

Abstract Book
of
The Meeting of the Polish Zebrafish
Society



Meeting of the Polish Zebrafish Society

September 26-27, 2019



International Institute of Molecular
and Cell Biology in Warsaw
Poland

<http://www.zebrafish.org.pl>



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Meeting of the Polish Zebrafish Society

September 26-27, 2019, Warsaw

Program

DAY I (26.09.2019):

from 12.30 Registration

12.45 General Assembly of the Polish Zebrafish Society (the first date)

13.00-14.30 General Assembly of the Polish Zebrafish Society (the second date)

14.30-14.45 Welcome and Introduction

14.45-15.15 Marta Gajewska, POLLASA: "Legal aspects of zebrafish work in Poland"

15.15-16.45 Session I (Neuroscience)

Keynote speaker: Savani Anbalagan, *Centre of New Technologies, University of Warsaw*: „Role of astroglia (pituicytes) in the hypothalamo-neurohypophyseal system - a major brain-to-blood neuro-endocrine interface. “

Justyna Zmorzyńska, *International Institute of Molecular and Cell Biology in Warsaw*: "Impaired commissural tract fasciculation is related to increased epileptogenesis and anxiety in the Zebrafish model of Tuberous Sclerosis Complex"

Eugene Gasanov, *International Institute of Molecular and Cell Biology in Warsaw*: "Voltage-gated potassium channel *Kncg4b* mutation affects zebrafish ear development.

16.45-17.15 Coffee break

17.15-18.45 Session II (Genetic disorders)

Keynote speaker: Cecilia Winata, *International Institute of Molecular and Cell Biology in Warsaw*: "Constructing the gene regulatory network underlying heart development using genomics"

Marta Migocka-Patrzałek, *University of Wrocław*: "The muscle glycogen phosphorylase (Pygm) in zebrafish"

Anna Sarosiak, *Institute of Physiology and Pathology of Hearing*: "Dissecting pathogenicity of genetic variants detected in HL patients - preliminary studies on the function of the *wfs1* gene in zebrafish"

Agnieszka Madejska, *Institute of Physiology and Pathology of Hearing*: "Application of gentamicin to develop the workflow for studying hearing and balance disorders in zebrafish model" - CANCELED

18.45-20.45 Poster session and "Get together party"

DAY II (27.09.2019):

9.00-11.00 Session III (Immunity and infection)

Keynote speaker: Tomasz Prajsnar, *The University of Sheffield*: "Using zebrafish to unravel the role of phagocytes in *Streptococcus pneumoniae* infection"

Krzysztof Rakus, *Jagiellonian University*: "Zebrafish as a model to study Tilapia Lake Virus (TiLV) infection"

Miriam Mojżesz, *Jagiellonian University*: „Activation of DExD/H-box RNA helicases during infection with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV) in zebrafish and common carp“

Magdalena Widziołek-Pooranachandran, *Jagiellonian University*: "Oral pathogen-mediated vascular damage in vitro and in vivo in a novel zebrafish systemic infection model"

11.00-11.30 Coffee break

11.30-13.00 Session IV (Pharmacology and Toxicology?)

Keynote speaker: Vladimir Korzh, International Institute of Molecular and Cell Biology in Warsaw, "Potassium channels and epilepsy".

Kinga Gawęł, *University of Oslo*: "AS-1, a new potent and broad-spectrum anticonvulsant, prevents seizures in mouse and zebrafish pentylenetetrazole (PTZ) seizure tests"

Agnieszka Michalak, *Medical University of Lublin*: „Acute toxicity and locomotor activity in zebrafish larvae treated with SL-327“

Monika Maciąg, *Medical University of Lublin*: "Examination of the toxicity profile of doxorubicin in zebrafish model"

13.00-13.15 Conclusions and closing remarks

ORAL PRESENTATIONS

1. Savani Anbalagan

Centre of New Technologies, University of Warsaw

Role of astroglia (pituicytes) in the hypothalamo-neurohypophyseal system - a major brain-to-blood neuro-endocrine interface

Role of astroglia (pituicytes) in the hypothalamo-neurohypophyseal system - a major brain-to-blood neuro-endocrine interface. The hypothalamo-neurohypophyseal system (HNS) regulates homeostasis through the passage of neurohormones and blood-borne proteins via permeable blood capillaries that lack blood-brain-barrier (BBB). The basic components of the HNS are the hypothalamic axonal projections, endothelial blood vessels and astroglial-like cells, termed pituicytes. Why do neurohypophyseal blood vessels become permeable while the neighbouring blood vessels of the brain form a tight BBB remains unclear. We present the first vertebrate pituicytes transcriptome and show that pituicytes express genes that are associated with BBB breakdown during neuroinflammation. We tested the hypothesis that pituicyte-derived signals instruct the developmental cellular decision to form a permeable neuro-vascular conduit that bypasses the BBB. Thus, we provide evidence that pituicyte-derived cues regulate normal development and maintenance of permeable neuro-vascular interfaces. Finally, the mechanism by which a permeable endothelial fate is maintained in the developing neurohypophysis resembles previously reported pathophysiological conditions in the brain.

Key words: astroglia, permeability, blood-brain barrier

2. Justyna Zmorzynska

International Institute of Molecular and Cell Biology

Impaired commissural tract fasciculation is related to increased epileptogenesis and anxiety in the Zebrafish model of Tuberous Sclerosis Complex

Introduction: Tuberous Sclerosis Complex (TSC) is an autosomal dominant disease caused by mutations in genes encoding for TSC1 or TSC2. These proteins form a complex that inhibits mTORC1 signaling, which activates multiple molecular pathways leading to growth and differentiation in neurons. Lack of TSC1-TSC2 functional complex due to mutations results in mTORC1 overactivation and in neurodevelopmental syndromes like epilepsy, intellectual disability, or autism spectrum disorder.

Materials and Methods: To investigate mechanisms underlying TSC disease, we use zebrafish mutant TSC2vu242 [Kim et al, 2011]. We examined TSCvu242 fish using various behavioral tests and by live brain imaging with light-sheet microscopy.

Results: We confirmed that homozygotic TSC2vu242 mutants were lethal at the early larval stage, underscoring the importance of maintaining proper mTORC1 signaling. Also, TSC2vu242 brains showed increased activation of the mTORC1 pathway and white matter thinning. We discovered improper axon development and axonal tract fasciculation, together with changes in the expression of genes involved in axon guidance. Live imaging showed neuronal hyperexcitability in the brain and epileptogenesis in the early development of the Tsc2-deficient fish. Together with decreased locomotion of TSC2vu242 mutants, these results suggest non-motor seizures. We also examined fish by multiple anxiety-testing behavioral tests and found an increase in anxiety in mutant fish. Moreover, we could rescue anxiety-related behavior and white matter thinning in the TSC2vu242 mutants using the same drug.

Conclusions: These results suggest that white matter disruption contributes to the neurodevelopmental syndromes like anxiety and epilepsy in the fish model of TSC disease.

Key words: neurodevelopmental disorders, Tuberous Sclerosis Complex, anxiety

3. Eugene Gasanov

International Institute of Molecular and Cell Biology in Warsaw

Voltage-gated potassium channel *Kcng4b* mutation affects zebrafish ear development

E.V. Gasanov¹, J. Jędrychowska^{1,2}, V. Korzh¹

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²Postgraduate School of Molecular Medicine, Medical University of Warsaw, Poland

Voltage-gated potassium channels (Kv-s) are the key regulators of polarization of plasma membrane and transduction of neuroelectrical impulse that regulates cell activity. Dysfunction of Kv-s has been linked to hereditary disease, including epileptic encephalopathy, they are highly expressed in the ear and play a key role in the hearing and vestibular reception. Kv-s consist of four α -subunits which could be encoded by different genes in dependence of cell type, allowing to modulate channel activity and localization. Hence, the Kv genes display tissue- and time-specific expression during development, meanwhile the exact cell function and molecular mechanisms of action of Kv subunits remain largely unknown.

Previously the pivotal role of Kv6.4 encoded in zebrafish (*Danio rerio*) by *kcng4b* gene was shown for development of brain ventricular system (Shen et al., 2016). *kcng4b* is expressed only in developing embryo, but not in adult fish, in cells lining the brain ventricular system, ear, and eye. Using CRISPR-Cas9-mediated mutagenesis we generated two mutants of *kcng4b* causing a reading frame shift and leading, first, to truncation of the polypeptide and, second, its prolongation. While both mutants feature defects in the development of the ear and brain ventricular system, their phenotypes are different. Current analysis of *kcng4b* mutants aims to reveal the molecular mechanism involving the role of Kv-s in ear development."

