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Wrocław, Poland
October 19–21, 2023

ABSTRACT BOOK

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Pursuant to the ordinance of the Rector of Wroclaw Medical University No. 12/XVI R/2023, from February 1, 2023, authors are required to pay a fee for each manuscript accepted for publication in the journal Advances in Clinical and Experimental Medicine. The fee amounts to 990 EUR for original papers and meta-analyses, 700 EUR for reviews, and 350 EUR for research-in-progress (RIP) papers and research letters.

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5th International Wroclaw Scientific Meetings

Wrocław, Poland

October 19–21, 2023

ABSTRACT BOOK

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Introduction

Investing in knowledge always yields the best returns.
Benjamin Franklin

We are delighted to welcome you to the 5th International Wroclaw Scientific Meetings (IWSM), a gathering that brings together researchers from around the world. This year, the Scientific Meetings are being organized by dedicated students from the Faculty of Pharmacy and the Faculty of Medicine. These students are actively engaged in various groups, including the Group of Cancer Cell Biology, the Group of Biomedical and Environmental Analyses, the Group of Flow Cytometry and Biomedical Research, the Cardiac Perfusion Laboratory, and the Group of Toxicology.

Our conference, the 5th International Wroclaw Scientific Meetings, is specially dedicated to young scientists. Furthermore, this edition of IWSM is dedicated to the memory of Prof. Jolanta Saczko (1964–2023), an esteemed initiator of Scientific Meetings, who made significant contributions to the field of experimental anticancer therapies. Prof. Saczko was not only a pioneer but also an inspiring mentor to countless young scientists. In keeping with tradition, this conference aims to facilitate the exchange of scientific knowledge among young researchers and science enthusiasts from various centers in Poland and abroad.

This multidisciplinary conference covers a wide array of topics, including cell biology, drug interactions in both cancer and normal cells, cell–drug interactions, cellular responses to oxidative stress, and cellular engineering, including computer modeling. Throughout the conference, young scientists will have the invaluable opportunity to present their research findings and engage in discussions with experienced and esteemed researchers in the world of science, who have graciously accepted our invitation to participate in this year's event. This year's plenary lectures will be delivered by world-renowned scientists from both Poland and abroad. In addition to these lectures, practical workshops on flow cytometry, quantum dots, electroporation, and cellular impedance will be organized, providing hands-on learning experiences for attendees.

We extend a warm invitation to all of you to participate in these enriching sessions and discussions. We sincerely hope that this conference will be a platform for fruitful exchanges and foster valuable connections among attendees. Moreover, it brings us immense joy to witness the growing interest of young science enthusiasts from Poland and other international centers in our conference.

We wish all conference participants engaging discussions and memorable moments in Wrocław.

Dr. hab. Eng. Julita Kulbacka
University Professor

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LECTURES

Ions, membranes and channels: From the quantum level to continuum modelling

Mounir Tarek

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Prof. Mounir Tarek (France) is a Senior Research Director at the CNRS-Université de Lorraine, a recipient of a PhD in Physics from the University of Paris in 1994. He joined the CNRS after a four-year tenure at the National Institute of Standards and Technology (Gaithersburg, Maryland, USA) following 3 years of tenure at the University of Pennsylvania. His research focus and expertise lie in studying cell membrane transport processes. It involves using computational chemistry methods to study membranes, proteins, ion channels, and membrane transport proteins. Over the last few years, he has studied many aspects of the electroporation of cell membranes subject to high electric fields.

Abstract

The application of short and intense electric pulses enables to transiently alter the properties of cell membranes, making them permeable to a wide range of chemical species. This phenomenon is routinely used in medical applications as well as in biotechnology and industrial processing. To date, most investigations of the processes involved have focused on the ability of intense electric fields to create pores within the lipid bilayers, allowing us to better understand and control the phenomenon termed "electroporation". On the other hand, our knowledge about the chemical processes enhanced as a consequence of the application of electric fields to cells is still sketchy. In this contribution, we enclose the capabilities of computational resources and the predictive power of advanced atomistic and quantum level molecular dynamics techniques to decipher key steps in several chemical and biophysical processes occurring during and following electric field stimulation of cell membranes. We show this phenomenon under low-voltage conditions, and predict that under sub-nanosecond pulse electroporation conditions, peroxidation of model cell membranes by potent reactive oxygen species (OH• and OOH•) is significantly enhanced. We quantify the permeability of the peroxidized membranes to a host of species including ions and molecules to demonstrate that electrically mediated chemical effects may play a significant role in several processes following exposure of cells to high electric fields. We discuss the relevance of these effects for cells subject to radio-frequency electromagnetic fields (RF-EMF) as well as for excitable cells subject to electrostimulation.

New modalities of electroporation for drug and gene delivery: MHz bursts versus contactless high magnetic fields

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Prof. Vitalij Novickij (Lithuania) is the Director of the Institute of High Magnetic Fields (VilniusTECH) and Head of the Department of Immunology (Centre for Innovative Medicine). He specializes in high-power electronics and electromagnetic radiation and its effects on biological objects, with a high focus on electroporation in the context of drug and gene delivery. Since 2013, he co-authored more than 90 scientific publications in peer-reviewed scientific journals with impact factor (CA WoS). Some of the developed generators right now are distributed to 7 laboratories worldwide including University of Ljubljana, Wroclaw Medical University, Old Dominion University, University of California, etc.

Abstract

Electroporation is commonly used in biomedical applications for both drug and gene delivery. When high intensity electric fields are applied, the polarization of cell membrane occurs, leading to the formation of hydrophilic pores allowing controlled intracellular delivery of exogeneous molecules. The talk will focus on the recent 2 new modalities of electroporation for drug and gene delivery. One is based on extremely high-power electromagnetic field pulses, which enable contactless treatment. Another one is based on high frequency nanosecond electric field bursts, which result in residual transmembrane potential accumulation significantly potentiating intracellular delivery of various molecules.

Elemental constituents of selected African medicinal plants for fibrosis tumor treatment

Gcobisa Manzane, Sekomeng Johannes Modise

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Prof. Sekomeng Johannes Modise (South Africa) is the Head of the Biotechnology and Chemistry Department at the Vaal University of Technology (Vanderbijlpark, South Africa). His research team focuses on the indigenous knowledge systems, which incorporate traditional medicine, clothing and food. The traditional medicine and its knowledge are embedded in the traditional healers and communities. The healers and various communities approach the researchers with different plant materials and concoctions for validation and further tests. The validation of various plants' capability of treating different ailments historically and in the current era is conducted by his team. The research involves treatments for tuberculosis, cancerous ailments and inflammatory diseases.

Abstract

Uterine fibroids are benign, non-cancerous growths that develop within the muscular wall of uterus in females. The growth of this tumor has a negative impact on women's health. Currently, there is no existing medication or remedy that is registered to treat it, but only a few surgical administrations and medication that only deal with its symptoms. The cause of uterine fibroids include hormonal and genetic reasons, growth factors, and extracellular matrix factors. Common symptoms of uterine fibroids include heavy and prolong menstrual bleeding which can lead to a high risk of anemia, lower abdominal pains, pelvic pressure, infertility, and pregnancy loss. Traditional health practitioners in South Africa claim to treat various illness (including fibroids) using medicinal plants. In this study, medicinal plants were considered because of their therapeutic capabilities. The study also focused on the safety of usage of these plants and their potential in remedying fibrosis. The plants used are *Gunnera perpensa* (GunPer) and *Albizia tanganyicensis* (AbiTan). The extracts were exposed to phytochemical screening, thin layer chromatography and elemental analysis. Mineral nutrients that have been studied for their potential managing of symptoms or reducing the risk of fibroids growth such as Fe, Mg, Se, and Zn were found at various levels.

Nanomaterials and their applications in biochemistry and environment in Asia

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Viet Hoai Nguyen, PhD (Vietnam), is the Head of Laboratory of Chemistry, Biology, and Environment, Research Center for Environmental Monitoring and Modeling, Vietnam National University. His research team specializes in materials and their applications in biochemistry and environment. He focuses on carbon nanomaterials, silver nanoparticles and carbon quantum dots.

Abstract

In recent past, it has been proved that evolution in nanomaterials (NMs) is the key factor in nanotechnology development. Nanomaterials have become prominent in technological breakthroughs due to their adjustable physical, chemical and biological characteristics and superior performance over bulk equivalents. The NMs can be divided into many categories based on size, composition, capping agents, form, and origin. The applications of NMs are spreading to almost all branches of science and technology, such as health, medicine, electronics, energy, and the environment. The lecture introduces NMs and their applications in biochemistry and the environment in Asia. Researchers from Japan, India, China, Singapore, Korea, and Vietnam are attracted by applications of NMs.

