletal muscles described as McArdle's disease – a genetic disease inherited in an autosomal recessive manner. The absence of a functional form of PYG in the muscles prevent obtaining energy from the glycogen. The clinical symptoms of the disease include intolerance to exercise, fatigue, cramps, and exercise-induced muscle pain.

Zebrafish *(Danio rerio*) was shown to have the ability to become an animal model of the disease. Orthologs of the human *Pygm* gene are present in zebrafish genome – namely *pygma* and *pygmb*. The morpholino oligonucleotides are able to temporarily knock down the gene expression, causing changes in muscle integrity in fish. Our goal is to find a bioactive compound able to mitigate disease symptoms. We plan to tests on selected molecules with the use of morphants. We will focus on molecules with drug-like properties such as triheptanoin, able to influence metabolic pathways and/or muscle performance. Triheptanoin is a triglyceride, which serve as source of calories and fatty acids for energy gain, thereby omitting deficiencies of fatty acid oxidation disorders (FAOD) enzymes.

Our preliminary results could be used for further, more systematic drug screening research with the use of a stable animal model of McArdle disease.

Acknowledgmens:

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The effect of long-term exposure of silver nanoparticles on the liver and germinal tissue of the butterfly splitfin (*Ameca splendens*)

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The aim of this study was to investigate the effects of silver nanoparticles (AgNP) on liver homeostasis and germ cell divisions of butterfly splitfin (*Ameca splendens*). Fish of both sexes aged about 4 months were exposed to aqueous solutions of silver nanoparticles at concentrations of 0.01 mg/l, 0.1 mg/l and 1.0 mg/l for 42 days. Fish from the control group were maintained in water without AgNPs. On the last day of the experiment, the fish were euthanized and their livers and gonads were dissected. The livers were frozen in liquid nitrogen and then enzymatic analyses were performed to evaluate the enzymatic activity of the following enzymes: alkaline phosphatase (ALP), acid phosphatase (ACP), leucine aminopeptidase (LAP) and amylase (AMYL). The gonads, on the other hand, underwent standard histological processing. Their morphology was then analyzed, and the distribution of oocyte diameter in the ovary and the area occupied by male germinal cells in the testis were measured. Enzyme analyses showed that the tested nanoxenobiotic reduced the activity of ACP, LAP and AMYL in the livers of fish exposed to AgNP. In contrast, histological analyses showed that silver nanoparticles inhibited meiotic divisions of oocytes in a non-linear manner. In the group of fish exposed to AgNP 0.01 mg/l, a lower percentage of early first-order oocytes was found, while the percentage of previtellogenic first-order oocytes, was higher compared to fish in the other experimental groups. In case of the male gonad, a reduction in the area occupied by spermatocytes in the gonad cross-section was observed in the 0.1 mg/l group. The results indicate that exposure to AgNP can lead to disruption of liver homeostasis and impair reproduction in naturally occurring populations, which can also cause disruption of demographic structure in populations of the endangered species butterfly splitfin.

Study on cumarin antiepileptic properties using zebrafish (Danio rerio) as a model organism

Dominika Kramm, Natalia Wiak

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Epilepsy is a chronic neurologic disorder that affects over 70 million people and animals worldwide. Patients with drug-resistant epilepsy (DRE) have increased risks of premature death, injuries, psychosocial dysfunction, and a reduced quality of life, so development of more effective therapies is an urgent clinical need. Drug resistance provides a major challenge in epilepsy management. Coumarins obtained from Apiaceae plants, using models of the central nervous system, both in vitro and in vivo, showed therapeutic properties in diseases with neurodegenerative disorders. Danio rerio (zebrafish) are a powerful experimental model for genetic and developmental studies. Adaptation of zebrafish to study seizures was initially established using the common convulsant agent pentetertazol (PTZ). By using this model, our laboratory developed simple locomotion-based assays to monitor and quantify seizures in larval zebrafish. In our study we used 5 days old, wild-type Danio rerio larva, 24h before the experiment we incubated them in coumarins with medium E3. Locomotor activity was tracked and analyzed by DanioVision and EthoVision XT. The purpose of our work is to determine the influence of coumarins on epileptic seizures. The studies showed a different spectrum of action of coumarin compounds, some tested coumarins showed stimulatory effects, while others inhibited locomotor activity in the PTZ model, which indicates their potential antiepileptic activity.