Key words: Potassium channel, *kcng4b*, ear, development

4. Cecilia Winata

International Institute of Molecular and Cell Biology in Warsaw

Constructing the gene regulatory network underlying heart development using genomics

5. Marta Migocka-Patrzałek

University of Wrocław

The muscle glycogen phosphorylase (Pygm) in zebrafish

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The muscle glycogen phosphorylase (PYGM) is an enzyme responsible for the first reaction in glycogenolysis. There are several known PYGM mutations leading to the inherited human McArdle disease, which symptoms include exercise intolerance with premature fatigue, muscle cramps and myalgia due to lack of efficient glycogen decomposition in muscle tissue. So far no efficient treatment was found except from appropriate diet and training [1]. Our experimental goal is the evaluation if zebrafish (*Danio rerio*) could be a good animal model for this human disorder. The zebrafish has many experimental advantages, such as transparent, externally developing embryos with easily visible muscles, which share high similarity to human tissue. Zebrafish was successfully implemented as an animal model of several human myopathies [2]. The zebrafish *Pygma* and *Pygmb* shares more than 80% of aminoacid sequence identity with human PYGM. Our experimental data revealed that *Pygm* expression level is changing both on mRNA and protein level during early (1-5dpf) zebrafish development and is correlated with glycogen level. The *pygma* and *pygmb* knockdown, performed using the morpholino technique, result in lowering *Pygm* quantity and alternated morphology including lower birefringence, and different muscle fibers shape and organization. The ultrastructure analysis shows disintegrated muscles with glycogen granules accumulation in subsarcolemmal region. The *Pygm* deficiency leads also to glycogen up-regulation. Concluding, we present results indicating that lower quantity of *Pygm* leads to morphological and biochemical changes in zebrafish, which are similar to the McArdle disease symptoms.

ACKNOWLEDGEMENTS: The work was supported by National Science Centre (the grant no. 2017/01/X/NZ4/00093) and the Polish State Committee for Scientific Research, Project No. 1068/S/IBE/2018.

Key words: McArdle disease, glycogenolysis, *Pygm*

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[2]. Plantié E, Migocka-Patrzałek M, Daczewska M, Jagla K (2015). Model organisms in the fight against muscular dystrophy: lessons from *Drosophila* and zebrafish. *Molecules* 20: 6237–6253.

6. Anna Sarosiak

Institute of Physiology and Pathology of Hearing

Dissecting pathogenicity of genetic variants detected in HL patients - preliminary studies on the function of the wfs1 gene in zebrafish

Anna Sarosiak^{1,2}, Agnieszka Madejska¹, Dominika Oziębło^{1,2}, Marcin Leja^{1,2}, Przemko Tylżanowski^{3,4}, Monika Ołdak¹

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2. Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland
3. Department of Development and Regeneration, University of Leuven, Leuven, Belgium
4. Medical University of Lublin, Lublin, Poland

Autosomal dominant hearing loss (ADHL) is the second most common form of hereditary hearing loss characterized by a large genetic heterogeneity. Detection rate of causative genetic variant in ADHL reaches approximately 50% leaving the remaining half unexplored. Due to evolutionary conserved features and a high accessibility for genetic manipulations zebrafish model can be used to investigate the pathogenicity of newly discovered genetic variants and their role in the development of HL.

Novel probably pathogenic variants of the WFS1 gene causative of low-frequency HL were found in families with ADHL. In preliminary phase of the study, morpholino oligonucleotides (MO) were used to obtain a zebrafish knock-down model of the wfs1 gene. In a small percentage of wfs1 morphants, different morphological defects were observed and distributed in a MO dose-dependent manner. Additionally, diverse otoliths malformations were observed. Gradual effect and phenotypic features were not observed in larvae injected with control MO. Due to a possible non-specific MO effects we plan to perform further phenotypic validation.

In the next stage, we plan to lead the research towards verification of pathogenicity of the detected variants based on evaluation of function and structure of the zebrafish hearing apparatus. To achieve this, we will evaluate morphology of morphants' auditory system, apply neuromast stainings and high-resolution neuromast imaging, prepare an overexpression model with an injection of mutant and wild type mRNA encoding human WFS1 gene and test the hearing responses assessing the zebrafish locomotor activity after the vibration stimuli.

Supported by: NCN Grant no. 2016/22/E/NZ5/00470 SONATA BIS6.

Key words: zebrafish, hearing loss, knock-down model, wfs1 gene

7. Agnieszka Madejska

Institute of Physiology and Pathology of Hearing

Application of gentamicin to develop the workflow for studying hearing and balance disorders in zebrafish model

Agnieszka Madejska¹, Anna Sarosiak^{1,2}, Dominika Oziębło^{1,2}, Przemko Tylżanowski^{3,4}, Monika Ołdak¹

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3. Department of Development and Regeneration, University of Leuven, Leuven, Belgium
4. Medical University of Lublin, Lublin, Poland

Introduction: Zebrafish model is used to study ototoxicity due to the presence of hair cells on the body surface. Hair cells are key structures that are responsible for maintaining hearing and balance in zebrafish. They form clusters called neuromasts which are localized on the head and along the lateral line. It is known that aminoglycosides have ototoxic properties and their administration cause apoptosis of hair cells in neuromasts leading to deafness. In our studies, we would like to obtain zebrafish with damaged neuromasts to focus on molecular and behavioral characterization of the hearing loss phenotype. We would like to use our observations to create a workflow for studying the mechanism leading to hearing loss in zebrafish.

Materials and Methods: For our experiments we have used 5 dpf zebrafish larvae. They were treated with gentamicin (GTM) for 6 hours. After incubation with GTM live cell staining with DASPEI

and Yo-Pro-1 was performed. Neuromast imaging was made with fluorescence microscope SteREO Discovery.V8. Locomotor activity after GTM administration was measured with the ZebraBox System using the Tracking mode. Changes in expression of genes related to apoptosis will be examined in real-time PCR.

Results and Conclusions: Vital dye staining was almost completely lost in neuromasts after GTM administration indicating cell death. Specific GTM doses have a different effect on larvae's activity.

Our results confirmed that we have obtained a hearing loss phenotype and this is a basis for planning our further analysis.

Supported by: NCN Research Grant no. 2016/22/E/NZ5/00470 SONATA BIS6.

8. Tomasz Prajsnar

The University of Sheffield

Using zebrafish to unravel the role of phagocytes in *Streptococcus pneumoniae* infection

Tomasz K. Prajsnar, Andrew K. Fenton, David H. Dockrell and Stephen A. Renshaw.

Streptococcus pneumoniae (pneumococcus) is a serious human pathogen causing multiple pathologies including severe community-acquired pneumonia which remains a leading cause of global mortality. Enhancing immune microbicidal responses could combat this problem but our understanding of how specific phagocytes kill bacteria is poorly understood, especially in vivo.

In this study, we use larval zebrafish to study the interaction between intravenously administered pneumococci and professional phagocytes such as macrophages and neutrophils in order to unravel and potentiate bacterial killing mechanisms employed by these innate immune cells.

We show that capsule protects pneumococcus from phagocytosis by macrophages which conversely occurs rapidly in response to non-encapsulated pneumococci. The encapsulated bacteria cause 100% mortality within 48 hours post infection (hpi) while the same dose of non-encapsulated pneumococci are avirulent. Time course analysis of the in vivo bacterial numbers revealed that while encapsulated pneumococcus proliferates reaching approximately 10^6 CFU at the time of host death, the non-encapsulated counterpart is unable to grow and is cleared within 20 hpi. Importantly, larvae devoid of macrophages succumb to non-encapsulated pneumococci even in the presence of neutrophils.

Additionally, we demonstrate that upon phagocytosis by macrophages, bacteria undergo rapid acidification, and chemical inhibition of vacuolar ATPase (V-ATPase) by bafilomycin leads to prevention of intracellular bacterial killing. The autophagic response and expression of pro-inflammatory cytokines within pneumococcus-infected phagocytes is currently under investigation.

Collectively, our data demonstrate that larval zebrafish are suitable to study pneumococcal infection and could help design novel immunomodulatory strategies to combat this disease.