Hydrazones: Simple yet powerful bioactive compounds

Robert Kaplánek

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Robert Kaplanek, Eng. PhD (Czech Republic), is a senior researcher at 1st Faculty of Medicine, Charles University in Prague/BIOCEV in the Laboratory of Medicinal Chemistry. He received PhD in organic chemistry at University of Chemistry and Technology in Prague. His research is focused on metal chelators and derivatives of natural compounds as novel cytostatics, epigenetically active compounds, anti-Alzheimer's, antimicrobial and antimycobacterial drugs.

Abstract

Along with relatively easy synthesis as well as structural diversity, hydrazones are an important class of biologically active compounds with a broad spectrum of biological activity, including antimicrobial, anti-inflammatory, anticonvulsant as well as anticancer activity or anti-Alzheimer's effect. Due to their metal-binding ability, they can serve as therapeutics in the treatment of iron overload diseases or metal poisoning. Their mechanism of action is based on several processes including chelation of metal ions, intercalation, inhibition of enzymes (ribonucleotide reductases, histone deacetylases, acyl transferases, caspases, TET proteins), disruption of cell-to-cell communication, or production of reactive oxygen species (ROS).

In an attempt to find new potent and selective agents, novel hybrid compounds bearing 2 or more pharmacophores are developed using molecular hybridization approach. It allows to prepare compounds that can affect multiple targets utilizing a combination of several different mechanisms of action.

Bioluminescent technologies for studying protein biology and cellular responses

Axela Johann

Promega GmbH, Walldorf, Germany

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Abstract

In the realm of molecular biology, understanding the intricacies of protein biology is paramount to unraveling the complexities of life itself. This seminar aims to shed light on cutting-edge technologies that have revolutionized how we explore the world of proteins. We will delve into the HiBiT Protein Tagging System, a groundbreaking approach ideally suited to monitor cellular protein levels and investigate protein function. When combined with CRISPR genome editing, this system provides a powerful toolkit for studying proteins at their native expression levels while preserving transcriptional regulation. Additionally, we will explore the innovative Lumit™ Immunoassays, a fast-track method that has transformed protein quantification and interaction analysis.

This seminar will equip attendees with the knowledge and tools necessary to explore protein biology with unprecedented precision and efficiency. By harnessing the HiBiT Protein Tagging System, CRISPR genome editing, and Lumit™ Immunoassays, researchers can advance our understanding of proteins and their roles in health and disease, opening up new avenues for scientific discovery and innovation.

Key words: protein biology, HiBiT Protein Tagging System, CRISPR genome editing, Lumit™ Immunoassays, protein quantification, interaction analysis

ORAL PRESENTATIONS

Application of additive manufacturing in the production of medigummies for children

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Conflict of interest
None declared

Abstract

Background. The current approaches for adjusting drug doses to pediatric patients involve segmenting drugs by opening capsules, cutting and crushing tablets as well as mixing the powders with food to mask the taste and texture. These approaches are both inconvenient and pose the risk of possible drug overdose or ineffective therapy. Additive manufacturing, commonly known as 3D printing (3DP), is an innovative method to produce personalized drugs with tailored dosage, shape and release profile.

Objectives. The aim of the proposed project was to obtain non-commercial filaments via hot melt extrusion (HME), which will be used as a matrix to incorporate the active pharmaceutical ingredient (API) and produce chewable pills and tablets in an acceptable form with personalized dose for pediatric population.

Materials and methods. Hydroxypropyl cellulose (HPC, Klucel EF HPC), polyethylene glycol (PEG 1000), hydroxypropyl methylcellulose (HPMC HME 15LV, Affinsol), Gelucire 48/16 (Gattefosse), food dye, and amlodipine were used. The prints were prepared using combined process of HME and fused deposition modeling (FDM) 3D printing. Prints (in the shape of a teddy bear and a heart) were prepared with 10%, 20% and 30% infill to increase their plasticity. The received materials were subjected to thermal stability studies using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The structural changes were analyzed using PXRD and ATR-FTIR. Texture Analyzer was used to assess the flexibility of produced filaments.

Results. During the study, blends of each polymer and plasticizer were extruded in the following compositions: 95:5, 90:10 and 90:20 wt% for HPC:PEG100, and 90:10, 90:20 wt% for HPMC:Gelucire. All formulations were tested to determine the formula with the most suitable properties for processing in 3DP. Optimal plasticity formulation had 5% PEG 1000 and was used as the matrix for the model drug. A filament with 0.32 wt% food dye was added for a child-friendly color.

Conclusions. 3D printing has made it possible to produce personalized flexible drug forms with a child-friendly appearance.

Key words: personalized therapy, 3DP, additive manufacturing

The usefulness of amphiregulin and endocan for the assessment of the degree of inflammatory activity and fibrosis in non-alcoholic fatty liver disease

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Conflict of interest
None declared

Abstract

Background. Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in developed countries, where it is becoming an increasingly common cause of cirrhosis and hepatocellular carcinoma (HCC). The disease is diagnosed when fat accumulation in at least 5% of hepatocytes is found on imaging or histopathology (with the exclusion of alcohol abuse). The NAFLD encompasses non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). Patients with NAFLD, especially those with NASH, experience premature mortality from cardiovascular causes; NAFLD has many features of the metabolic syndrome and is associated with increased levels of markers of inflammation and endothelial dysfunction.

Objectives. The aim of the study was to check the possibility of using the amphiregulin and endocan concentrations for diagnostics in patients with NAFLD in relation to healthy people, and to determine the possibility of using amphiregulin and/or endocan to distinguish between NASH and NAFL in a group of patients.

Materials and methods. The study included 58 patients with NAFLD (27 NASH patients; 21 NAFL patients; 10 patients in the control group). Serum plasma was used from Wroclaw Medical University patients. The concentrations of amphiregulin and endocan were determined with the enzyme-linked immunoassay (ELISA) tests.

Results. Amphiregulin and endocan levels in NAFLD, NASH and NAFL patients were significantly higher than those in the control group ($p < 0.01$). The reactive oxygen species (ROC) analysis (NAFLD compared to control group) showed the area under the ROC curve (AUC) >0.8 and >0.7 for amphiregulin and endocan, respectively, while the ROC analysis of NASH compared to NAFL showed AUC > 0.55 for both parameters tested. The correlation in NASH and NAFL groups of patients between amphiregulin and endocan was statistically significant ($p < 0.05$).

Conclusions. Amphiregulin and endocan concentrations can be helpful in assessing inflammatory activity and fibrosis in NAFLD. The ROC analysis (NASH compared to NAFL) allowed to exclude the randomness of the results for both amphiregulin and endocan.

Key words: amphiregulin, endocan, NAFLD, NASH, NAFL

Studies of stem cell lysates in potential use as a factor regenerating nerve cells

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Conflict of interest
None declared

Abstract

Background. New factors regenerating the cells of the nervous system are constantly being sought. The use of stem cells, as already shown in various studies, can lead to the activation of the carcinogenesis process. For this reason, the idea arose to investigate stem cell lysates as potentially inducing the regeneration of nerve cells and at the same time being a safer alternative to stem cells.

Objectives. The aim of the study was to evaluate the effect of MIC-1 stem cell lysate on neuron-like cells.

Materials and methods. Studies were performed using the differentiated cell line PC12 (medium containing 100 ng/mL nerve growth factor (NGF) for 72 h), which was treated with MIC-1 cell lysate, and viability (MTT and LDH assays) and cell morphology (length and density of spikes) were assessed.

Results. Cell lysates in the range up to 250 µg/mL presented no cytotoxic effect on PC12 cells in both the MTT and LDH assays. In the mean concentration range of 50–150 µg/mL, an increased effect on length and density was observed.

Conclusions. Stem cell lysates from MIC-1 have a positive effect on neuron-like cells (they do not indicate cytotoxic activity and at the same time they extend the length of the neurite).

Key words: steam cells, neurons, PC12

The analysis of the influence of selected factors on changes in α-synuclein structure

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Conflict of interest
None declared

Abstract

Background. Research on the development of Parkinson's disease has indicated that the hallmark of the disease is the appearance of Lewy bodies containing mainly α-synuclein. In Lewy bodies, α-synuclein occurs in a misfolded form and may cause neuronal death. Although quite a lot of research on the structure of α-synuclein has already been performed, there are still many questions concerning changes in the secondary structure of this protein and the source of its toxicity.

