EPITHERAPY IN ALZHEIMER'S DISEASE: HOPE ARISING FROM EPIGENETIC RESEARCH

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Introduction: Alzheimer's disease (AD) is the most prominent form of dementia in the general population. Several pathophysiological mechanisms responsible for development and progress of AD have been proposed; however, its complex etiology is still unclear. Currently available treatment alleviates the symptoms without disease course alteration. A growing body of evidence proposes epigenetic mechanisms as potential targets for future AD therapy. Literature research was conducted in order to investigate the epitherapeutic potential of certain molecules.

Materials and methods: MEDLINE and PubMed databases were searched for English-language articles containing keywords "Alzheimer's disease“, "epigenetics“, "epigenetic treatment“, “epitherapy“. No time of article type constraints were applied. Extensive analysis of selected articles was performed and data was then interpreted in an overview.

Results and Discussion: Curcumin demonstrated effects in prevention and mitigation of AD symptoms with a proposed mechanism of action regarding histone acetyltransferases (HATs) activity reduction, histone deacetylases expression, NF-κB expression and DNA methyltransferases inhibition. Vitamin D (VD) has also shown various interactions with the epigenome. VD increased genomic Vitamin D receptor binding, regulated the binding of pioneer transcription factors, formation of topologically associated domains, histone modifications and chromatin accessibility. Citicoline, when metabolized into cytidine and choline, served as a precursor of phospholipids and acetylcholine synthesis. When used parallelly with Acetylcholinesterase inhibitors it improved AD symptoms by sirtuin 1 (SIRT1) expression increment. Resveratrol is also recognised for potential epigenetic treatment of AD due to its neuroprotective properties against amyloid beta toxicity, through increased expression of SIRT1 and microglial NF-κB inhibition.

Conclusion: Research of molecules acting on epigenetic levels may lead to new targets for AD therapy. Many studies showed that some commonly available compounds might have beneficial effects in AD by acting at the epigenetic level. However, to confirm current findings further research is required.

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POTENTIAL NEUROPROTECTIVE EFFECTS OF DEHYDROEPIANDSTERONE (DHEA) AND ITS SULPHATE (DHEAS) IN BRAIN INJURY MODEL

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Introduction: Previous research has shown that dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) alleviate the effects of excitotoxicity and damage caused by oxidative stress and have potentially protective effects in the case of ischemic brain injury. The aim of this study was to elucidate the potential effects of the neurosteroids DHEA and DHEAS (DHEA/S) in preventing neuronal death and promoting neuronal survival using an oxygen and glucose deprivation and reperfusion (OGD/R) model that mimics ischemic injury and related pathological conditions very well.

Materials and methods: The potential neuroprotective effects of DHEA and DHEAS were examined in two cell models, in human neuroblastoma cells, the SH-SY5Y cell line, and in primary culture of mouse neurons. Both cell cultures were treated with appropriate concentrations of DHEA/S (10, 100, 500, and 1000 nM) 24 hours before injury (pretreatment) or 24 hours after injury (posttreatment). To induce OGD/R, cells were given glucose and serum-free medium and placed in an air-insulated modular chamber filled with N_2 alone for 16 hours at 37°C. Changes in cell viability were investigated using the MTT test and the commercial Muse® Count & Viability Kit.

Results and discussion: DHEA and DHEAS showed neuroprotective activity in an *in vitro* model of ischemic-reperfusion (OGD/R) brain injury. Metabolic activity (MTT test) and viability (Muse® Count & Viability test) of SH-SY5Y cells and neurons, after pretreatment and post-treatment with DHEA/S, were higher than injury (OGD/R). However, they did not reach the level of metabolic activity and viability of control, i.e., uninjured cells. On both cell cultures, pretreatment with DHEA/S was observed to be significantly more effective than post-treatment.

Conclusion: The results of the study suggest that the studied neurosteroids have potential in the treatment and prevention of injuries caused by ischemia, as well as those caused by neurodegeneration.

The research was conducted as part of the project "Therapeutic potential of neurosteroids and neurotrophins in dementia (TePoNeDe)", IP-2019-04-6100, funded by the Croatian Science Foundation.