Key words: Zebrafish Infection Bacteria Pneumococcus Autophagy

9. Krzysztof Rakus

Jagiellonian University

Zebrafish as a model to study Tilapia Lake Virus (TiLV) infection

Krzysztof Rakus¹, Miriam Mojżesz¹, Magdalena Widziołek¹, Niedharsan Pooranachandran¹,
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The essential immune mechanisms, receptors and pathways are often well conserved in vertebrates and zebrafish represent a relevant model for the study of core immune mechanisms activated by viruses. Here, we have evaluated the possible use of zebrafish to study immune response and host-pathogen interactions during infection with Tilapia Lake Virus (TiLV). TiLV (genus Tilapinevirus, family Orthomyxoviridae) belongs to the same family as the influenza virus and its genome is 10-segment, negative-sense RNA. It causes massive mortality of tilapia fish and is responsible for high losses in aquaculture.

We developed an infection model of zebrafish with TiLV in both adult and larval fish. Increased viral load was observed in spleen, kidney and liver of adult fish at 1, 3, 6, and 14 days post infection (d.p.i.) as well as in larvae at 1 and 2 d.p.i. We also demonstrated TiLV-induced the up-regulation of the gene expression of several immune related molecules in spleen and kidney of adult fish. The changes in the expression was observed for genes encoding pathogen recognition receptors involved in sensing of viral RNA (tlr3, tlr22, rig-I), transcription factors (irf3, irf7), type I interferons (inf ϕ 1, inf ϕ 2, inf ϕ 3), anti-viral proteins (mx α), pro-inflammatory cytokines (il-1 β , tnf- α , il-8, ifn γ 1-2), anti-inflammatory il-10, T-cell markers (cd4-1, cd4-2, cd8) and IgM (igm). Moreover, tropism of TiLV and histopathological changes were analyzed in selected organs of adult zebrafish.

Our results indicate that zebrafish is a good model to study mechanisms of TiLV infection and to follow anti-viral response.

This work was supported by National Science Center of Poland (2015/18/E/NZ6/00516).

Key words: Tilapia Lake Virus, anti-viral immune response, interferons type I

10. Miriam Mojżesz

Jagiellonian University

Activation of DExD/H-box RNA helicases during infection with spring viraemia of carp virus (SVCV) and chum salmon reovirus (CSV) in zebrafish and common carp

Miriam Mojżesz¹, Katarzyna Kłak¹, Mikołaj Adamek², Piotr Podlasz³, Małgorzata Chmielewska-Krzesińska³, Magdalena Chadzińska¹, Krzysztof Rakus¹

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The host response to viral infection involves sets of receptors and immune pathways which evolved to afford protection against viruses. Recognition of the non-self signature of invading viruses is a crucial step for the initiation of the anti-viral response. The non-RLR (non-RIG-I-like receptors) DExD/H-box RNA helicases have been implicated as additional receptors for viral nucleic acid detection and/or to be part of innate immune signaling pathways that induce type I interferon (IFN) response in mammals.

In the present work we aimed to study, the anti-viral role of DExD/H-box RNA helicases: DDX1, DDX3, DHX9, DDX21 and DXH36, which are known to sense a viral RNA in mammals. The expression of DExD/H-box RNA helicases and genes involved in INF type I pathway were studied *in vitro* in zebrafish ZF4 cell line during SVCV or CSV infection as well as *in vivo* in zebrafish and common carp during SVCV or CSV infections.

In vitro SVCV infection does not affect RNA helicases gene expression. However CSV infection up-regulates gene expression of *ddx3* and *dhx9*. *In vivo* SVCV infection in zebrafish induced up-regulation of *ddx1* and *dhx36* expression in kidney while CSV infection induced up-regulation of *ddx1* and *dhx9* expression in spleen. Moreover, we observed a slight but significant up-regulation of *ddx1*, *dhx9* and *ddx21* in head kidney of SVCV-infected carp.

Our *in vitro* and *in vivo* study demonstrated, for the first time in fish that selected non-RLR DExD/H-box RNA helicases might be involved in sensing of viral infection.

This work was supported by National Science Center of Poland (2015/18/E/NZ6/00516).

Key words: DExD/H-box RNA helicases, interferons, *vig-1*, SVCV, CSV

11. Magdalena Widziołek-Pooranachandran

Jagiellonian University

Oral pathogen-mediated vascular damage in vitro and in vivo in a novel zebrafish systemic infection model

Magdalena Widziołek^{1,2,3}, Cher Farrugia¹, Tomasz Prajsnar^{4,5}, Robert Wilkinson⁶, Krzysztof Rakus², Jan Potempa³, Graham P Stafford¹, Craig Murdoch¹

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Abstract

Porphyromonas gingivalis (Pg) is the main pathogen involved in the development of chronic inflammatory disease; periodontitis. Reports show that during dental procedures Pg enters the bloodstream contributing to cardiovascular diseases. The main virulence factors of this microbe are cysteine proteases; gingipains which contribute to pathogenesis. We established a novel zebrafish larvae systemic infection model for Pg that allows real-time visualisation of host-pathogen interactions. In this study, we used this model alongside monolayers of endothelial cells to investigate the mechanism of Pg dissemination and interaction with host cells.

Wild-type (WT) Pg W83 and gingipains null mutant were labelled and injected into transgenic zebrafish embryos containing a fluorescently-tagged vasculature. Bacterial interaction with host cells was visualised using light-sheet microscopy. The efficiency of phagocytosis by immune cells was assessed using a pH sensitive dye. The ability of Pg to increase endothelial permeability was evaluated using fluorescent dextrans both in vivo and in vitro.

Pg penetrated the zebrafish vasculature causing oedemas and cardiac damage in a gingipain-dependent manner. Zebrafish injected with WT Pg displayed significantly reduced phagocytosis and increased survival compared to those injected with mutants lacking gingipains. Pg reduced expression of the endothelial cell junction proteins and increased the permeability of endothelial monolayers in vitro. In agreement to this, intra-vascular Pg also affected zebrafish vasculature

In conclusion, infection with Pg markedly affects the zebrafish vascular system causing decreased systemic immune cell activity and increased endothelial damage. Therefore Pg may be a significant contributor to oral microbe-related cardiovascular damage leading to long-term systemic disease.

This work was supported by National Science Centre of Poland (Etiuda V, nr 2017/24/T/NZ6/00300).

Key words: oral pathogen, host-pathogen interaction, cardiovascular disease

12. Vladimir Korzh

International Institute of Molecular and Cell Biology in Warsaw

Potassium channels and epilepsy

13. Kinga Gawel

University of Oslo

AS-1, a new potent and broad-spectrum anticonvulsant, prevents seizures in mouse and zebrafish pentylenetetrazole (PTZ) seizure tests

14. Agnieszka Michalak

Medical University of Lublin

Acute toxicity and locomotor activity in zebrafish larvae treated with SL-327

Agnieszka Michalak¹, Barbara Budzyńska², Monika Pilaszkiwicz¹, Grażyna Biała¹

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Zebrafish is a comprehensive animal model that can be easily used in order to evaluate acute toxicity of drugs and their impact on locomotor activity. Our previous studies focused on determining the role of the MEK-ERK pathway in anxiety-related behaviours in mice. We are seeking for new, efficient animal models and our interest has been directed recently toward zebrafish.

We used the zebrafish model to evaluate acute toxicity and estimate LD50 of SL-327, a selective MEK1/2 inhibitor. To establish the toxicity of SL-327 we used the Fish Embryo Acute Toxicity (FET) test, in accordance with the OECD Guidelines for the Testing of Chemicals (Test No. 236, 2013). Then, based on the results of toxicological studies, a range of concentrations of SL-327 has been chosen to assess its influence on locomotor activity. Changes in locomotor activity may serve as an interesting indicator of anxiety-related behaviours in zebrafish larvae. Thereby, additional analysis of the locomotor activity in zebrafish under light-dark challenge was performed. Locomotor activity of 5 dpf zebrafish was evaluated using the ZebrafishBox system by ViewPoint

LC50 for SL-327 was determined to a value of 14.73 μ M. No changes in locomotor activity were observed in 5 dpf zebrafish under continuous light. However, SL-327 significantly decreased locomotor activity in dark phase in zebrafish subjected to light-dark challenge, which suggests potential anxiolytic properties of the tested compound.

This work was supported by the Internal Fund of the Medical University of Lublin 2018/2019.

Key words: SL-327, acute toxicity, locomotion

15. Monika Maciąg

Medical University of Lublin

Examination of the toxicity profile of doxorubicin in zebrafish model

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(2)Independent Laboratory of Behavioural Studies, Medical University of Lublin, Poland

Doxorubicin is a powerful anthracycline antibiotic used for the treatment of cancer. However, its use as a chemotherapeutic agent is limited by its toxicities. Most especially observed is cardiotoxicity, but other organ systems are also degraded by doxorubicin use.