Objectives. An important but poorly understood effect of protein misfolding is adsorption, which causes changes in conformation due to the protein's interactions with the surface. Therefore, studies aimed at the structural analysis of the adsorbed protein were carried out using several physicochemical methods, including the characteristics of the protein in solution and on the adsorption surface.

Materials and methods. Experiments were conducted using α-synuclein from Merck. A number of analytical methods were used to determine the structural stability of the protein (CD, DLS UV-vis, LDV) and the adsorption efficiency on the gold surface (FTIR, QCM-D, MP-SPR).

Results. Using circular dichroism (CD) and infrared spectroscopy (IR), it was confirmed that the interaction of the protein with gold induces changes in its conformation, and the direction and intensity of structural changes, which depend on the pH of the environment in which the adsorption was carried out. Complementary experiments using MP-SPR and QCM-D showed that the adsorption conditions and the structure of molecules have a stronger effect on the hydration of formed films than the degree of surface coverage.

Conclusions. The presented research focuses on determining the influence of molecular interactions on the stability of intrinsically disordered proteins on the gold surface and the efficiency of deposition of proteins on the negatively charged surface. The research will identify the mechanism of internally disordered protein folding toward toxic aggregates and amyloid fibers.

Key words: α-synuclein, adsorption, secondary structure, CD, FTIR, MP-SPR, QCM-D

Anticancer activity of isolated bioactive compounds produced by fungal endophytes from the Mokrzański Forest

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Conflict of interest
None declared

Abstract

Background. Cancer, a disease of pathophysiological changes related to the process of cell division, is responsible for a large number of deaths worldwide, proving to be a significant disorder. Multidrug resistance of cancer cells and side effects of drugs are still the main obstacle to effective treatment. Additionally, research into the sources of new drugs indicates that natural products continue to play an important role in drug discovery and development.[1]

Objectives. Endophytes, a new type of microbial resource that can produce a variety of biological components, have potential for research.[2] In this project, the anticancer activity of compounds produced by endophytic fungi from the Mokrzański Forest in Wrocław was assessed.

Materials and methods. In this project, the anti-cancer activity of compounds synthesized by endophytic fungi from various species of trees from the Mokrzański Forest was assessed. The research models used in the project were melanoma (A375), lung cancer (A549) and breast cancer (MCF-7) cell lines, as well as keratinocyte (HaCaT) cells functioning as a comparative model. In order to investigate the anticancer activity, cell-viability assay (PrestoBlue), in vitro wound healing tests, anti-aging tests, and anti-mutagenic activity tests were performed. The samples were analyzed using thin layer chromatography (TLC) and tandem mass spectrometry analysis (LC-MS/MS).

Results. Studies indicate a strong anticancer activity of compounds produced by endophytic fungi isolated from the leaves of Norway maple (*Acer platanoides* L.). After separation of the compounds with the use of the TLC technique, it was shown that the compound produced by endophytic fungi from silver birch (*Betula pendula*) bark also has strong anticancer properties.

Conclusions. Endophytic fungi are a source of new bioactive molecules with a large variety of applications. Understanding the interactions of endophytic fungal metabolites and the cellular mechanisms that are responsible for their biological activity will reveal their full value so that they can be used in the medical industry in the future.

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Key words: endophytic fungi, natural products, anticancer activity, cytotoxicity

Methods of mitigating viruses: FIV, FeLV and SARS-CoV-2

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has proved that animal-borne viruses are able to threaten human health and life. Despite increased hygiene practices, interspecies transmission of viruses can still occur. The RNA viruses undergo diverse mutations within a short period, making it challenging to develop effective vaccines and targeted treatments. The tendency of viruses to mutate, their virulence and ease of spread within populations necessitate the development of methods for effective virus neutralization and drugs active against these pathogens. The feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are among the most common pathogens in the cat population, with prevalence ranging from a few percent to as high as several dozen percent depending on the region. Due to the potential life-threatening nature of infections caused by these viruses, they raise concerns among cat owners. Although FIV shares significant similarities with its human counterpart, the human immunodeficiency virus (HIV), there have been no documented cases of FIV transmission from cats to humans, unlike the case of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). While there is no evidence yet to suggest that SARS-CoV-2 poses a threat to cats similar to FIV or FeLV, these animals can serve as reservoirs for the virus. Some literature sources also hypothesize that SARS-CoV-2 can be transmitted from animals to humans. In light of the threat posed by these feline viruses, it is necessary to develop methods to mitigate their presence in the environment through appropriate and effective disinfection. This is crucial not only for managing local infection outbreaks that may occur in shelters or veterinary clinics, but also for protecting cat owners in the face of potential interspecies transmission.
This paper presents a review of active substances effective in disinfecting surfaces potentially contaminated by viruses transmitted by domestic cats, such as FIV, FeLV and SARS-CoV-2. Ethanol, propanol, isopropanol, octenisept 4-phenoxyethanol, IV ammonium salts, and mixtures of these substances effectively eliminate feline viruses. The action of these substances can be enhanced by detergents. The paper also discusses drugs used in the treatment of infections caused by these viruses and highlights misconceptions propagated in the cat-owner community that are inconsistent with current knowledge, as encountered by the authors themselves. Vaccines, which are used to protect the cat against the development of diseases caused by viruses are also presented.

Key words: disinfection, COVID-19, FeLV, FIV, SARS-CoV-2

Functionalized liposomes for targeted drug delivery

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Abstract

Background. In recent years, liposomes have gained attention as potential drug delivery systems, which may help to overcome side effects and systematic toxicity of many modern drugs. Surface of the liposomes can be modified with specific ligands that allow binding to a selected receptor located on the cell membrane. Despite the fact that the range of nanoparticles used in therapies is growing, still little is known about how the surface structure of nanomaterials affects their cellular uptake and drug delivery. The nuclear magnetic resonance (NMR) spectroscopy enables gaining a detailed understanding of the surface structure of the synthesized materials, the degree of their functionalization, as well as the conformation and dynamics of functional groups.

Objectives. The aim of the study was to synthesize and characterize liposomal formulations with and without surface modification (e.g., saccharides, PEG, PEG-folate) using NMR spectroscopy.

Materials and methods. Liposomes were synthesized using the thin lipid film hydration method, with phospholipids (DOPC, DOPS, DSPE) at different molar ratios. Surface functionalization of the liposomes was achieved by changes in lipid composition. The size of the particles was determined using dynamic light scattering (DLS) and transmission electron microscopy (TEM). Composition of the liposomes was confirmed with NMR.

Results. Liposomes of about 100 nm in size were synthesized. Surface modification did not affect the morphology of the prepared liposomes. The successful functionalization was confirmed using NMR spectroscopy.

Conclusions. Liposomes differing in surface composition (e.g., galactose, lactose, PEG, PEG-folate) were prepared. All liposomal formulations were characterized using NMR spectroscopy. The use of NMR spectroscopy enabled us to characterize the particles in detail, which may provide further experimental advances in studying the interactions of functionalized nanoparticles with complex biological systems.

Key words: nanoparticles, liposomes, functionalization, NMR

Innovative 3D-printed scaffolds with complex porosity for tissue engineering applications

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Abstract

Background. Regenerative medicine is an interdisciplinary field which integrates principles of tissue engineering and molecular biology. Its primary aim is to promote healing and repair of damaged tissues and organs. 3D printing (3DP) shows great potential in regenerative medicine due to its flexibility, which enables individualization of drug dosage, customization of scaffold dimensions and adjusting the drug's porosity according to the patient's needs.[1]

Objectives. The aim of the proposed study was to design new materials with an incorporated active substance based on a polymer that is a blend of 2 biodegradable polyesters with significantly different melting points, which could be used in fused deposition modelling (FDM) 3DP technology.

Materials and methods. Biodegradable polymers hydroxyapatite (2 different particle sizes) and meloxicam were used to prepare the scaffolds of different porosity. Filaments were fabricated using hot melt extrusion (HME) and reprocessed via FDM 3DP to obtained scaffolds. The raw substances, filaments and scaffolds were tested for thermal stability and structural changes that may occur during processing. Thermal and structural analysis of the obtained materials used the following methods: differential scanning calorimetry (DSC), thermogravimetry (TGA), powder X-ray diffractometry (PXRD), Fourier transform interference spectrum spectroscopy (FTIR), high-performance liquid chromatography (HPLC), X-ray computed tomography, and scanning electron microscopy (SEM). The elasticity of the filaments was tested using a 3-point bending test. Materials were analyzed for cytotoxic effects with flow cytometry using mouse fibroblasts.