USE OF PHARMACOLOGICAL SEDATIVES AMONG BIOMEDICAL STUDENTS DURING THE SARS-CoV-2 PANDEMIC

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Introduction: The pandemic has led to an increase in the number of mental health problems in the general population, especially among health professionals. Studies show that biomedical college students reported worsening mental health during the pandemic. Also, Croatia was hit by several earthquakes that could have worsened the situation.

The aim of this study was to explore habits of taking pharmacological sedatives during pandemics/earthquakes among biomedical and non-medical students.

Materials and methods: The questionnaire of 35 questions was created in the Google forms and distributed electronically. 1403 students completed the questionnaire (627 respondents from biomedical and 776 from non-medical faculties). Statistical processing was performed in IBM SPSS 25 (IBM Corp, Armonk, USA). To test the hypotheses, the $\chi 2$ test was used with the effect strength by the p test.

Results and discussion: A significant association was found in usage of pharmacological sedatives before and after the onset of pandemic for all subjects ($p < 0.05$). Within the group of biomedical fields, medical students use statistically significantly more pharmacological sedatives, both before and after the onset of pandemic ($p < 0.034$). Biomedical students observed together do not differ statistically in terms of taking sedatives either before or after the onset of pandemic when compared to non-medical faculties ($p > 0.05$). Most respondents take sedatives by prescription.

Conclusion: Although an increase in the use of sedatives due to pandemics and earthquakes was expected, students were using sedatives equally before and after stressful events. Regardless of events, medical students take more pharmacological sedatives when compared to students of non-biomedical fields and when compared to students of other biomedical fields. Usage of this type of medicine among medical students has already been recognized.

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THE INFLUENCE OF RECOMBINANT HUMAN ERYTHROPOIETIN ON SIRT1 EXPRESSION IN RATS EXPOSED TO FOCAL CEREBRAL ISCHEMIA

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Introduction: Cerebral stroke is one of the leading causes of mortality and long-term disability in humans but treatment options are still very limited. Almost 80% of human strokes are of ischemic origin and most of them are caused by middle cerebral artery occlusion (MCAO). The aim of our study was to investigate the effects of recombinant human erythropoietin (rhEpo) on the expression of SIRT 1 protein (*silent information regulator two protein 1*) such as on iNOS and GFAP expression in the parietal cortex and hippocampus of rats exposed to MCAO. In addition, activation of the microglia in the parietal cortex and/or hippocampus of rats exposed to MCAO was analyzed.

Methods: Adult male Hannover Wistar rats were exposed to the focal cerebral ischemia by right MCAO for 1 hr. Ischemic animals received either vehicle or rhEpo (5000 IU/kg, i.p.), 3 h after induction of ischemia. Sham operated, vehicle treated animals served as the control group. Rats were sacrificed 24 h after the onset of the ischemic or sham experimental procedure. All procedures were approved by the Faculty's Ethical Committee.

Results: Administration of rhEpo caused a statistically significant increase in the expression of SIRT1 protein in the parietal cortex of ischemic animals compared to the control group. No significant changes in the level of iNOS and GFAP expression were observed in the examined brain regions. In the gyrus dentatus and CA1 hippocampal region of ischemic animals, a decrease in the number of activated microglial cells was observed due to rhEpo administration.

Conclusion: Post-ischemic administration of rhEpo could exert a neuroprotective potential in rats exposed to focal cerebral ischemia via modulation of SIRT1 expression.

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HYAURONIC ACID IN DENTAL MEDICINE

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Introduction: Hyaluronic acid is a linear polysaccharide found in almost all vertebrate organs. Most of the cells have the ability to synthesize hyaluronic acid. Hyaluronic acid is biocompatible and non-immunogenic. It has an anti-inflammatory and bacteriostatic properties, and promotes healing. The aim of our work was to systematically review the published literature about potential effects of HA as an adjuvant treatment for chronic inflammatory disease, in addition to its use to improve healing after common dental procedures.

Materials and methods: Relevant published studies were found in PubMed, Google Scholar, and Researchgate using a combined keyword search or medical subject headings.