The ability of zebrafish to determinate parameters such as lethal dose, teratogenicity and specific-organ toxicity, in particular of the heart and the nervous system make it an attractive model for anticancer drug examination.

In this study the zebrafish embryos were exposed to different concentrations of doxorubicin and development toxicity were observed. We evaluated the effects of doxorubicin exposure on lethality, hatching rate, body length and morphological alterations. Due to the cardiotoxicity of doxorubicin, we examined its effects on heart rate and the diameter of the dorsal aorta. Moreover, because of doxorubicin fluorescence, we could observed tissue-specific distribution of this compound.

Our results indicate that doxorubicin in higher concentrations mainly caused acute lethal effects, and lower concentrations caused malformations, decreased heart rate and the diameter of the dorsal aorta in zebrafish larvae.

The unfortunate consequences of doxorubicin have promoted the search for new drugs that can prevent or reverse the damage after exposure to this anticancer drug using the zebrafish model.

The authors acknowledge financial support from the National Science Center (grant 2017/25/B/NZ7/02654).

Key words: Doxorubicin, chemotherapeutic agent, toxicity

POSTER SESSION

1. Katerina Makarova

Medical University of Warsaw

Optimization of spin probe/spin trap system for ESR studies of the oxidative stress in the zebrafish embryos (in vivo)

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EPR spectroscopy is one of the techniques which provide direct information about free radicals in the biological systems. However, X-band EPR studies often require sophisticated sample preparation protocols that could potentially affect oxidative stress effects in biological samples. Furthermore, due to the EPR resonator dimensions, application of X-band EPR to in vivo studies is limited, and usually the ex vivo studies using tissues and their homogenates are performed.

Here, an application of X-band EPR for the studies of the oxidative stress in vivo in zebrafish embryos is reported. The 4hpf zebrafish embryos were incubated in the 1mM - 10mM solution of spin probes (TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl) and 3-carbamoyl-PROXYL (3-Carbamoyl-2,2,5,5-tetramethyl-1-pyrrolidineoxy)) and spin traps (DMPO (5,5-dimethylpyrroline-N-oxide), POBN (α -(4-pyridyl N-oxide)-N-tert-butyl nitron) and PBN (α -phenyl N-tert-butyl nitron)). We have observed triplet EPR signal in 1-3 dpf zebrafish embryos from 1mM and 2.5 mM of 3-carbamoyl-PROXYL (3-CP) and TEMPOL spin probes, respectively. In case of DMPO spin trap we were able to observe EPR singlet line. The optimal EPR spectrometer parameters, both safe for zebrafish embryos and sufficient to get EPR spectrum, were 4 scans by 20s, 100G sweep and 0.03 mW power. The maximum non-toxic concentration of spin agent were 10mM for DMPO, PBN and 3-CP, respectively. Hence the proposed method may be used in oxidative stress and potentially antioxidant screening studies.

Key words: in vivo EPR, zebrafish, spin probes, spin traps

2. Piotr Podlasz

University of Warmia and Mazury in Olsztyn

Study of the role of galanin during inflammation, innate immunity and bacterial infection using zebrafish as a model organism

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Immune response occurs in an organism during various pathological conditions and is becoming clear that immune system does not act separately, but is involved in complex processes between different systems during an infection including nervous system. Galanin is a neuropeptide with many different functions and a candidate molecule to be involved in such a cross talk. To explore further the function of this gene, we chose the experimental model system based on zebrafish. Our previous studies revealed that the galanin gene is very conserved across species, including zebrafish. This strong evolutionary conservation may suggest important and similar roles of galanin in all vertebrates. To study the function of galanin during inflammation, innate immunity and bacterial infection we used genetically modified zebrafish lines. Firstly, transgenic line with inducible overexpression of galanin: Tg(hsp70l:galn) and recently generated in our lab CRISPR-cas9 zebrafish mutant which lacks of expression of the galanin (galn -/-). Additionally, we used transgenic lines with fluorescent labeled neutrophils and macrophages. Our results show that galanin expression is significantly upregulated after chemical wounding. Also, we discovered that overexpression of galanin influences the innate immunity, showing a protective way of action during a *Mycobacterium marinum* and *Staphylococcus aureus* infection. On the contrary, downregulation of galanin results in increased bacterial burden. Our data from RNA-seq and qPCR confirmed key role of galanin during immune response. The obtained results suggest that galanin or its analogues can be used in the future for modulation of inflammation or in the treatment of bacterial infections.

Key words: galanin, inflammation, infection, *Mycobacterium marinum*, *Staphylococcus aureus*

3. Kinga Gawel

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AS-1, a new potent and broad-spectrum anticonvulsant, prevents seizures in mouse and zebrafish pentylenetetrazole (PTZ) seizure tests

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Epilepsy is one of the most common and debilitating neurological diseases affecting about 1–2% of the population worldwide. The pharmacotherapy of epilepsy is highly unsatisfying, thus the search for more effective anticonvulsants is still clinically unmet need. In our recent studies, we developed compound AS-1 as a promising candidate for a new potent and broad-spectrum anticonvulsant. This substance revealed potent protection across the most important animal acute seizures models such as the maximal electroshock test (MES), the subcutaneous pentylenetetrazole test (scPTZ), and the six-hertz test (6 Hz, 32 mA) in mice. Herein, we aimed to assess the effect of repeated administration of AS-1 (at doses of 15, 30 and 60 mg/kg) on the progression of kindling induced by repeated injection of PTZ (40 mg/kg, intraperitoneally) in mice. Also, we evaluated the effect of 24 hours-long incubation with AS-1 (5 mM) on the number and duration of EEG epileptiform-like discharges, induced by acute PTZ (20 mM) administration in 7 days post-fertilization zebrafish larvae. Our study revealed that AS-1 decreased seizure severity score and thereby suppressed kindling progression in mice, which suggests that the tested compound may possess the ability to prevent epilepsy development. Also, incubation of larvae with AS-1 substantially decreased the number and cumulative duration of epileptiform-like events in EEG assay. In conclusion, AS-1 may be regarded as a novel anticonvulsant agent.

Acknowledgments The studies were supported by the Polish National Scientific Centre grant UMO-2017/27/B/NZ7/00249 (for KK) and start-up funds of NCMM (for CVE). KG has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 798703-GEMZ-H2020-MSCA-IF-2017.

Key words: AS-1, seizures, pentylenetetrazole, mice, zebrafish

4. Adrianna Skiba
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Swertiamarin – potent seco-iridoid glycoside against epilepsy developed by scn1lab zebrafish mutants

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Dravet syndrome (DS) is form of rare, drug resistant epileptic encephalopathy, that starts in early years of life and is characterized by high mortality. 85% of DS patients have mutation in SCN1A gene [1]. Scn1lab zebrafish mutants also called “didy” have homologous mutation in sodium channel voltage gated type I α subunit. These channel activity is involved in processes such as regulation of locomotor rhythm, neuronal action potential and metabolism and energy. Didys are used to study Dravet syndrome but also anxiety disorder and epilepsy [2].

Serotonin regulate in human central nervous system main adaptive reactions and responses to environmental changes such as mood, anxiety, aggressiveness, feeding behaviour and its associated with mood affective disorders – autism, bulimia, obesity, cognitive deficits. Latest studies shows that serotonergic pathways may be also connected to seizures in DS. [3]

Swertiamarin is seco-iridoid glycoside that possess a similar pharmaceutical properties as 5-HT₂ receptor modulators. It was reported to penetrate blood-brain barrier and as antidepressant in mice and rats. Modulation of 5-HT suggest that swertiamarin may be good candidate to inhibit seizures in scn1lab zebrafish. Swertiamarin was tested against seizures in PTZ zebrafish model, but does not shows significant activity. Further study will include anti-epileptic bioassay on scn1lab mutants and anxiety-like test on wild-type zebrafish as well on scn1lab mutants.

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Key words: zebrafish, epilepsy, seizure, Dravet syndrome

5. Barbara Budzynska
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Zebrafish and rodents as complementary models for evaluation anxiety-like effects of xanthotoxin

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ABSTRACT

Xanthotoxin (8-methoxypsoralen, 8-MOP), is a furanocoumarin found in many medicinal plants and is used in the treatment of psoriasis, vitiligo, and cutaneous T-cell lymphoma. This drug also possesses slight antioxidative activity as evidenced from in vitro studies as well as interacts with the benzodiazepine binding site of the GABA-A receptor, and may exert anxiolytic activity.