Results. Six formulations for processing via HME were prepared. Two scaffold designs with different geometries (1000 µm and 500 µm porosity) were developed and printed using the FDM for each formulation. The produced materials showed uniformity of drug content and thermal stability under process conditions. Gradual degradation of the scaffolds in PBS was observed during 7 weeks of conducting the experiment. Low cytotoxicity of the materials was confirmed.

Conclusions. All the results of the structural and cellular analysis indicate a promising use of prepared 3DP materials in reconstructive medicine, as these materials may find applications in the treatment and regeneration of bone tissue.

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Key words: tissue engineering, personalized therapy, 3DP

The influence of asymmetrical electric fields with calcium ions or bleomycin on ovarian carcinoma

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Conflict of interest
None declared

Abstract

Background. The utilization of electrical pulses with negative polarity (↓) subsequent to positive polarity pulses (↑) may trigger a phenomenon known as bipolar cancellation (BPC). This physiological reaction is thought to be particularly associated with nanosecond electroporation (nsEP). Existing scientific literature has yet to comprehensively examine the effects of bipolar electroporation (BP EP) utilizing protocols characterized by dissimilarities in pulse durations, encompassing both nanosecond and microsecond ranges. Moreover, the influence of intervals between reversed polarity pulses needs consideration. The authors decided to analyze the influence of the combination of asymmetrical pulses with calcium ions (Ca^{2+}) and bleomycin (BLM) on the effectiveness of the BPC phenomenon.

Objectives. The study aimed to analyze the effectiveness of asymmetrical electroporation protocols on the viability of ovarian carcinoma cells. Moreover, the influence of interpulse intervals, Ca^{2+} and BLM usage on the BPC phenomenon was observed.

Materials and methods. The authors utilized the ovarian clear cancer cell line (OvBH-1, MDAH-2774, SKOV-3) model to investigate the BPC. Cells were exposed to pulses delivered in bursts but as uni- or bipolar, symmetrical or asymmetrical sequences with a duration of 500 ns or 50 µs and electric field strength equal to 4.0 kV/cm or 14 kV/cm, respectively. The authors analyzed cell viability using an MTT assay. Cell membrane permeabilization was examined using Yo-Pro-1 uptake. Fluorescent staining was also performed.

Results. The acquired outcomes have additionally been explored within the domain of calcium and BLM electrochemotherapy. Diminished permeabilization of the cellular membrane and affected cellular viability have been noted. The impact of temporal gaps during interphases (1 µs and 10 µs) on the BPC phenomenon was documented.

Conclusions. The results indicate that the modulation of the BPC phenomenon is achievable through the manipulation of pulse asymmetry or the background of a temporal gap between the positive and negative polarities of the pulse.

Key words: asymmetrical pulses, electroporation, pulsed electric fields, ovarian cancer

Strategic pre-treatment planning for electroporation-based biomedical applications on superficial tumors

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Abstract

Background. Non-invasive electrodes offer a promising avenue for electrochemotherapy targeting superficial tumors, minimizing the need for invasive procedures while effectively delivering therapeutic benefits. However, such electrodes are commonly linked to diminished electric field penetration, primarily attributed to the composition of the skin and limited electrode contact area.

Objectives. In this study, we suggested modifying the non-invasive electrode composition by incorporating a passive needle (sterile syringe for drug administration). The proposed electrode composition was used for electrochemotherapy of B16 tumors in vivo. As a reference, treatment without a passive needle was performed.

Materials and methods. The three-dimensional superficial murine melanoma B16 tumor model with non-invasive plate electrodes, passive needle (4×10^6 S/m) and conductive gel (1.1 S/m) was developed in COMSOL Multiphysics (COMSOL, Stockholm, Sweden). A 420-kV terminal voltage was selected for 4-mm gap distance plate electrodes. Dirichlet and Neumann boundary conditions were selected. Conductivity step function was included. Tumors were then treated using ESOPE pulsing protocol (1.3 kV/cm × 100 µs × 8 (1 Hz)) and calcium chloride ($CaCl_2$). The survival rate was evaluated in 3 groups (untreated and treated with and without passive needle).

Results. Simulation results show that the passive needle amplifies electric field within the tumor, ensuring homogenous treatment, yet diminishing was observed at non-contact tumor boundaries. The use of $CaCl_2$ for treatment yielded superior results compared to the untreated group, with a noticeable two-fold increase in median survival days on average. The highest survival rate was observed in the group treated with a passive needle.

Conclusions. The developed electrode structure with a passive electrode was efficiency-wise better when compared with the group in which a passive needle was not provided, which is in agreement with the simulation results.

Key words: electrochemotherapy, electrodes, superficial tumor, passive needle, calcium, spatial electric field distribution

Fantastic γδ T cells and where (and how) to find them

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Abstract

Background. Gamma delta (γδ) T cells might be genuinely fantastic. They are unconventional and rare in the natural environment, usually hidden and showing themselves only when needed. Their diverse phenotype and activity are not explored enough. Their cultivation may usually be a cumbersome process, and even a slight change in the setting can have an enormous impact on their characteristics. Finally, when properly handled, γδ T cells can fight like beasts against versatile miscreant (cancer) cells.

Objectives. Therefore, to uncover their nature, our research involves the expansion and comparison of effector functions of 2 major subtypes of human γδ T cells, Vδ1+ and Vδ2+, toward one of the most aggressive brain tumors – glioblastoma multiforme (GBM), and to determine which subset is most suitable for the use in immunotherapy.

Materials and methods. Cultures of γδ T cells were started from buffy coat-isolated PBMC or pure γδ T cells, and maintained in medium with human serum, antibiotics and: 1) for Vδ1+ type – with mix of cytokines and OKT-3 antibody; and 2) for Vδ2+ type – with IL-2 and zoledronic acid (ZA). The γδ T cell phenotype was verified using flow cytometry. Cytotoxic activity of γδ T cells toward GBM cell cultures was assessed with LDH viability assays and fluorescent imaging. The synapse between cancer and γδ T cells was visualized using correlative light and electron microscopy (CLEM).

Results. Obtained in vitro data show high variability of phenotype and functional properties of γδ T cells between chosen expansion protocols. Both γδ T cell subtypes were able to destroy GBM cells, but their effectiveness varied between lines. Imaging results showed that γδ T cells swarm around tumor cells and quickly induce destructive morphologic changes in attacked GBM cells.

Conclusions. Our findings expand knowledge about the potential of γδ T cells for their application in cellular immunotherapy. However, further understanding of γδ T cell biology cell culture expansion techniques is still required.

Key words: glioblastoma, gamma delta T cells, immunotherapy

The effect of calibration technique on the stability of liposomes in formulation against the infection progress of SARS-CoV-2

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Conflict of interest
None declared

Abstract

Background. Applying liposomes as drug delivery platforms remains challenging, as formulations must be characterized by high homogeneity and excellent long-term stability. In the current study, we use 2 liposome calibration techniques to synthesize a liposome-based drug inhibiting the infection progress of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Objectives. The study aimed to investigate whether the high-throughput homogenization technique enables the acquisition of homogeneous liposomal suspensions with stability comparable to liposomes obtained with the extrusion method.

Materials and methods. Liposomes were composed of the following lipids: hydro-soy PC, cholesterol, DSPC, various PGs, DSPE-$PEG_{1000,}$ and DSPE-PEG_{2000}-Maleimide. After initial hydration, liposomes were calibrated via homogenization with LM20 Microfluidizer (Microfluidics) or pressure extrusion (patent pending). Further, the peptide sequence directed against SARS-CoV-2 was conjugated to liposomes. The excess peptide was separated from preparation via dialysis. Each preparation was characterized in terms of lipid and peptide content. Stability and homogeneity were assessed by changes in particle size distribution and zeta potential, measured with ZetaSizer Nano (Malvern). Measurements continued for 6 months or until the homogeneity was lost. Accelerated aging tests were also performed in samples incubated with a culture medium at 37°C.

Results. Liposomes subjected to homogenization were less stable than those obtained with pressure extrusion. The type of PG exerted the greatest influence on long-term stability. Extruded liposomes with DPPG were stable for at least 6 months, whereas homogenized liposomes lost their stability after 30–45 days. Liposomes with DOPG lost their stability within a month, even when subjected to extrusion. Finally, POPG liposomes showed high stability regardless of the calibration method, which was confirmed with the accelerated aging tests.