Results and discussion: It has been in use in dental medicine since the beginning of the 21st century. In the treatment of periodontitis, it is applied topically in combination with non-surgical and surgical periodontal therapy and leads to significant clinical improvement. Topical application of hyaluronic acid gel has been observed to have a positive effect in patients with gingivitis. It inhibits the pathological effect of dental plaque on peri implant tissue and improves soft tissue healing. Hyaluronic acid gel injections lead to a reduction of 'black triangles' even after a single application, but better and longer lasting results are achieved by multiple injections of hyaluronic acid into the area of the interdental papilla. In the treatment of temporomandibular disorders, it leads to improved temporomandibular joint function and pain relief, but further research is needed to further investigate its application. In patients with recurrent aphthous ulcers, it is a good alternative to corticosteroids because it leads to faster healing, relieves pain and is safe for use in infants and pregnant women. Relief of symptoms has been observed in patients with burning mouth syndrome.

Conclusion: Administration of HA as topical agent play a promising key role in the postoperative care of patients undergoing dental procedures, it also has a beneficial effect in most applications for chronic inflammatory gingival and periodontal disease and oral ulcers.

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DISINHIBITION OF BRAINSTEM MOTOR NEURONS AND NEUROPEPTIDE EXPRESSION

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Introduction: Tetanus toxin, a neurotoxin that prevents synaptic transmitter release, induces overt activity of motor neurons by synaptic silencing of nearby inhibitory neurons. Our previous serendipitous observation suggested that the protein level of calcitonin gene-related peptide, a peptide normally present in high levels in primary sensory neurons, might be increased in ipsilateral facial motoneurons after peripheral whisker pad TeNT injection. Herein, we sought out to confirm this preliminary observation, and examined whether TeNT-induced rise in CGRP is dependent on disinhibition of facial motor neurons by counteracting its spastic activity with botulinum toxins types A (BoNT/A) and C (BoNT/C).

Materials and methods: Wistar rats were treated into the right vibrissal pad with 1 ng TeNT, and then, after mild spastic paralysis of ipsilateral whiskers was already present, with low local doses of BoNT/A (50 pg) and BoNT/C (70 pg), or saline vehicle. On the sixth day following the TeNT injection animals were perfused for immunohistochemical analysis of CGRP in ipsilateral and contralateral facial motor nuclei.

Results: Both BoNT toxins counteracted the spastic paralysis (whiskers spreading out) induced by TeNT, and induced a flaccid paralysis resulting in the whisker pad positioned backwards. The level of CGRP was augmented 4-6 times in all TeNT-treated animals, compared to untreated side. The TeNT-induced upregulation of CGRP levels was not altered by BoNTs.

Conclusion: Herein, we found that significant up-regulation of CGRP can be a reliable marker of TeNT activity in facial motor neurons. Mentioned neuropeptide up-regulation by TeNT was not counteracted by BoNTs, suggesting that the TeNT-induced up-regulation of CGRP in motor neurons does not necessarily involve its post-synaptic action in inhibitory neurons. Other options might involve yet unknown cellular action of TeNT within motoneurons, resulting in altered gene expression.

EFFECT OF CENTRAL INHIBITION OF GIP RECEPTORS ON LIVER OXIDATIVE STRESS AND INSULIN SIGNALING IN RAT MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease is the most common type of dementia with increasing incidence and unknown etiopathogenesis. Recent studies indicate the importance of peripheral organs' role, such as the liver and intestine, in the neurodegeneration process. Incretin peptides are being investigated for their potential neuroprotective effects. The aim of the study was to explore changes in liver oxidative stress and insulin signaling after central inhibition of glucose-dependent insulinotropic polypeptide (GIP) receptors in the streptozotocin-induced rat model of Alzheimer's disease (icv-STZ).

Materials and methods: Three-month-old male Wistar rats (n=40) were intracerebroventricularly treated with streptozotocin (3 mg/kg) or vehicle. 1 month after model induction the animals received either GIP inhibitor [Pro^3]-GIP (85 μg/kg) or vehicle. Liver redox homeostasis was analyzed by quantification of thiobarbituric acid reactive substances, protein and low molecular weight thiols, oxidation-reduction potential, nitrocellulose redox permanganometry, and activity of superoxide dismutase. Insulin signaling and apoptotic enzymes were analyzed by SDS-PAGE followed by western blot. The experiments were approved by the Croatian Ministry of Agriculture (EP186/2018) and the Ethical Committee of the University of Zagreb School of Medicine (04-1343-2006).