The purpose of our experiment was to examine the influence of xanthotoxin on anxiety-like behaviors in *Danio rerio* larvae and male Swiss mice. The elevated plus maze (EPM) test was used to evaluate the anxiety level in rodents. Zebrafish larval behaviour analysis was evaluated using the ZebraBox system manufactured by ViewPoint. Thigmotaxis was used as an index of anxiety. Zebrafish larvae displaying thigmotactic behaviour avoid the centre of an arena (inner zone) and prefer to stay close to the boundaries of a well (outer zone). Moreover, larval locomotion was measured using simple locomotor assay as well as light on/off assay (stressful conditions).

Our results indicate that xanthotoxin in dose-dependent manner influences on thigmotaxis in larval zebrafish defined as anxiety-related behaviours. We also revealed that xanthotoxin exerts marked influence (anxiolytic at the doses of 1 and 2,5 mg/kg and anxiogenic at the dose of 5 mg/kg) observed in the EPM test in mice.

In summary, our studies confirm that thigmotaxis observed in 5 dpf zebrafish larvae may serve as a very useful tool for screening the influence of natural products on anxiety level. This model can be preliminary for rodents models of anxiety behaviors. Also, the results of our research suggest xanthotoxin, administered in low doses, to be an interesting therapeutical option for disorders with a high level of anxiety.

This study was supported by National Science Centre, Poland, Grants: 2014/13/B/NZ4/01249 and 2017/25/N/NZ7/01899

Key words: Anxiety, coumarins, zebrafish, mice

6. Dodzian Joanna

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Health monitoring in zebrafish research facilities

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Zebrafish as a model organism has become increasingly popular in Poland. Tremendous potential in developmental, biochemical and biomedical studies causes the need to maintain *Danio rerio* in scientific centers in accordance with European and Polish law. One of the elementary regulation during establishing a zebrafish research facility is the implementation of a health monitoring program. The control of the health status of fish should be provided on multiple steps: from introducing new lines into the facility, until their humane endpoint of life. The daily observation of fish, elimination of abnormal and sick individuals, as well as internal/external specimens examination needs to be done. Animals welfare and proper health status must be a concern of all, due to the fact that only individuals in good condition could provide repeatable results, unified for different institutes. Moreover, it is well known that diseases and subclinical infections may affect the interpretation of experimental results. The potential risk of zoonoses is another factor that needs to be considered. Despite that knowledge, health monitoring of zebrafish is still not so propagated among research institutions in the whole world. Thus open discussion and information flow might be a way to increase awareness of the importance of the health status of fish kept in the facility.

Key words: Zebrafish, Health Monitoring

7. Magdalena Góra
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Zebrafish sperm cryopreservation as an effective and valuable technique for preserving genetic resources

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Danio rerio is a popular and valued model organism used in research in various fields of science. Consequently, research laboratories and zebrafish facilities are faced with a challenge of the constant maintaining of an increasing number of zebrafish lines, which is impractical due to high costs and space limits. A common way to deal with this kind of limitation is cryopreservation of sperm and in vitro fertilization (IVF). In order to improve the quality of frozen sperm and post-thaw fertilization rates after cryopreservation, we adapted the protocol taken from University College London (UCL) Zebrafish Facility. Our 3 years' experience shows that (i) collecting sperm from one male instead of pooling from several males helps to avoid mixing good and poor quality of sperm samples. It also shortens the time spent by fish outside the water and, thus, fish stress. (ii) there is no significant correlation between storage period of frozen sperm samples and IVF success (number of viable embryos produced in an IVF divided by the total number of eggs fertilized by a single sperm sample) (iii) there is a significant negative correlation between age of the males and IVF success.

Key words: zebrafish, husbandry, cryopreservation, IVF

8. Niedharsan Pooranachandran
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CRISPR/Cas9 technology to efficiently generate zebrafish mutants with high mutagenesis rate

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CRISPR is a family of DNA sequences evolved as a defence mechanism in prokaryotes to find and destroy DNA of viruses. CRISPR/Cas antiviral defence mechanism was adapted for gene editing purposes in living organisms, one such example is the generation of null mutants in zebrafish. CAS9 enzyme is responsible for cleavage of the target DNA, while the CRISPR RNA acts as a guide and is designed to target a specific DNA sequence. Currently, we are using the latest methods of CRISPR technology to generate a series of zebrafish mutants knocked-out for selected DExD/H box RNA helicases. The plethora of CRISPR design tools available online allows for efficient design of CRISPRs to any desired gene. Subsequently, it is now possible to directly order the RNA sequence and inject alongside the CAS9 protein, thus bypassing a number of steps. Injections are ideally performed on one-cell stage embryos for maximal efficiency, and in many cases we observed a mutagenesis rate of over 90 %. Here we present an example of a CRISPR generated mutant; DDX1 that has over 90 % mutagenized cells in 100 % of injected embryos.

This work was supported by National Science Center of Poland (2015/18/E/NZ6/00516)

Key words: CRISPR, Immunology, DExD/H, Infection

9. Monika Gawrońska-Grzywacz

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THE EFFECT OF NOVEL HYDRAZIDE-HYDRAZONES ON THE CENTRAL NERVOUS SYSTEM OF ZEBRAFISH LARVAE – PRELIMINARY STUDY

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Zebrafish (*Danio rerio*) proved its efficacy in the high-throughput screening of novel chemical compounds as a cost effective vertebrate model organism. The hydrazide-hydrazone analogues show the wide range of activities, among others: anti-inflammatory, analgesic, anticonvulsant, but also anticancer, antibacterial, antidiabetic or cardioprotective. In this study, three novel chemical compounds were tested: 2,4-dihydroxy-N-[(2-nitrophenyl)methylidene]benzhydrazide (T1); 2,4-dihydroxy-N-[(3-nitrophenyl)methylidene]benzhydrazide (T2) and 2,4-dihydroxy-N-[(4-nitrophenyl)methylidene]benzhydrazide (T3) in zebrafish larvae. The locomotor activity of 4-dpf larvae were monitored after the end of exposure to test compounds using an automated video system (ZebraBox ZEB512-2, ZebraLab version 3.4, ViewPoint Life Sciences Inc.). The study was also carried out for control groups, i.e. larvae in growth medium (negative control) and larvae in 1% DMSO (solvent control). The information on the distance covered by larvae during slow movements (0.2-0.8 cm/s) and fast (> 0.8 cm/s) was analyzed. The statistical analysis was performed using ANOVA followed by Bonferroni post-hoc test and p values less than 0.05 were considered significant. The impact on the central nervous system of 4-dpf zebrafish larvae was observed only for compound T1 with 2-nitrophenyl group. Depending on the concentration, it significantly increased (10 μ M, 3.02 mg/l) or decreased their locomotor activity (2000 μ M, 0.60 g/l) in comparison with negative and solvent controls. The last mentioned effect may suggest potential neurotoxicity. However, this hypothesis requires further research, e.g. using 7-dpf larvae with a formed blood-brain barrier. It may be concluded that zebrafish model is a valuable innovative model for predicting the central effects and even potential neurotoxicity of novel chemical compounds.

Key words: zebrafish larvae, locomotor activity, hydrazide-hydrazones

10. Monika Maciąg
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Examination of the toxicity profile of doxorubicin in zebrafish model

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Doxorubicin is a powerful anthracycline antibiotic used for the treatment of cancer. However, its use as a chemotherapeutic agent is limited by its toxicities. Most especially observed is cardiotoxicity, but other organ systems are also degraded by doxorubicin use.

The ability of zebrafish to determinate parameters such as lethal dose, teratogenicity and specific-organ toxicity, in particular of the heart and the nervous system make it an attractive model for anticancer drug examination.

In this study the zebrafish embryos were exposed to different concentrations of doxorubicin and development toxicity were observed. We evaluated the effects of doxorubicin exposure on lethality, hatching rate, body length and morphological alterations. Due to the cardiotoxicity of doxorubicin, we examined its effects on heart rate and the diameter of the dorsal aorta. Moreover, because of doxorubicin fluorescence, we could observed tissue-specific distribution of this compound.

Our results indicate that doxorubicin in higher concentrations mainly caused acute lethal effects, and lower concentrations caused malformations, decreased heart rate and the diameter of the dorsal aorta in zebrafish larvae.