Conclusions. We demonstrated that liposome stability strongly depends on their composition and the calibration technique. The pressure extrusion supports the long-term stability; however, with a thorough optimization of the lipid composition, it is possible to obtain a stable, highly-homogeneous formulation based on the high-throughput homogenization technique.

Key words: liposomes, extrusion, microfluidization, SARS-CoV-2

ATP depletion-mediated cell death during calcium electrochemotherapy

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Conflict of interest
None declared

Abstract

Background. Calcium electroporation is based on electric field-mediated delivery of cytotoxic doses of calcium into the cells and has gained significant interest in the field of electrochemotherapy (ECT) to treat cancer. The biophysics of the process is intensively studied and optimal pulsed electric field parameters are constantly researched and optimized.

Objectives. In this work, we used a CHO-K1 luminescent cell line for the estimation of cell membrane permeabilization, adenosine triphosphate (ATP) depletion and cytotoxicity without the use of additional markers and methodologies, which is a new approach in the area of electroporation.

Materials and methods. The 5 kV/cm and 10 kV/cm sub-microsecond (100 ns and 600 ns) pulses were delivered in a sequence of 10–100 pulses and then compared to European Standard Operating Procedures on Electrochemotherapy (ESOPE) protocols in the context of calcium electrochemotherapy.

Results. Reversible electroporation is accompanied by ATP depletion associated with membrane damage, while during calcium ECT, the ATP depletion is several-fold higher, which results in cell death.

Conclusions. It was possible to derive sub-microsecond protocols that are efficacy-wise equivalent or better than ESOPE, which in turn introduces flexibility in parametric protocols design allowing for a reduction of input energy and minimization of potential muscle contractions, and ensures a more uniform treatment.

Key words: electrochemotherapy, MHz, membrane permeabilization, high frequency

Isolation of *Pelargonium alchemilliodes* L. L'Her active compounds and their effect on bacterial growth and keratinocytes in vivo

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Abstract

Background. *Pelargonium alchemilliodes* L. L'Hér is an evergreen shrub, cultivated principally for the medicinal essence and decoction in Southern Africa for the treatment of skin problems and wounds.

Objectives. The aim of the study was to optimize the extraction of phenolics and flavonoids from *P. graveolens.*

Materials and methods. The proliferative and cytotoxic effects of phenolics and flavonoids on human keratinocytes, as well as their antioxidant and antibacterial activities were studied. Active compounds were isolated.

Results. Total antioxidant capacity and reducing power were comparable to standard gallic acid, while the antiradical activity had IC50 values of 0.18 ±0.03–8.98 ±0.15 mg/mL. The MIC value of 1.56 mg/mL for extracts was registered against *Staphylococcus aureus* and *Salmonella typhi* comparable to chloramphenicol. Two triterpenoid compounds, namely 1-hydroxy-30-norlanosta-6, 8-diene and 1,2,3,4,4a,8,9,10,10a-octahydro-2-(2-hydroxypent-4-enyl)-4a-vinyl-1H-benzo[c]chromen-6(10bH)-one were isolated from the methanol extracts.

Conclusions. The results showed a significant ($p < 0.05$) increase in cell proliferation and viability when the extract was administered at concentrations ≤50 µg/mL.

Key words: *Pelargonium alchemilliodes* L. L'Her, keratinocytes, minimum inhibitory concentration, cell proliferation, cytotoxicity

Decontamination of tetracycline from wastewater using magnetite-silica-polydopamine (Fe_3O_4-SiO_2-PDA) nanocomposite

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Abstract

Background. Water pollution by pharmaceuticals is a global concern and its remediation is vital. Tetracycline is one of the most enduring micro-pollutants in the environment due to its widespread misuse. Hence, numerous approaches have been studied for the removal of antibiotics from wastewater.

Objectives. In this study, magnetic nanocomposite was synthesized by coating magnetite with silica (SiO_2) using the modified stober route. The synthesized Fe_3O_4-SiO_2 was polymerized with dopamine in a basic solution to obtain Fe_3O_4-SiO_2-PDA.

Materials and methods. The successful synthesis of Fe_3O_4-SiO_2-PDA nanomaterial was confirmed through scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared (FTIR), and X-ray diffraction (XRD) techniques.

Results. Batch adsorption studies were conducted to investigate whether the synthesized nanocomposite could adsorb tetracycline from an aqueous solution. The maximum tetracycline adsorption capacity of the Fe_3O_4-SiO_2-PDA nanocomposite was 310.99 mg/g.

Conclusions. The results indicate that the as-obtained hybrid core-shell Fe_3O_4-SiO_2-PDA is the most efficient for the removal of tetracycline from wastewater.

Key words: water pollution, magnetic nanocomposite, silica, adsorption

Reversal of doxorubicin resistance in human colon cancer cells using microbubble-assisted sonoporation

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Conflict of interest
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Abstract

Background. Multidrug resistance (MDR) in cancer, driven by membrane proteins responsible for drug efflux, may be affected by ultrasound-mediated sonoporation. Previous studies have demonstrated reduced Pgp expression in the blood–brain barrier in rats (Aryal et al., 2017), with effects lasting over 72 h. Similar findings were reported by Bjånes et al. (2020), who investigated the gemcitabine efficacy in sonoporated pancreatic cancer cells.

Objectives. To explore this phenomenon further, we determined the effect of microbubble-assisted sonoporation on human colon cancer cells sensitive and resistant to doxorubicin.

Materials and methods. We employed ultrasound (2 W/cm^2, DC 50%, 1 MHz, 30 s) with or without SonoVue microbubbles, followed by doxorubicin treatment at various timepoints (0 h, 2 h, 4 h, 8 h, 16 h, 24 h, 48 h, 72 h) in LoVo and LoVoDx cells. We assessed cytotoxicity, intracellular doxorubicin concentration, Pgp activity, and drug resistance-related gene expression. Additionally, we measured the level of ABCB1, ABCC1 and ABCG2 proteins in LoVo and LoVoDx cells using flow cytometry following the US exposure.

Results. Microbubble-assisted sonoporation effectively altered drug resistance in both LoVo and LoVoDx cells. The highest intracellular doxorubicin concentration and toxicity occurred when doxorubicin was administered within 0–16 h post-exposure, coupled with reduced Pgp activity. The ABCB1, ABCC1 and ABCG2 protein levels were diminished for up to 24 h in doxorubicin-resistant LoVoDx cells. Ultrasound exposure also influenced the expression of drug resistance-related genes, with the most significant reductions observed in ABCC1, ABCG2 and TOP2A mRNA levels up to 24 h post-treatment. Notably, microbubble-assisted sonoporation downregulated FOXO3, NFKB1 and NFKB2 transcription factors in both LoVo and LoVoDx cells.

Conclusions. This study reveals an effective approach for mitigating drug resistance in cancer cells through microbubble-mediated sonoporation. Ultrasound influences MDR-related protein levels, activity and gene expression, enhancing intracellular doxorubicin delivery in both sensitive and resistant colon cancer cells. The reduced Pgp levels and activity are attributed to ultrasound-induced alterations in membrane proteins and DNA damage during sonoporation, inhibiting transcription and translation.

Key words: sonoporation, ultrasound, multidrug resistance, colon cancer

Pulse burst compression for calcium electrochemotherapy of C57BL/6J carcinoma tumors

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Conflict of interest
None declared

Abstract

Background. Calcium electroporation (CaEP) is an innovative cancer treatment approach involving the internalization of higher-than-normal amounts of calcium through electroporation, resulting in cell death. It provides an alternative to conventional chemotherapeutics, such as bleomycin.

Objectives. In this study, for the elimination of carcinoma tumors, we introduce novel sub-microsecond pulsing protocols based on the pulse compression into MHz burst (4 kV/cm × 700 ns × 200, 1 MHz). The results are compared to the standard microsecond (μsCaEP) clinical protocol.

Materials and methods. In C57BL/6J mice, the tumor was induced by subcutaneously injecting 1×10^6 LLC1-Luc cells. When tumors reached ~100 mm^3, treatment was applied. Afterward, mice tumor growth dynamics and survival were assessed. Moreover, mice tumors, lymph nodes, spleens, and circulating blood were collected and used for further investigation with flow cytometry in order to characterize the immune response.

Results. Our study showed CaEP efficacy to eliminate tumors and increase mice survival rates. Additionally, calcium EP-based treatment resulted in an increased percentage of $CD4^+$ and $CD8^+$ central memory T cells and decreased splenic myeloid-derived suppressor cells (MDSC). Moreover, increased levels of antitumor IgG antibodies after CaEP treatment were detected.