Results and discussion: Liver redox homeostasis was not perturbed by either STZ-icv or central [Pro^3]-GIP. Insulin signaling alterations were observed upon central administration of both STZ and [Pro^3]-GIP, and STZ-icv was a moderator of the effects of [Pro^3]-GIP on liver insulin signaling. Liver insulin receptor and insulin-degrading enzyme were decreased by both STZ-icv and [Pro^3]-GIP. Phosphorylation of the insulin receptor substrate-1 and 5' AMP-activated protein kinase expression in response to acute [Pro^3]-GIP administration was qualitatively different in STZ-icv pretreated animals. Expression of apoptotic enzymes (Caspase 3 and cytochrome C) was unchanged in both STZ and [Pro^3]-GIP treatments.

Conclusion: Intracerebroventricular administration of GIP inhibitor alters insulin signaling but does not affect redox homeostasis and apoptosis in rat liver. Related changes in insulin signaling differ in magnitude in Alzheimer's disease rats (STZ-icv).

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EVALUATION OF THE INFLUENCE OF CHEMICALLY FUNCTIONALIZED SINGLE-WALLED CARBON NANOTUBES ON THE LEVELS OF OXIDATIVE STRESS AND INFLAMMATION PARAMETERS IN ASTROCYTES EXPOSED TO *IN VITRO* TRAUMATIC INJURY

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Introduction: Each year, traumatic brain injury (TBI) affects nearly 15 million people with high mortality and disability rates. Astrocytes are key cells of the central nervous system (CNS) in response to trauma with significant roles in the CNS recovery after injury. The lack of effective pharmacological therapy has prompted the idea of using nanomaterials in TBI treatment. We aimed to explore some effects of the application of single-walled carbon nanotubes (SWCNTs), chemically functionalized with poly-m-aminobenzene sulfonic acid (PABS) on primary mouse astrocytes in an *in vitro* model of severe TBI (sTBI).

Materials and methods: Primary mouse astrocytes were grown on 6-well plates with deformable membranes and subjected to severe stretch injury. PABS-SWCNTs were applied at 1 h post-injury and the astrocytes were collected 24 h following *in vitro* TBI. Non-injured and injured, untreated cells were also collected. The dot blot method was used to detect the levels of oxidatively damaged proteins. Western blot analyses were used to determine the expression levels of the proteins of interest.

Results and discussion: Stretch injury, with or without PABS-SWCNTs treatment, had no effect on the levels of oxidatively damaged proteins in astrocytes, compared to the levels determined in the control conditions. The application of PABS-SWCNTs to the injured astrocytes increased the glial fibrillary acidic protein (GFAP) expression levels in relation to the ones recorded in the samples from the control but also injured, untreated cells. Contrary, PABS-SWCNTs did not affect the expression levels of inducible nitric oxide synthase (iNOS), nor the levels of excitatory amino acid transporter 1 (EAAT1).

Conclusion: Results of this study suggest that PABS-SWCNTs do not cause significant oxidative protein damage in the injured astrocytes. The effect of PABS-SWCNTs on increased GFAP expression has also been shown. However, further research is needed to determine if PABS-SWCNTs are effective, but also safe for use in the treatment of TBI.

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AGE-DEPENDENT EFFECTS OF ORAL GALACTOSE ON THE ENERGY BALANCE IN THE HYPOTHALAMUS OF TRANSGENIC TG2576 MICE

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Introduction: One of the most used transgenic mice model of Alzheimer disease (AD) is the Tg2576[1]. Literature data shows changes in the brain energy metabolism before development of Aβ plaques and galactose showed potential therapeutic effect in this model [1,2,3] Therefore, we aimed this study to investigate changes in the hypothalamic (HPT) brain energy status before and after galactose treatment. For this purpose we were investigating the expression of insulin receptor (IR), cytochrome c oxidase subunit 4 (COXIV), AMPK , pAMPK and its ratio.

Material and methods: Adult male B6; SJL-Tg(APPSWE)2576Kha transgenic (TG) mice and wild types (WT) aged 5 (5M) and 10 (10M) months were used in experiments. Oral galactose therapy was given to half of WT (WT+G) and half of TG (TG+G) group for 2 months (200mg/kg/day), while others received tap water ad libitum. Protein expression of IR, AMPK, p-AMPK , p/t AMPK and COXIV in HPT was measured by Western blot. Data were analyzed by Kruskal-Wallis and Mann-Whitney U-test ($p<0,05$).

