Science Fund
of the Republic of Serbia

3rd Symposium in Biomedicine: Basic and Clinical Neuroscience

June 29, 2022
University of Belgrade,
Faculty of Pharmacy

ABSTRACT BOOK

PROMIS grant - PsyCise project

The symposium is funded by the Science Fund of the Republic of Serbia, PROMIS-PsyCise grant (No. 6066800).

PROGRAMME

08:45 **Registration & coffee**

09:00 **Psychiatry session**

Magnus Ingelman Sundberg, Karolinska Institute
Novel aspects of genetic variations explaining interindividual differences in response to antidepressants and antipsychotics

Nađa Marić Bojović, Uni. Belgrade – Faculty of Medicine
Distress during the COVID-19 pandemics – Probable overestimation due to sampling methodology issues

Marin Jukić, Uni. Belgrade – Faculty of Pharmacy & Karolinska Institute
Utility of CYP2C19 genotyping and therapeutic drug monitoring of plasma levels on escitalopram treatment – A prospective cohort study

Bojana Pejušković, Uni. Belgrade – Faculty of Medicine
Bidirectional associations between COVID-19 and depression – The possible anti inflamatory effects of selective serotonin reuptake inhibitors

11:30 **Coffee break**

12:10 **Neurology session**

Oscar Jungholm, Karolinska Institute
Novel antibody-based treatments for pain and cancer
VIA ZOOM

Maja Tomić, Uni. Belgrade – Faculty of Pharmacy
How metformin relieves pain – an uncomplete puzzle

Aleksandra Tomić Pešić, Uni. Belgrade – Faculty of Medicine
Structural brain alterations in patients with dystonia

Filip Milosavljević, Uni. Belgrade – Faculty of Pharmacy
Humanized CYP2C19 transgenic mouse as an animal model of cerebellar ataxia

PROGRAMME

13:25 **Lunch break & Poster session**

14:40 **Neuroscience session**

Kent Jadermark, Karolinska Institute
Pharmacological treatment of schizophrenia – pre- and postpsychosis
CANCELLED

Miroslav Savić, Uni. Belgrade – Faculty of Pharmacy
Preclinical prediction of mood and cognitive adverse effects of pharmaceuticals

Vesna Pešić, Uni. Belgrade – Faculty of Pharmacy
Novel research in understanding ketamine effects in depressive-like behaviour models

Jasmina Đuretić, Uni. Belgrade – Faculty of Pharmacy
Age-dependent effects of memantine in experimental autoimmune encephalomyelitis

16:30 **Poster awards**

ABSTRACTS

ANTICANCER EFFECT OF NEW CARBONIC ANHYDRASE 9 INHIBITORS IN GLIOBLASTOMA CELLS

Mirna Jovanović [1], Sofija Jovanović-Stojanov [1], Miodrag Dragoj [1], Ana Kostić [1], Ema Lupšić [1], Ana Podolski-Renić [1], Jelena Dinić [1], Milica Pešić [1]

[1] Institute for Biological Research "Siniša Stanković"- National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia, mirna.jovanovic@ibiss.bg.ac.rs.

Introduction: Carbonic anhydrase 9 (CA9) is a membrane enzyme, a regulator of intracellular and extracellular pH, overexpressed in cells in a hypoxic environment [1]. Solid tumors, adapted to hypoxia, have large quantities of the CA9 and the increased expression correlates with tumor patients' poor prognosis, tumor malignancy, and resistance to drugs [2]. In glioblastoma, hypoxia promotes the spreading of cancer cells into the brain tissue, to evade the environment with low oxygen levels [3]. Inhibitors of CA9 have previously been investigated for anticancer drugs [2]. In the present study, we evaluated the anticancer properties of three CA9 inhibitors (AFA-30, AFA-40 and AFA-49), phosphonium salts derived from coumarin, in sensitive (U87) and chemoresistant (U87-TxR) human glioblastoma cell lines.

Materials & Methods: The effect of CA9 inhibitors on cell growth, either alone or in combination with tariquidar was determined by sulforhodamine B assay. Flow cytometry was used for the assessment of change in intracellular pH by BCECF staining, and the rhodamine 123 assay of P-gp activity. Changes in the expression of *CA9*, *CA12*, and *ABCB1* genes were analyzed by qPCR.

Results: The three compounds inhibited cell growth of both sensitive (U87) and resistant (U87-TxR) cells in 48 h treatments, in both hypoxic (1% O_2) and normoxic (20% O_2) conditions. However, compared to U87 (IC_{50} range 1 - 5 μM), the U87-TxR were less sensitive to the compounds' growth inhibition effect (IC_{50} range 8 – 30 μM). U87-TxR cells are characterized by the increased expression of the P-gp extruding pump. When tariquidar, a P-gp inhibitor, was applied in combination with CA9 inhibitors, U87-TxRcells were sensitized to these compounds. In the P-gp activity assay, we demonstrated that compounds (5 – 50 μM) increase a P-gp substrate accumulation – rhodamine 123. Further, gene expression of *ABCB1* was increased 2 – 8 times in U87, following treatment. In 24 h treatments, these CA9 inhibitors decreased intracellular pH. Moreover, the 24 h treatments resulted in decreased expression of *CA9* and *CA12*.

Conclusion: The three CA9 inhibitors here described have significant anticancer effects in glioblastoma cells and show potential for further pre-clinical investigation, especially in tumors with emphasized hypoxic zones contributing to increased malignancy, such as glioblastomas.

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1. Mussi, S., et al., Antiproliferative effects of sulphonamide carbonic anhydrase inhibitors C18, SLC-0111 and acetazolamide on bladder, glioblastoma and pancreatic cancer cell lines. J Enzyme Inhib Med Chem, 2022. 37(1): p. 280-286.

2. Kalinin, S., et al., Carbonic Anhydrase IX Inhibitors as Candidates for Combination Therapy of Solid Tumors. Int J Mol Sci, 2021. 22(24).

3. Monteiro, A.R., et al., The Role of Hypoxia in Glioblastoma Invasion. Cells, 2017. 6(4).

Faculty of Pharmacy –
University of Belgrade

CERTIFICATE

OF POSTER PRESENTATION AT THIRD SYMPOSIUM IN BIOMEDICINE: BASIC AND CLINICAL NEUROSCIENCE

This certificate is presented to:

Mirna Jovanović

Prof. Dr Marin Jukić

June 29, 2022

Date