Conclusions. The experimental results demonstrated that the administration of CaEP led to tumor growth delay as well as increased survival rates and stimulated immune response, indicating a potential synergistic relationship between CaEP and immunotherapy.

Key words: cancer, calcium, nanosecond, electroporation, immunology, immunomodulation, anticancer, in vivo

Effect of natural NF-κβ inhibitors on normal muscle cells and tumor cells of muscle origin: In vitro studies

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Conflict of interest
None declared

Abstract

Background. The NF-κB signaling pathway plays a crucial role in cancer progression, including muscle-derived cancers such as rhabdomyosarcoma or sarcoma. Several natural compounds have been studied for their ability to alter NF-κB signaling in these types of cancers.

Objectives. The aim of the research is to assess the therapeutic potential of selected natural cytostatic substances in the treatment of neoplastic diseases of muscle origin in cellular models.

Materials and methods. The cytotoxic effect of selected cytotoxic substances of natural origin was analyzed – cucurbitacin E (CurE), biochanin A, caffeic acid phenethyl ester (CAPE), berberine, and curcumin. Tests on the effect of the substance were carried out in vitro (on the control L6 muscle line and the tested line WEHI-164). The MTT test determined the viability of cells after treatment (24 h and 48 h) with the tested cytotoxic substances. This assay evaluates mitochondrial activity, which is a cell viability marker. The results were expressed as the percentage of viable cells relative to untreated control cells. Based on the results of the MTT test, the IC_{50} values were determined for each of the substances. Additionally, molecular docking simulation of cucurbitacin E (CurE), biochanin A and caffeic acid phenethyl ester (CAPE) to cytoskeleton proteins was performed using Swissdock software, and the results were evaluated in Chimera. Additionally, the comet assay was performed, which is a method of measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells.

Results. The MTT assay results show a stronger cytotoxic effect on WEHI-164 cells than on L6 cells. Our results indicate that CAPE, CurE and biochanin A demonstrated anticancer activity. Molecular docking showed that CAPE, CurE and biochanin A inhibit actin polymerization. Comet assay results showed more DNA damage in single WEHI-164 cells than in L6 after treatment with natural cytotoxic substances.

Conclusions. The results of the MTT test indicate that the analyzed substances have a strong cytotoxic effect on fibrosarcoma cells from the WEHI-164 line with a gentle effect on L6 control cells. In addition, the comet assay revealed an increased number of cells with damaged DNA in the case of WEHI-164 cells after treatment with natural cytostatics. The tested compounds are inhibitors of the NF-κβ factor and the cell cycle, and therefore can be considered in the treatment of muscle-derived tumors.

Key words: muscle cancers, NF-κβ, MTT test viability, molecular docking simulation, comet assay

Effect of tau protein phosphorylation on the process of its adsorption on the neuronal membrane

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Abstract

Background. The abnormalities in the tau protein are thought to initiate a pathological cascade in Alzheimer's disease (AD) manifesting as neuronal damage. Soluble tau oligomers (TauOs), which are formed in association with protein fibrils that are the main component of amyloid, are of great interest. The mechanism of tau transport across membranes has been analyzed and verified in many cell lines and animal models; nevertheless, the details, including the role of tau structure and its post-translational modification remain unclear.

Objectives. The phosphorylation at Ser262, 356 and 258 leads to non-fibrillary structures of the tau protein, which limits fibril growth and leads to TauOs. Using fully atomistic molecular dynamics, we study the interaction of a K18 monomer on the surface of a neuronal cell membrane with a lipid composition similar to the AD state. These simulations provide a detailed understanding of the interactions between the protein and membrane, explaining the crucial role of the degree of protein phosphorylation.

Materials and methods. Folding simulations of K18 form of tau AD protein (AD, PDB:7NRQ) were performed in the presence of a three-dimensional membrane model of the neuronal cell. Conformational changes of the tau protein and its adsorption ability were investigated for 3 models: wild-type (WT), hyperphosphorylated Tau (HP) and tau phosphorylated at 3 positions: Ser258, Ser262 and Ser356 (3P). The CHARMM-GUI tools were used for modeling and phosphate modifications. Simulations were prepared in Gromacs using a force field dedicated to intrinsically disordered proteins (IDPs).

Results. Our results demonstrated that phosphorylated tau, specifically the 3P variant, can adsorb to the membrane surface. The optimal degree of phosphorylation (3P) facilitates flexible domain penetration into the membrane while maintaining a balance between chain stiffness and charge. Excessive phosphorylation (HP) leads to electrostatic repulsion and prevents adsorption. Furthermore, the negatively charged lipids in the membrane do not stiffen the flexible domains through electrostatic interactions.

Conclusions. Our research focuses on elucidating how tau protein phosphorylation, particularly in the non-fibrillary 3P variant, influences its ability to adsorb to the neuronal cell membrane with an AD-like lipid composition. This methodology can be extended to study interactions between the neuronal membrane and various tau protein variants, potentially leading to a deeper understanding of TauO formation in AD.

Key words: Tau K18, TauOs, phosphorylations, Gromacs, MD, cluster analysis, lipoprotein profile

Study of the reaction of benzyl alcohol with atomic chlorine

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Funding sources
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Conflict of interest
None declared

Abstract

Background. The work is devoted to examining the stability of benzyl alcohol – a substance commonly found in cosmetics – on interaction with atomic chlorine, which is becoming more and more common as a result of pollution of the Earth's atmosphere. The reaction products of atomic chlorine with benzyl alcohol and their amounts in the post-reaction mixture are presented.

Objectives. The main objective of the work was to qualitatively and quantitatively identify reaction products formed in benzyl alcohol as a result of reaction with atomic chlorine generated in the gas phase.

Materials and methods. The reagents used are: benzyl alcohol for synthesis (Sigma-Aldrich), molecular chlorine >99.5% (Sigma-Aldrich) and molecular nitrogen 5.0 (Linde).

Results. In the post-reaction mixture, the following compounds have been identified: benzyl aldehyde in the amount of 0.64%, benzyl chloride in the amount of 0.62%, benzyl alcohol in the amount of 96.42%, dichloromethylbenzene with the amount <0.02%, 2-chlorobenzyl alcohol in the amount of 0.04%, 3-chlorobenzyl alcohol in the amount of 0.05%, dibenzyl ether in the amount of 1.65%, and benzyl benzoate in the amount of 0.31%. After reaction, the investigated sample became acidic.

Conclusions. The reaction produces benzyl chloride in amounts that may have a carcinogenic effect on the skin.

Key words: benzyl alcohol, chlorine atoms

Nanosecond pulsed electric fields: Reshaping immune checkpoints and cytokine landscape in melanoma cells

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Conflict of interest
None declared

Abstract

Background. Currently, the involvement of immune checkpoint receptors, including PD-1, LAG-3, and TIM-3, in suppressing the immune response in cancer is being investigated. Diverse strategies are being employed to address the challenge of studying the undesirable immune response.

Objectives. This study explored the impact of the nanosecond pulsed electric field (nsPEF) treatment on the expression of immune checkpoint receptors in melanoma cells (A375 and C32).

Materials and methods. Cell viability was evaluated using MTT and Presto blue® assays, while the permeability of cell membranes was gauged through the utilization of YoPro-1 dye. Holotomography microscopy was utilized to assess the influence of the nsPEF treatment on cellular structure. The effect of nsPEF on cytokine secretion was determined using the enzyme-linked immunosorbent assay (ELISA). The presence of PD-1, LAG-3 and TIM-3 antigens was investigated using the western blot technique. Additionally, confocal microscopy imaging was conducted to analyze alterations in the expression pattern of PD-1 and MHC-II in cells.

Results. Our findings demonstrated that the nsPEF treatment displayed considerable potential for augmenting cell membrane permeability and inducing morphological changes in the cell membrane, all without cytotoxic effects. We observed that the nsPEF treatment prompted the emergence of vesicles from within the cell to its exterior, cellular contraction, and the migration of lipids from the cell's interior to its periphery. This treatment elevated the expression of PD-1 molecules. Moreover, we also noted the possible co-localization or clustering of MHC class II and PD-1 molecules on the cell surface, along with the secretion of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6.

Conclusions. These findings suggest that the nsPEF treatment could be a viable approach to enhance the delivery of therapeutic agents to cancer cells and modulate the tumor microenvironment to promote an antitumor immune response. Further studies are needed to explore the mechanisms underlying these effects and their impact on the antitumor immune response, and investigate the potential of the nsPEF treatment in combination with immune checkpoint inhibitors to improve clinical outcomes for cancer patients.