Results: IR expression in TG (vs.WT) was decreased both in 5M (-48%,p=0,007) and 10M (-23%,p=0,022) mice and stayed decrease after galactose therapy. No differences in expression of COXIV was noted between the groups both in 5M (KWtest,p=0,105) and 10M(KWtest,p=0,324) mice. pAMPK/AMPK ratio (used as indirect marker of AMPK activity) was increased both in younger WT and TG (5M) mice after galactose therapy (WT+G;+83%,p=0,014 ,TG+G;+65%,p=0,022) but in older WT (10M) mice pAMPK/AMPK was decreased after galactose therapy (WTvs.WT+G, -58%, p=0,036).

Conclusion: TG mice showed the decrement in IR level. Galactose increased the AMPK activity only in younger TG and WT mice, but did not change the IR and COXIV level. The potential therapeutic impact of galactose seems to be connected to maintaining the energy balance and depend on the age and stage at which AD occurs.

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METHYLMOLNUPIRAVIR (CROCITIVIR) - ORALLY ACTIVE NUCLEOSIDE ANALOGUE RNA-DEPENDENT RNA POLYMERASE INHIBITOR FOR TREATMENT OF COVID-19

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Introduction: Coronavirus disease 2019 (COVID-19) is the highly contagious infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Viral RNA-dependent RNA polymerase plays a crucial role in viral life cycle, facilitating synthesis of negative-strand subgenomic RNA, structural protein-related mRNAs, and viral genomic RNA, therefore allowing all other biological activities of the virus. This, alongside being highly conserved and lacking a counterpart in mammalians, makes RdRp a promising drug target for COVID-19 treatment. This project aimed to develop and characterize nucleoside analogue RdRp inhibitor that could be orally administered.

Materials and methods: In vitro studies were conducted using Vero E6, Calu-3 and HAE cell cultures. Citotoxicity was determined using CCK8 test on Vero6 and Cell-Titer Glo test on Calu-3 cells. Antiviral activity was examined by using qRT-PCR and immunofluorescence microscopy on Vero6 and plaque test on both Calu-3 and HAE cell cultures. In vivo studies were conducted on Ces1c−/− mice and Rhesus monkeys, measuring weight loss, lung titer of virus and antigens immunofluorescence in mice, while clinical signs, viral titer and severity of lung lesions were monitored in monkeys. Pharmacokinetics and toxicokinetics were tested on Sprague-Dawley rats and Rhesus monkeys. After obtaining regulatory approval, clinical studies followed.

Results: Out of molecules chosen through rational drug design, methylmolnupiravir showed best potency and oral bioavailability in silico. While toxicological screening tests showed possibility of hepatotoxicity, further studies and subsequent clinical trials combined with short period of drug use negate serious liver harm. Prodrug showed an acceptable pharmacokinetic profile after oral application. Both in vitro and in vivo studies showed improvement of all tested parameters. Methylmolnupiravir was 19% more effective in reducing lung viral titers of the virus than remdesivir, as well as 24% more effective in reducing lung lesions in trials on rhesus monkeys. Phase 1 clinical trials showed adequate tolerability after p.o. 600 mg dose. In Phase 2 methylmolnupiravir decreased mortality, number of days needed for normalisation of blood oxygen saturation and number of days until hospital discharge. Additionally, patients' overall medical state improved.

Conclusion: Methylmolnupiravir is the first orally active cytidine analogue which acts as RdRp inhibitor, showing significant efficacy and good tolerability in the treatment of COVID-19.

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EFFECTS OF ARONIA MELANOCARPA EXTRACT ON THE ASTROCYTES EXPOSED TO 6-HYDROXYDOPAMIN

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Introduction: Parkinson's disease (PD) is the most common movement disorder and the second most frequent progressive neurodegenerative disease in the world. Neurotoxin 6-hydroxydopamine (6-OHDA) is used in *in vivo* and *in vitro* models of PD and may contribute to elucidating the pathogenesis of this disease. Therapeutic options in the treatment of PD are limited, and there is growing scientific evidence of the neuroprotective effects of antioxidants such as *Aronia melanocarpa* (chokeberry). The aim of this study was to determine the effects of chokeberry extract treatment on the astrocytes exposed to 6-OHDA.