The unfortunate consequences of doxorubicin have promoted the search for new drugs that can prevent or reverse the damage after exposure to this anticancer drug using the zebrafish model.

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Key words: Doxorubicin, chemotherapeutic agent, toxicity

11. Katarzyna Zawada
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Application of spin trap EPR, DFT calculations, HPLC - MS and Danio rerio model to study the atrazine degradation path

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Abstract

Atrazine is a widely-used herbicide of the triazines class. It is a known xenoestrogen. Many methods are used to remove atrazine from the environment. We have examined the mechanism of atrazine degradation by peracetic acid (PAA) homolysis catalyzed by MnO₂ using EPR spin trapping, DFT calculations and HPLC-MS. Atrazine degradation under PAA/MnO₂ system led to additional reaction paths to those observed under standard oxidation processes, i.e. Fenton reaction. DFT calculations revealed that reactions with CH₃CO• radicals were the most energetically favorable for the first step of atrazine degradation, followed by H-atom abstraction reactions. The least probable reactions were predicted for the CH₃COO• radicals. EPR spin trapping results also suggested different reaction pathway for the degradation of atrazine in Fenton and PAA/MnO₂ system. Also, FDMPO spin trap was able to trap new O-centered free radical probably originating from atrazine molecule. HPLC-MS/MS confirmed that the degradation of atrazine occurred via different reaction paths and led to different degradation products in Fenton and PAA/MnO₂ systems. Our results indicate that systems where CH₃CO• radical is generated could be more effective for the atrazine degradation than systems with hydroxyl radicals. However, the toxicity tests on zebrafish embryo model revealed that products of atrazine degradation in PAA/MnO₂ system were more toxic than in Fenton system.

Key words: Fenton reaction, Peracetic Acid Homolysis, Gibbs free energy, enthalpy, triazines, zebrafish

12. Karolina Romaniuk

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Genome editing in zebrafish: generation of the mutant line with a truncated Tollip protein by the CRISPR/Cas9 method

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The CRISPR/Cas9 method is successfully used to introduce changes within the genome of numerous research models, including cell lines, invertebrates and vertebrate organisms. Due to the ease of manipulation, rapid development and well-characterized biology, zebrafish is an attractive model to investigate protein functions in a complex system.

Here, we describe the utility of CRISPR/Cas9 technique to target the specific site of tollip gene. Tollip is a multitasking endosomal protein that possesses a C-terminal ubiquitin-binding CUE domain involved in protein degradation. The purpose of this study is to generate a zebrafish line with a truncated Tollip protein lacking the CUE domain.

To introduce modification within tollip gene, one-cell stage embryos were injected with guide RNA (gRNA) and mRNA of Cas9 nuclease. Among the P0 mosaic progeny, we identified two founder fish which offspring (F1) inherited two and seven different mutations, respectively. However, in silico analysis of F1 embryos revealed that only two mutations (deletion of four and ten nucleotides) led to a premature stop codon upstream of the CUE domain. After reaching the sexual maturity by heterozygous mutants, appropriate F1 fish were incrossed to obtain the homozygous genotype. Next, to verify our in silico assembly and to ensure that the length of Tollip protein in zebrafish mutant lines was changed as predicted, we performed Western-blot analysis. The protein band of Tollip was shifted, indicating the presence of a protein lacking the CUE domain.

The obtained zebrafish lines will be further used to investigate the role of the CUE domain in protein homeostasis in the organism.

Key words: zebrafish, Tollip, CRISPR/Cas9, CUE domain

13. Suelen Baggio
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Glutamatergic neurotransmission system is impaired in an adult zebrafish FASD model

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Background: Fetal Alcohol Spectrum Disorder (FASD) is a syndrome related to ethanol (EtOH) exposure during development with neurological impairment. Glutamatergic neurotransmission is modulated by EtOH exposure, which affects synaptic plasticity and cognitive processes. Adult zebrafish is a known model to evaluate long-lasting impairments of milder forms of FASD, also used to investigate the glutamatergic system. Aim: Evaluate brain glutamatergic system of adult zebrafish exposed to ethanol during development. Methods: zebrafish larvae (24 h post-fertilization), were exposure to EtOH (0.0%, 0.1%, 0.25%, 0.5% and 1%) for 2 hours. After 4 months, the animals were euthanized and their brain was used to access: glutamate transport uptake activity; glutamate binding in enriched membrane fraction; glutamine synthetase (GS) activity; Na⁺/K⁺ ATPase activity; and high-resolution respirometry. Results: Animals exposed to EtOH 0.5% and 1% presented a 50% reduction of brain glutamate uptake compared to control ($p < 0,001$). Ceftriaxone, a positive modulator of glutamate uptake, rescued 50% of this drop ($p < 0,0001$). Both groups presented reduced levels of glutamate binding compared to control (43% and 60%, respectively, $p = 0,0041$). Both groups presented 32% reduction in Na⁺/K⁺ ATPase activity compared to control ($p = 0,0003$). One-fifth of GS activity was reduced on EtOH 1% group compared to control ($p = 0,0032$). No alterations were observed in high-resolution respirometry. Conclusion: Embryonic alcohol exposure disrupts adult zebrafish glutamatergic neurotransmitter system.

Key words: FASD; glutamate; neuroscience; ethanol

14. Sebastian Jurczak
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Uptake and distribution of microplastics in zebrafish embryos and larvae

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Over the last few decades, plastic waste has become an increasingly serious environmental problem because it accumulates on our planet. Although the risk of macroplastics is well studied, degradation of macroplastic residues in the ocean and beach litter in microplastics and nanoplastics has recently become a serious problem and an increasingly important area of research. So far it has been perceived as a macroscopic issue, plastic contamination is also visible at smaller scales. In fact, while macroplastics is the most studied, microplastics may actually be more widespread in marine environments. Many studies have shown the widespread presence of MP (microplastic) at all marine trophic levels, and both ocean research and laboratory manipulation clearly indicate MP transfer between trophic levels. To date, the molecular responses induced by plastic particles are not sufficiently tested in aquatic vertebrates. Therefore, there is still a need to supplement and detail the actual effect of microplastics on the body. Microplastics are defined as a plastic debris smaller than 5 mm. In 2013, global plastic production has risen nearly 300 million tons and it's still growing. Due to their high durability, flexibility and resistance to corrosion, PS (polystyrene) products are widely used in the packaging, construction and food industries. They are also intentionally added to cosmetic and household products. They are generally referred to as Styrofoam (PS). Due to the high incidence in everyday life, PS has become the main contamination of soils, rivers, lakes and oceans and is a source of microplastics.

This study aims to understand the impact of microplastic survival and efficiency of freshwater fish at their early stages of life. The developmental effect on zebrafish embryos during exposure to microplastics and subsequent uptake was examined. The exposure experiment for the collection of microplastic plastics in zebrafish larvae (Tübingen line) was carried out on 6-well cell culture plates at concentrations of 0, 100, 10, 0.1, 0.01 µg/L respectively of green fluorescent polystyrene microspheres (468/508 nm) with diameter 500 nm. A total of 15 embryos for each treatment group were used for analysis (n=15 embryos). The exposure time lasted from 4 hpf to 120 hpf. At 72 hpf, the embryos were anaesthetized with 0.024% tricaine (Sigma-Aldrich, USA) to examine the length of the body, the yolk sac, and the presence of a microplastics in larvae under the Zeiss stereo microscope (V8, Göttingen, Germany). The distribution of microplastics in the embryo generally increased with increasing concentrations of microplastics, and were most abundant in the 100 µg/L treatment group.

Key words: zebrafish, microplastic, uptake, larvae development

15. Oksana Palchevska

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Hydroxide peroxide action on zebrafish brain: light sheet microscopy perspective

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Introduction. Zebrafish is an amazing model for neurobiological research due to its unique features, such as extracorporeal development and availability of transparent mutants available. In our research we use this animal model to establish the role of Stim proteins in central nervous system under stress conditions (such as oxidative stress). STIMs are the ER-resident transmembrane proteins that are involved to Store-Operated Calcium Entry (SOCE). STIM2 is predominant isoform in mammalian brains, and it was shown to be protective for mouse neurons under hypoxia stress. We analyse the effect of hydroxide peroxide on activities of cells in brains of wild type and Stim2 knockout zebrafish.