Key words: melanoma, nsPEF, immune checkpoint receptors

Exploring the impact of electroporation-induced extracellular vesicles on melanoma cell biology

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Conflict of interest
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Abstract

Background. Electroporation (EP) is a technique used mainly in oncology, thanks to which we can influence the permeability of biological membranes. The selection of appropriate parameters helps to achieve a specific clinical goal, e.g., facilitates the delivery of drugs or even leads directly to the death of cancer cells. Electroporation itself is an exposure of the cell to stress, which reacts to the release of various types of transmitters, including extracellular vesicles (EVs). These cell-derived particles contain valuable, biologically active cargo, protected by their lipid bilayer membrane, that can be carried a long distance.

Objectives. Our main goal was to determine how EVs isolated from cells previously exposed to an electrical field affect other cells, including their ability to proliferate, migrate and be invasive.

Materials and methods. The research was conducted on 2 melanoma cell lines (A375 and Me45) and immortal keratinocytes (HaCaT). The main methods that allowed to evaluate the effect of EVs on the examined cells were the assessment of cell impedance and wound healing assays.

Results. The use of EP (800–1600 V/cm; 0.1-ms long; 1 Hz) resulted in the release of EVs that can slow down the cell growth and migration with different intensity depending on the tested cell line. The EVs isolated from non-treated cells showed no biological effect or the opposite effect than EVs from electroporated cells.

Conclusions. There was a significant slowdown in the proliferation and migration of cells after adding EVs isolated following EP to their medium, visible in all tested parameters. This phenomenon is called the 'bystander effect', which, in our case, was induced by EP. Cells exposed to electrical impulses may transmit a signal in the form of EVs to non-electroporated cells, thereby reducing the viability of non-treated cells.

Key words: electroporation, extracellular vesicles, melanoma

In vivo microscopy imaging of 2 model plants *Iris domestica* and *Scutellaria baicalensis* after pulse electric field (PEF) treatment

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Conflict of interest
None declared

Abstract

Background. Electroporation (EP) is a method of biological membrane permeabilization, i.e., creating pores in the cell membrane (plant, animal or bacterial cells) immersed in a conductive medium using electrical impulses.

Objectives. The aim of this work was to develop and optimize a pulsed electric field (PEF) treatment for the vital microscopy imaging and reversible EP from the roots and rhizome of *Iris domestica* (syn. *Belamcanda chinensis* L. DC. Iridaceae) and *Scutellaria baicalensis* L. cultivated in aeroponic system.

Materials and methods. Seven-week-old aeroponic cultivars of both plants were electroporated (4-mm gap cuvette) under applied different pulsed electric field strength of 1, 3 and 7.5 kV/cm, with constant pulse duration (50 µs) and repetition (n = 50) of individual pulse. Roots were electroporated in the presence of propidium iodide (PI) and directly imaged after the EP on the Leica SP8 confocal microscope. The PI was excited with a 552-nm laser line and the collected emission range was 560–680 nm. From 6 to 8 areas were imaged with volumes up to 146 µ m and a 2-µm Z step. Nuclei were marked with a paintbrush tool in a separate empty channel and, upon channel binarization, counted with the Analyze Particle function. Then, the plants were cultivated in aeroponic systems and observed over the next 4 months in comparison to control.

Results. Our study proved that live roots EP of 1 kV/cm and 3 kV/cm is reversible (plants survived and still grow for the next 4 months) but the EP of 7.5 kV/cm is non-reversible (not one plant survived). *Iris domestica* is less sensitive to PEF treatment than *S. baicalensis*.

Conclusions. We can conclude that 1 kV/cm electric field improved plants growing when compared to controls. A simple linear relationship of stained cell nuclei shown by confocal microscopy was proportional to the strength of the electric field and nuclear membranes were observed at 3-mm depth of the root.

Key words: electroporation, plant roots, confocal microscopy

Phytochemical screening of selected African medicinal plants identified for use against fibrosis tumor

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Abstract

Heavy ovarian bleeding may be because of presence of fibrosis tumors. The American Society for Reproductive Medicines endorsed a number of fertility fertilization inclusive of cryopreservation of embryo, mature oocyte ovarian tissue, etc. Even though usually benign, a uterine fibroid is essentially a tumor. In recent years, demand for fertility preservation for oncologic and nononcologic indications, as well as personal reasons, has risen dramatically and will prove a major challenge in the coming years.

In this study, preliminary phytochemical determination by chemical test reflects the presence of various bioactive compounds responsible for the therapeutic properties in medicinal plants such as Alkaloids, Phenolic compounds, Terpenoids, and proteins. A subsequent Thin Layer Chromatography profiling of these plants were carried out using different solvents of extracts and they revealed various compounds which were separated at different Rf value. *Gunnera Perpensa* (GunPer) and *Abizia tanganyicensis* (AbiTan) had a number of active compounds which could be associated with potency for minimizing fibrosis. This paper will discuss the findings for this research work.

Key words: bioactive compounds, phytochemical screening, fibrosis tumor

POSTER PRESENTATIONS

Non-psychoactive cannabinoids as potential inhibitors of tet1 protein

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None declared

Abstract

Background. Cannabinoids can modulate physiological processes by modulating specific cannabinoid receptors. Nowadays, market with products containing some of the non-psychoactive cannabinoids is expanding and it is necessary to study their biological properties and mechanisms of potential side effects. Cannabinoids could also cause harmful side effects due to the dysregulation of the epigenome, whereas some of these side effects could be explained by interference with TET1 protein activity.

Objectives. To study the cannabinoid effect on the activity of TET1 protein.

Materials and methods. Chelation ability of non-psychotropic cannabinoids (cannabidiol (CBD), cannabinol (CBN) and cannabigerol (CBG)) towards iron(II) ions using UV/Vis spectroscopy was studied. The inhibition of TET1 was determined using a fluorometric TET hydroxylase activity quantification kit. Molecular docking was performed with the CB-Dock web server.

Results. All tested cannabinoids displayed a strong affinity for Fe(II) ions; CBD and CBN exhibited potent inhibitory activities (IC50 = 4.8 μM and 6.27 μM) towards the TET1 protein, whereas CBG had no effect on the enzyme activity. An in silico molecular docking study revealed marked binding potential within the catalytic cavity for CBD/CBN, but some affinity was also found for CBG; thus, the total lack of activity remains unexplained.

Conclusions. We observed that the CBD and CBN, which exhibit affinity for Fe(II) ions, displayed a potent inhibitory effect on the TET1 protein, based on their Fe(II) affinity. We used molecular docking for in silico studies. Data showed different binding between CBD, CBN and CBG and suggest a higher potential of CBD and CBN to interact with amino acid residues in the active center. The determined IC50 values imply that under chronic exposure, some of the non-psychoactive cannabinoids could cause a reduction in TET1 protein activity.

Key words: cannabinoids, TET1 protein inhibitors, iron chelation

In vitro studies on the activity of terpenes present in *Cannabis sativa L.* against colorectal cancer stem cells

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Abstract

Background. Cancer stem cells (CSCs) play a key role in the formation, progression, metastasis, and recurrence of cancers, including colorectal cancer. Current chemotherapies rapidly eliminate proliferating tumor cells, but are not effective against CSCs. Potential good candidates for targeted drugs are compounds of natural origin, due to their ability to simultaneously affect multiple signaling pathways involved in cancer cell function and survival. Promising compounds are *Cannabis* terpenes.

Objectives. The objective of this study was to evaluate the efficacy of BOR and GUA in eliminating tumor cells and CSCs in colorectal cancer. We aim to assess the effects of the compounds alone and their combinations with irinotecan (IRY), a standard cytostatic used in the treatment of colorectal cancer.

Materials and methods. In vitro studies were conducted using colon cancer cells with standard sensitivity to cytostatics (LOVO cell line) and a cellular model of aggressive and resistant colon cancer enriched in CSC – LOVO/DX. The 2D and 3D cell cultures and Presto Bleu were used to study anticancer effects. The CSCs were isolated based on MACS technology using anti-CD44 and anti-CD133 antibodies conjugated with magnetic beads.