Materials and methods: Experiments were performed on primary mouse astrocytes, untreated or pretreated for 24 h with different concentrations of chokeberry extract (10, 50 or 100 μg/mL). 6-OHDA was added to the cell culture medium during the next 72 h for lactate dehydrogenase (LDH) test or 24 h for western blot (WB) analyses.

Results and discussion: In this study, the addition of different concentrations of chokeberry extract alone to the cell culture media did not affect LDH activity in the samples taken from the uninjured astrocytes. Application of 6-OHDA significantly increased the LDH activity, while the cells treated with the higher concentrations of chokeberry extract had significantly lower activity of this enzyme. Furthermore, heat shock protein 70 (HSP70), brain derived neurotrophic factor (BDNF) and inducible nitric oxide synthase (iNOS) expression levels were measured in astrocytes exposed to 6-OHDA. Chokeberry extract treatment did not significantly affect the levels of the protective proteins HSP70 and BDNF but increased the level of iNOS expression in injured astrocytes, albeit not statistically significantly.

Conclusion: This study suggests possible protective effects of chokeberry extract in an *in vitro* model of PD. The exact mechanism of the observed protective effect is still unclear and could be the subject of further research.

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RECEPTION AND RELEASE OF FLUORIDE FROM ACRYLIC RESIN SURFACE TREATED WITH TWO FLUORIDE GELS OF DIFFERENT COMPOSITION

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Introduction: Wearing mobile acrylic replacements makes it difficult to maintain oral hygiene which results in increased risk of caries. The aim of this study was to determine the uptake and release of fluoride from acrylic resin treated with highly concentrated fluoride gels.

Materials and methods: By mixing powder and liquid, 18 acrylic tiles were made and divided into three groups of six samples. The group (A) was treated with sodium fluoride gel (Mirafluor K-gel), the group B was treated with sodium fluoride and amine fluoride gel (Elmex gelee), and the group (C) was without treatment. Samples were weighed, gel-treated for 30 minutes, washed with deionized water for 3 minutes and placed in 5 ml of deionized water in an incubator at 37 °C. After 24 hours, the first measurements of fluorine release were made with an ion-selective electrode (ORION EA 940). Subsequent measurements were performed after 48, 72, and 144 hours, after which the weighing was repeated.

Results and discussion: The A group of acrylic tiles released 0,0365 ppm F/g mm^2 while group B released 0,0128 ppm F/g mm^2. Statistically significant concentration of fluorine was released after 24 hours, when compared to other time points. Measurements at later time points showed a constant low rate. There was no statistically significant difference in fluorine release when NaF and NaF+amine F treatments were compared. The change in mass of the acrylate was nonsignificant.

The incorporation of fluoride within the acrylate structure shows an initial increased release over 3 days and significantly lower values in the remaining measured time (1). Topical NaF and NaF+amine F treatment of acrylic surface ensures the release of fluoride ions within first 24 hours, avoiding acrylate structural fluoride incorporation risks.

Conclusion: The acrylate releases all fluorine within 24 hours of application. Both NaF gel and NaF+amine fluoride gel are equally suitable.

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ASSOCIATON OF COGNITIVE DEFICIT WITH GLUTAMATE AND INSULIN SIGNALLING IN A RAT MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease, in addition to being considered a motoric disorder, can be manifested by the appearance of cognitive deficit. Glutamate and insulin signalling dysfunction is involved in the development of cognitive deficit in Alzheimer's disease. One of the main goals of this thesis is to investigate cognition in rat model of Parkinson's disease, and its association with brain glutamate and insulin signalling, induced by bilateral intrastriatal injection of 6-hydroxydopamine (toxin used for the selective depletion of dopaminergic neurons).

Materials and methods: Three months after 6-hydroxydopamine treatment, Wistar male rats were subjected to motoric and cognitive tests (RotaRod, Passive avoidance, Morris Water Maze) and later sacrificed. Expression of tyrosine hydroxylase and proteins involved in insulin and glutamate signaling were assessed in hippocampus, hypothalamus and striatum by immunofluorescence, Western blot and ELISA methods. The national regulatory body, the Croatian Ministry of Agriculture, approved the experiment (license number EP 186 /2018).