Methods. To examine neurons activity under oxidative stress we focus on live imaging of zebrafish telencephalon using light sheet Z1 SPIM microscope (Zeiss). We use transgenic zebrafish Tg(elavl3:GCaMP5G) line, which express calcium probe in neurons and its offspring crossed with knock-out of *stim2b*^{-/-}. This allows monitoring calcium related neuronal activity in neurons with and without Stim2. The ROS production was monitored using CellROX (Invitrogen).

Results. We have estimated the dynamics of hydroxide peroxide action on the brain of 5 dpf larvae and found that the safe and still effective was an incubation with 2 mM H₂O₂ during 40 minutes. At this time point we measured neuronal activity of zebrafish neurons. Our results show that lack of Stim2 changes brain neuronal activities upon H₂O₂ treatment. These studies will help to elucidate the effect of Stim2 proteins on brain function and its adaptation to oxidative stress.

Key words: zebrafish, brain, light sheet microscopy

16. Anna Przyborowska
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The effect of galanin on the melanogenesis in zebrafish zebrafish, galanin, melanogenesis

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The zebrafish (*Danio rerio*) is one of the most important vertebrate model organism. The characteristic external pigment pattern of zebrafish is generated by an array of three types of pigment cells. These include melanophores (melanin-containing melanocytes), xanthophores and iridophores. The combination of xanthophores and iridophores makes the yellowish-silver interstripes, while melanophores contribute to the longitudinal dark stripes of the epidermis. Developmentally, melanin in melanophores can be visualized in the dorsolateral skin and retina at approximately 24 h post-fertilization.

Galanin is a neuropeptide widely distributed in central and peripheral nervous system in vertebrates which has been implicated in several physiological functions, such as nociception, cognition, feeding, mood, neuroendocrine regulation and probably in pigmentation.

In this project, we used the CRISPR-Cas9 gene editing tool to knock out galanin gene in zebrafish. Wild-type strain and galanin mutants at defined stages after fertilization were used for melanophores observation. Adult fish of these strains were used for light-black background adaptation test to study its effect on the pigmentation. The expression of genes relevant for the melanogenesis and melanosomes aggregation was analyzed.

Pigment pattern disruption was one of the most noticeable phenotypic consequences of galanin loss. At early stages of zebrafish development there were some differences in melanophores pattern between wild-type strain and galanin mutants. In later stages a reduced number of melanophores in galanin mutants was noticeable. What is more the disruption in melanosomes aggregation during the background adaptation was visible. Changes in expression of genes implicated in melanogenesis in galanin mutants were also shown.

Our preliminary results have shown that galanin can be relevant in melanogenesis and melanosomes aggregation in zebrafish. We anticipate that the galanin mutants will be useful in loss of function analyses that will increase our understanding of the role of this gene in diverse biological and pathological processes.

Key words: zebrafish, galanin, melanogenesis

17. Małgorzata Chmielewska-Krzesińska
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**The liquorice extract impact, on modulation of induced inflammatory response in Zebrafish larvae.
Preliminary study of using Zebrafish as an avatar in cancer development process**

Licorice extract (*Glycyrrhiza glabra*), possesses anti-inflammatory activities. In the study, 3-day post-fertilization (dpf) zebrafish larvae of genetic line Tg(MPX:GFP) with GFP-labelled neutrophils were used to investigate an inhibitory role of Licorice on chemical-induced inflammation associated with damaged hair cells of the lateral line. The study consisted of 3 groups: (1) Control, (2) 10 µg and (3) 100 µg of Licorice extract. Half of the larvae in each group was exposed for forty minutes to 10 µM CuSO₄ solution in the aim to provoke inflammation. As a result, neutrophils migrating towards damaged hair cells were counted, with the use of a stereoscopic microscope. In control and licorice exposed individuals, the migration of neutrophils was not observed. In animals exposed to CuSO₄, a dramatical increase of migrating neutrophils was seen only in the control group (3 fold increase to control group), licorice treated group(10 µg) slight increase (1 fold to control), and no migration in Licorice group (100 µg) was seen. To confirm, this results on a molecular level, the Real-Time PCR analysis for several genes (IL-1β, IL-6, IL-8, TNF-β) was performed. The most significant results were decrease of genes expression in control and licorice treated group in comparison to the CuSO₄ treated group. What is more important the licorice treated larvae exposed to CuSO₄ have the same pattern as the control group. The present study shows that licorice block chemically-induced inflammatory response. This is a promising result for the study of inflammatory processes in embryonic zebrafish xenograft assay of cancer.

Key words: Licorice, chemical-induced inflammation, neutrofil poster

18. Natalia Kasica-Jarosz
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The influence of pituitary adenylate cyclase – activating polypeptide (PACAP-38) on neutrophil migration towards free-radical damaged zebrafish hair cells

PACAP-38 is a pleiotropic neuropeptide, playing a potential regulatory role in the immune system. The present study deals with hair cells, which are sensory receptors responsible for signal transduction in the inner ear of vertebrates, including zebrafish. Hair cells are sensitive to oxidative stress which causes their apoptosis, necrosis or inflammation. Preventing oxidative stress-induced damages to hair cells of the inner ear would represent a great therapeutic value. However, no investigations have been carried out as yet dealing with factors exhibiting comprehensive anti-inflammatory action against free radical damages to the inner ear. We used 5 dpf zebrafish larvae to investigate an inhibitory role of PACAP-38 in inflammation associated with damaged hair cells of the lateral line. 40 min exposure to 10 μ M CuSO₄ was used to evoke free radical-derived necrosis of hair cells and consequent inflammation. The investigations were done *in vivo* under a confocal microscope by counting neutrophils migrating towards damaged hair cells in Tg(MPX:GFP) larvae. In CuSO₄-treated individuals, the number of neutrophils associated with hair cells was dramatically increased, while PACAP-38 co-treatment resulted in its over 2-fold decrease. qPCR analysis showed that CuSO₄ exposure up-regulated the expression of IL-8, IL-1 β , IL-6 and ATF3, while after PACAP-38 co-treatment expression of these genes was significantly decreased. Moreover, the presence of transcripts for all PACAP receptors in zebrafish neutrophils was revealed. The obtained results suggest that PACAP-38 is a factor playing an important regulatory role in inflammation and it cannot be excluded that this interesting property has more universal significance.

Key words: hair cells, PACAP, neutrophils

From frutis to anxiolytic activity of compound tested on zebrafish model: case of devenyol

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Devenyol is coumarin aglicon, belonging to class of simple coumarins and isolated for the first time from mature fruits of *Seseli devenyense* Simonk. Except antimicrobial and cytotoxic activity, it exhibits Central Nervous System activity.

The aim of our experiment was to determine the anxiolytic activity of devenyol on animal model represented by 5-dpf larvae of *Danio rerio*. Zebrafish larval behaviour analysis (locomotor activity and thigmotaxis behaviour) was evaluated using the ZebraBox system produced by ViewPoint company. As an index of anxiety thigmotaxis ("wall hugging") was used. Active compounds (with anxiolytic activity) decrease thigmotactic behaviour, which expresses as increasing of time spent in the centre of an arena (inner zone) compared to the boundaries of a well (outer zone). In addition, larval locomotion was measured as well as light on/off assay (anxiogenic conditions). For determination of the statistical differences between concentrations of investigated compound, the Prism software (GraphPad) was used. Obtained data were analysed using analysis of variance (ANOVA) one-way or two-way followed by post-hoc tests.

The results show that devenyol has anxiolytic activity in all tested concentrations: 1.5; 3.0, 6.0, 9.0 and 15.0 μM .

In summary, our research confirms activity of new coumarin derivative and fact of usefulness of 5 dpf zebrafish larvae model as powerful method for screening of the plant extracts and isolated natural compounds on anxiety level.

This study was supported by National Science Centre, Poland, Grants: 2017/25/N/NZ7/01899.

Key words: devenyol, anxiolytic activity, *Seseli*

20. Costantino Parisi

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ESTABLISHING A MODEL OF HYPERGLYCEMIA-INDUCED CONGENITAL HEART DISEASE IN ZEBRAFISH

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Maternal diabetes mellitus (MDM) is a pathophysiological condition hallmarked by glucose intolerance occurring during pregnancy. Elevated glucose levels during gestation may have teratogenic effects leading to abnormal embryonic development and organ malformations, amongst which, congenital heart disease (CHD) is the most prevalent. The exact molecular mechanism of MDM-induced CHD is not well understood. Zebrafish (*Danio rerio*) has emerged as an attractive model to study organ morphogenesis due to its rapid external development and embryo transparency. To characterize the gene regulatory networks and epigenetic determinants of glucose-induced CHD, we developed a model of D-glucose-induced heart malformations in Tg(myh7:EGFP), a zebrafish transgenic line with GFP-labelled cardiomyocytes. In brief, embryos were exposed to D-Glucose and D-Mannitol as negative control from 0-120 hpf. Heart morphology and topology were assessed using light sheet microscopy. After 48 hours of exposure, D-glucose-treated embryos exhibited developmental delay, inactive behaviour and cardiac malformations including impaired lack of cardiac looping, hypertrophy and bradycardia, phenotypes which have been reported in human cases of MDM. These results show the zebrafish embryo is a suitable model to investigate glucose-induced CHD. In perspective, we plan to employ genomics methodology to better understand the molecular mechanism behind this pathology.