Results and discussion: Cognitive and motor deficit was observed three months after the administration of 6-hydroxydopamine, while protein expression involved in insulin signalling remained largely unchanged. Motor and cognitive deficit was observed as well as a decrease in tyrosine hydroxylase expression and AMPAR activation in the hippocampus and striatum, while dopaminergic nuclei in substantia nigra were preserved.

Conclusion: These results suggest a possible association between glutamate signalling dysfunction and cognitive deficit in a rat model of Parkinson's disease.

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METABOLOMICS APPROACH IN PHARMACOLOGY OF DEPRESSION

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Introduction: Depression is one of the most prevalent neuropsychiatric conditions, and represents a significant burden to both the individual and society. Individuals with depression have a reduced quality of life, especially those resistant to treatment. Therefore, alternatives for conventional antidepressants are being studied. To further understand pathophysiology and drug metabolism in depression, efforts are being made in the prospective new field of pharmacometabolomics. This approach unifies pharmacology, the study of chemicals with biomedical properties, and metabolomics, which deals with chemical processes involving metabolites. The aim of this review is to summarize current knowledge of the field.

Materials and methods: The data presented in this literature review has been collected from articles found via PubMed and Mendeley databases. The keywords used for the literature search were: depression, metabolomics, pharmacology, pharmacometabolomics, metabolic markers, major depressive disorder.

Results and discussion: Various metabolites can cross the blood-brain barrier, resulting in complex interactions whose mechanisms are not yet fully elucidated. Recent studies have identified inflammatory processes as a potential conduit to neurodegenerative and neuropsychiatric disorders, including depression. Metabolites from the gut microbiome can activate immune cells and increase permeability of the blood-brain barrier. Furthermore, genetic predisposition can affect drug metabolism, causing some patients to be resistant to certain medication. Thus, pre-treatment screening might be helpful in individualizing therapy. The most relevant inter-individual genetic differences have been found in genes for mitochondrial enzymes. Finally, specific metabolites found in blood can be used as biomarkers of depression, and indication of therapy efficiency.

Conclusion: The synergy of pharmacology and metabolomics represents a significant tool for understanding exact pathways of antidepressants *in vivo*, as well as gaining insight on the etiology of the disorder. Novel discoveries made in pharmacometabolomics are paving the way for improving subsequent therapy, both by personalizing treatment according to metabolic type, and finding new targets for medication.

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PATIENT KNOWLEDGE AND AWARENESS OF ANTIBIOTIC RESISTANCE -pilot study

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Introduction: Antibiotic resistance is becoming one of the biggest problems in public health because it compromises infections treatment possibilities and it aggravates dental procedures as well as medical. The purpose of this research is to investigate the knowledge and awareness about antimicrobial resistance among patients of the School of Dental medicine, University of Zagreb.

Materials and methods: For the purpose of this study, a World Health Organization (WHO) survey was used. First it was translated from English to Croatian, and then distributed to patients at the Department's waiting rooms. The survey consists of questions about subjects general information, knowledge about antibiotics and awareness of the antibiotic resistance.

Results and discussion: The survey completed 30 patients, of which 22 (73.3%) were women and 8 were men. The proportions in the age groups was equal. 60% of respondents were from the city of Zagreb, and the majority of all respondents have completed high school as their highest level of education (50%). 73.3% of respondents claim that the last course of antibiotics they received from a doctor or nurse, while an equal share of respondents denies this or do not remember (13.3%). 83.3% of respondents received advice on how to take antibiotics properly. 42% of respondents believe that sore throat can be treated with antibiotics. The term "antibiotic resistance" (ABR) was heard by 85% of respondents, of which 45.6% from a doctor or nurse, and 25% through the media. 33.3% of respondents believe that ABR cannot affect them and their families, while 26% believe that ABR is a problem in other countries, but not in the Republic of Croatia.

Conclusion: The results of this pilot study indicate the importance of patients education about the emerging problem of antibiotic resistance and importance of raising ABR awareness which is not a local but a global problem.

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ACUTE EFFECT OF ORAL GALACTOSE AND GLP1R ANTAGONIST ON PROTEIN LEVELS IN THE HYPOTHALAMUS OF SPORADIC ALZHEIMER'S DISEASE RAT MODEL

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Introduction: Intracerebroventricular (icv) application of streptozotocin (STZ) causes mytochondrial dysfunction, oxidative stress and other metabolic effects, which lead to accumulation of τ-protein and Aß plaques that makes it suitable for animal model of sporadic Alzheimer's disease (sAD). Literature data shows that oral galactose prevented the early-developed cognitive deficits in this rat model. We aimed to explore whether acute effect on protein levels in hypothalamus of single oral galactose is mediated by glucagon-like peptide-1 (GLP-1) in sAD rat model.

Materials and methods: Three months old Wistar rats were injected icv with STZ (3 mg/kg) or vehicle only (CTR). One month after the injection, animals were divided in three experiments. In first experiment, we have tested the effect of 200 mg/kg oral galactose dose at different time points (30 min, 60 min, and 120 min) in CTR animals. In the second experiment, we have tested the effect of single acute icv dose of GLP-1R antagonist (Exendin 9-39, 85 μg/kg) in CTR and STZ rats, 30 min after the injection. In third experiment CTR and STZ rats, 30 min prior to oral galactose load, were injected icv with GLP-1R antagonist, and animals were sacrificed 120 min after galactose load. PDH, COXIV, CytC and Casp-3 protein levels were measured by Western blot method and concentrations of lactate, ATP and SOD were measured by ELISA in dissected HPT. The national regulatory body, the Croatian Ministry of Agriculture, approved the experiment (license number EP 186 /2018).

Results and discussion: Changes in HPT protein levels were most pronounced 120 min after oral galactose load in CTR animals. Administration of GLP-1R antagonist led to alterations in HPT protein levels and lactate concentration seen only in STZ rats. GLP1-R antagonist abolished the effect of galactose, only in Casp-3 levels in CTR animals.

Conclusion: We can conclude that oral galactose might have an effect on apoptosis in HPT, seen as reduced Casp-3 and CytC levels, while GLP-1R antagonist if given icv causes changes in HPT only in STZ animals. Further research is needed to elucidate if the effect of oral galactose in STZ animals is mediated through GLP-1 signaling.

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REPURPOSING A DIGITAL KITCHEN SCALE FOR NEUROSCIENCE RESEARCH

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Introduction: In recent years, an increasing number of open-source platforms are being developed by scientists intent on creating modular, robust, and affordable tools tailored to the requirements of their everyday work (Oellermann et al., 2021). This work aims to explore the potential of an ordinary kitchen scale for i) assessing startle response and prepulse inhibition sensorimotor gating, and ii) measuring grip strength in rodents.

Materials and methods: The developed platform, PASTA (Platform for Acoustic STArtle) consists of a hacked USB-enabled kitchen scale capable of outputting 80 readings of applied force per second to a computer. The platform was used for the analysis of acoustic startle and prepulse inhibition sensorimotor gating in rats treated intracerebroventricularly with streptozotocin (STZ-icv) (Virag et al., 2021). Furthermore, the platform was adapted for grip strength measurements and tested for assessment of rigidity in a rat model of Parkinson's disease induced by intrastriatal administration of 6-hydroxydopamine (6-OHDA-is) (Homolak et al., 2020). Open-source tools for data collection were prepared in Python, and for data analysis in R.

Results and discussion: Proof-of-concept experiments provide solid evidence that PASTA can be used to obtain high-precision quantitative data from startle response, prepulse inhibition, and grip strength testing in rats. STZ-icv demonstrate increased startle response but show no difference in prepulse inhibition indicating enhanced stress response, but no evident synaptic dysfunction 1 month after model induction. Muscular rigidity is significantly increased in 6-OHDA-is regardless of the dose used or reboxetine pretreatment and neither trial speed nor animal mass were recognized as important confounders.

Conclusion: PASTA provides a cheap, easy, precise, and reliable way to measure startle response, prepulse inhibition sensorimotor gating, and grip strength in rodents using widely available equipment and open-source software. Unlike commercial instruments, the flexibility and modularity of PASTA enable researchers to collect raw data and screen for potential confounders encouraging more reliable and transparent research practices.

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