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Key words: Diabetes mellitus, Congenital Heart Disease, disease models, Zebrafish

21. Przemko Tylzanowski
University of Leuven

Zebrafish is an attractive model to study many different processes including the formation of musculoskeletal system

One of the important aspects of such studies is the visualization of the soft and hard tissues at the histological level. Techniques typically used for such analysis are either based on classical histology or micro computer tomography (micro-CT). The former technique is laborious and the latter does not offer a good spatial resolution (around 20um). To overcome both limitations we have optimized the Nano-CT and different staining protocols to accomplish zebrafish embryo imaging at 2um voxel size. Embryos at 5dpf have been collected, and processed for imaging. This high-resolution method can be used to quickly evaluate zebrafish phenotypes in soft and hard tissues.

Key words: imaging, bone, muscle

22. Anna Lewicka
University of Wrocław

Ultrastructure of skeletal muscle tissue depleted of muscle glycogen phosphorylase

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Myogenesis is a very complex and multiorchestrated process which requires well-coordinated cooperation of many factors. Cells with the lineage of origin in mesoderm must be subjected to a plenteous repertoire of initiators to finally evolve in to competent, collective of skeletal muscle tissue.

While signaling leading to specification of dermomyotome cells to muscle cells by the transcription factors network is well-established, more complexed landscape emerges from formation of syncytial muscles. Using zebrafish muscle tissue, which is highly conserved with human tissue, we demonstrate the influence of depletion of glycogenolysis enzyme on muscle structure. In the zebrafish genome, muscle form of glycogen phosphorylase is found as two distinguishable sequences encoded by different genes *pygma* and *pygmb*. Both are expressed in developing embryo. In our experiments using morpholino oligos we tested the influence of *pygma* and *pygmb* expression knockdown on developing skeletal muscle tissue. The results we obtained may shed a light on glycogenolysis enzymes as new players in elaborate process of muscle formation in vertebrates.

The zebrafish embryo and larvae with *pygm* knockdown were compared to the wild type control. Animals were fixed, embedded and sectioned. Ultra-thin sections were analyzed using the Zeiss transmission electron microscope.

Zebrafish with lower *Pygm* level exhibit high accumulation of glycogen granules in the muscle tissue which leads to sarcomeres disorganization. The observations were confirmed by glycogen quantitative analysis, which revealed higher glycogen level in zebrafish with *pygm* knockdown when compared to the control.

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Key words: glycogen phosphorylase, skeletal muscle tissue, ultrastructure

23. Dafina Fondaj
University of Prishtina

Anxiolytic activity of methanolic extract *Seseli libanotis* (L.) W.D.J. Koch tested on *Danio rerio* model

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Seseli genus, belonging to Apiaceae family is rich source of coumarins and essential oils. Chemical composition of plant from mentioned genus determine its antimicrobial, antiinflammatory, carminative, stomachic and many others. Some species of *Seseli* are reported in book of ancient authors, like Hippocrates.

The main aim of our research was to determine of anxiolytic activity of methanolic extract obtained from mature fruits of *Seseli libanotis* (L.) W.D.J. Koch on animal model of anxiety (on 5-dpf larvae of *Danio rerio*).

Locomotor activity and thigmotaxis behaviour (Zebrafish larval behaviour analysis) was determined using the ZebraBox system created by ViewPoint company. As an index of anxiety thigmotaxis ("wall hugging") was used. Extract with anxiolytic activity has caused decrease thigmotactic behaviour (increasing of time spent in the centre of an arena).

For determination of the statistical differences between concentration of investigated compound, the Prism software (GraphPad) was used. Obtained data were analysed using analysis of variance (ANOVA) one-way or two-way followed by post-hoc tests.

The results show that methanolic extracts from mature fruits *Seseli libanotis* (L.) W.D.J. Koch in tested concentrations: 1.5; 3.0, 6.0, 9.0, 12.5 and 25.0 µg/mL showed anxiolytic activity.

In conclusion, extracts from mature fruits of *Seseli libanotis* (L.) W.D.J. Koch tested on animal model (5 dpf larvae) showed interesting anxiolytic activity. The above results, that investigated species should be subjected to further testing and isolation of compounds responsible for the activity.

This study was supported by National Science Centre, Poland, Grants: 2017/25/N/NZ7/01899.

Key words: *Seseli*, anxiolytic activity, *Danio rerio*

24. Joanna J. Sajkowska-Kozielewicz
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Geissospermum reticulatum bark – in vitro and in vivo activities of the extracts and alkaloidal fractions

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Amazonian species from the family Apocynaceae are characterized by presence of alkaloids, and are used as infusions or tinctures in traditional medicine in the treatment of malaria, hard-to-heal wounds or stomach problems, to name a few. Within this family, some genus have deserved special attention e.g. *Geissospermum reticulatum* A. Gentry – tree widely distributed throughout the Amazon. However, there is a knowledge gap regarding the biological action of these preparations.

The aim of this project was to determine the antioxidant, cytotoxic and toxic activities of infusions, tinctures, extracts and alkaloidal fractions of *G. reticulatum* barks in relation to the composition.

Seven samples (B1-B7) of dried barks of *G. reticulatum* were obtained from Peru. Chemical profile was analyzed using GC-MS. Ethanolic extracts exhibited cytotoxic activities on THP-1 and HL-60 cells. However, these extracts did not display toxic activities on zebrafish embryos.

Studied fractions (10 and 30 µg/ml) showed cytotoxic properties against THP-1 and Daudi cells. The cell-based study revealed that the B2 was the most effective whereas the B1 was the least potent. These fractions (B1 and B2) were added to the 5 hpf zebrafish embryos and their development was monitored over 72H. Both fractions were toxic to the embryos at 300 µg/ml and caused development delays rate of 70% and 100%, respectively. The fractions were lethal to all studied subjects after 72H. However, zebrafish embryos treated with lower concentrations (100 µg/ml) of B1 and B2 were able to develop during the 72-hour treatment. *G. reticulatum* bark alkaloids could be potentially used as therapeutic agents.

Key words: *Geissospermum reticulatum*, alkaloids, cytotoxicity, toxicity, zebrafish

25. Ewelina Koziół
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Daphnoretin alleviates pentylenetetrazol-induced seizure activity in zebrafish larvae

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Daphnoretin is a natural coumarin derivative that occurs in different Thymelaeaceae species like *Wikstroemia indica* C.A. Meyr, or *Daphne tangutica* Maxim. It has been shown that the compound has antibacterial, antiviral and anticancer agent. Since some coumarin derivatives are also known for their anticonvulsant properties, we tested the effect of daphnoretin in a zebrafish pentylenetetrazol (PTZ) seizure model. PTZ is GABA_A antagonist inducing increased locomotor activity, seizure-like behavior and epileptiform activity in the brain of zebrafish larvae. The seizure-like behavior was measured as the distance travelled in lardist units with an automated tracking device (Zebrabox, Viewpoint, France), whereas the epileptiform brain activity was monitored by non-invasive local field potential (LFP) recordings. The percentage of seizure inhibition by daphnoretin at 200 μM amounted to 63.03±0.76% and 82.55±0.65% in case of the locomotor and LFP measurements, respectively. In conclusion, this study revealed that daphnoretin exhibited antiseizure activity in a PTZ zebrafish model and therefore might be suitable for further testing in rodents as a possible anticonvulsant drug candidate.

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Polish Zebrafish Society
Polskie Towarzystwo "Zebrafish"
<http://www.zebrafish.org.pl>

The main task of the Society is to promote cooperation among people performing research on zebrafish (*Danio rerio*) in Poland and abroad. Besides the scientific collaboration, the Society promotes science education, popularization and promotion of zebrafish model organism for the general public.

The Society accomplishes its mission by (co)organizing workshops and high quality meetings devoted to zebrafish research.