Results. The BOR and GUA alone induced 4–86% and 5–85% cytotoxicity of LOVO cells and 3–14% and 1–39% cytotoxicity of NKM-enriched LOVO/DX cells. In LOVO cells, BOR increased the cytotoxic effect of IRY by 11–23% and GUA by 8–54% depending on concentration. The combination of GUA and IRY reduced LOVO/DX cell viability by 5–52% after a 48-hour incubation. The combination of BOR and IRY did not show increased cytotoxicity against LOVO/DX cells. In 3D culture studies, all combinations of BOR and GUA with IRY showed 39–84% better efficiency than IRY in $CD133^+$ spheroids, and 6–74% better efficiency in $CD44^-$, $CD44^+$ and $CD133^-$ spheroids. Irinotecan at concentrations of 5 μM and 10 μM caused a reduction in the number of colonies by 63–82%, 53–77% and 50–74% in whole populations, $CD44^+$ and $CD133^+$ subpopulations, respectively. The BOR and GUA more strongly inhibited colony formation up to 23–26% (BOR) and 11–21% (GUA) compared to IRY. The combination of BOR/GUA with IRY showed a significantly better antiproliferative effect than IRY alone, reducing the number of colonies to 8–19% (BOR) and 7–16% (GUA).Combinations of BOR or GUA with IRY induced apoptosis of 42–61% of LOVO cells.

Conclusions. The results of the study indicate significant anticancer properties of BOR and GUA against colorectal cancer cells, including CSCs. Both BOR and GUA enhance the anticancer effects of IRY.

Key words: cancer stem cells, flow cytometry, colon cancer, *Cannabis sativa*, terpenes

The influence of microplastic particles on the development of breast cancer cells

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Conflict of interest
None declared

Abstract

Background. Microplastic particles (MPs), ranging in size from 0.1 µm to 5 mm, pose a significant threat to the natural environment. They are created as a result of human activities, both during the production of certain products and the degradation of plastic materials. Breast cancer is the most common form of malignancy and the 2nd leading cause of cancer-related deaths among women. The relationship between microplastic particles and breast cancer is a subject that requires further scientific investigation.

Objectives. The aim of the project is to determine the cytotoxic impact of microplastic particles by examining the level of oxidative stress markers. Microplastic particles may affect cell morphology and intercellular communication as well as induce cell death. An intriguing aspect is to investigate the impact of microplastic particles on breast cancer cells compared to normal (non-cancerous) cells.

Materials and methods. Cytotoxic and oxidative stress mechanisms will be proposed as an effect of microplastic particle activity. Additionally, the impact of MPs on the antioxidant defense markers and cell death (apoptosis/necrosis) will be presented. The available literature data suggest that the interaction with MPs can also initiate changes in cell morphology, particularly in cancer cells.

Results. Components of plastics, such as bisphenols, including phthalates, heavy metals like cadmium, PFAS, or flame retardants, are all leached from microplastics. Many of these substances are endocrine-disrupting chemicals (EDCs), which can mimic estrogens at low concentrations and disrupt hormonal balance in the body, affecting the development and progression of hormone-dependent breast cancer types. Microplastic particles can also induce inflammation and oxidative stress in tissues, which may be associated with an increased risk of cancer development, including breast cancer.

Conclusions. Microplastic particles can interact with the immune system, potentially affecting its ability to detect and eliminate cancer cells. Disrupted immune function can impact breast cancer development.

Key words: microplastic particles, breast cancer

Exploring the relationship between antioxidant enzymes, oxidative stress markers and clinical data in relapsing–remitting multiple sclerosis

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None declared

Abstract

Background. The background of multiple sclerosis (MS) is complex and not entirely understood. There is considerable evidence suggesting that oxidative stress and imbalances in the pro/antioxidant system play a significant role in the neurodegenerative component underlying development and progression of the disease. Understanding the role of the pro/antioxidant profile in MS may encourage investigations into potential therapeutic approaches that target these pathways.

Objectives. Our study aimed to assess the alterations in specific oxidative stress parameters and antioxidant enzymes in the blood of patients with relapsing–remitting multiple sclerosis (RRMS). We also aimed to investigate the relationships between parameters of the pro/antioxidant balance and demographics and MS-related variables.

Materials and methods. A total of 161 patients with RRMS and 29 healthy individuals were included into the study. We measured the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) as well as the concentration of lipid peroxidation, total oxidant status and total antioxidant capacity using commercially available test or a procedure presented earlier. Analyzed measures were compared between MS patients and controls and referred to age, gender, MS duration, level of disability, and type of disease-modifying treatment (DMT).

Results. The activity of SOD did not show any significant differences between the study groups. In contrast, significantly decreased GPx activity and increased CAT activity were observed in the blood of patients with RRMS compared to the control group. We found a negative correlation between SOD and GPx activity as well as between GPx and CAT activity. Furthermore, a receiver operating characteristic (ROC) analysis (area under the ROC curve (AUC) = 0.831; p = 0.0000) indicated that the activity of GPx could be the most differentiating parameter between patients with RRMS and healthy subjects. Additionally, the activity of CAT showed the association with gender and the use of DMT. Significant relationships were also found between the type of DMT and total oxidative stress, lipid peroxidation and total antioxidant capacity value.

Conclusions. The alterations in pro-oxidative capacity are demonstrated in patients with RRMS. The enhanced GPx activity may be more beneficial to provide potential therapeutic strategies aimed at modulating antioxidant defense.

Key words: relapsing–remitting multiple sclerosis, pro/antioxidant balance

Higher level of novel advanced glycation end-product (AGE10) is observed in some complications of type 2 diabetes

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Conflict of interest
None declared

Abstract

Background. Advanced glycation end-products (AGEs), due to their stability and resistance to the action of enzymes, are considered potential markers of various metabolic diseases, e.g., type 2 diabetes (T2DM), and their complications. Although AGEs are formed in the reaction of reducing sugars or reactive alphaoxaldehydes with the amino groups of proteins, lipids and DNA, many of them remain unknown.

Objectives. To quantitatively analyze unique AGE10 epitope in T2DM patients' sera, the competitive enzyme-linked immunosorbent assay (ELISA) was developed by our team. The AGE10 has recently been identified in human serum using synthetic melibiose-derived AGE (MAGE).

Materials and methods. First, we have developed a competitive ELISA test in which the reaction of synthetic MAGE with anti-MAGE antibodies was inhibited by physiological AGE10, present in blood serum, or synthetic low molecular weight AGE10 (LMW-MAGE) used to prepare a standard curve. In this test, we used anti-MAGE monoclonal antibodies obtained by our team, which do not recognize previously known AGEs. Subsequently, we have quantified the concentration of AGE10 in patients with T2DM and micro- and macroangiopathies.

Results. The concentration of AGE10 was significantly higher in patients with microangiopathies in comparison to sera of patients without microangiopathies (158 µg/mL compared to 98 µg/mL), but it depended on the treatment method – a lower level of AGE10 was observed in patients with microangiopathies treated with aspirin. The AGE10 concentration correlated positively with the estimated glomerular filtration rate ($r = 0.34$, $p = 0.005$). The AGE10 as a marker for chronic kidney disease or microangiopathy showed moderate overall accuracy (69% and 71%, respectively) and good sensitivity (82.6% and 83.3%, respectively), but poor specificity (58.1% and 57.8%, respectively).

Conclusions. The level of AGE10 can be determined using a competitive ELISA test. Microangiopathy is associated with AGE10 elevation, which, however, can be reduced using aspirin.

Key words: advanced glycation end-products (AGEs), type 2 diabetes (T2DM), microangiopathy, aspirin

The possible outcome of variable saturation on the morphological, inflammatory and biochemical changes of blood parameters of patients with COVID-19 who did not undergo pre-hospitalization oxygen therapy, in context of the incidence of type 2 diabetes/insulin resistance

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Abstract

Background. To date, 770 million cases of coronavirus disease 2019 (COVID-19) have been diagnosed worldwide, of which almost 7 million have resulted in death. A more severe course of COVID-19 (and higher mortality) was observed among patients with type 2 diabetes. This was due to a weakened immune system, associated with defects in both the innate immune response, such as neutrophil dysfunction, and a defect in the adaptive immune response. Moreover, it has been noticed that in some people, COVID-19 causes damage to pancreatic cells and disturbances in carbohydrate metabolism.

Objectives. The aim of the study was to check the influence of pre-hospitalization oxygen therapy on the values of morphological, inflammatory and biochemical parameters of the blood serum of patients with type 2 diabetes/insulin resistance compared to the controls, in the course of COVID-19.

Materials and methods. The presented retrospective study was based on medical documentation from the COronavirus in the LOwer Silesia (COLOS) registry, which contained the records from 2139 patients admitted and treated at the university and temporary COVID-19 hospital organized by the University Medical Hospital in Wrocław (Poland) in the period from February 2020 to June 2021. The study group was further divided according to a 3-stage scale, taking into account type 2 diabetes/prediabetes, the use of prediabetic oxygen therapy, and in the absence of this oxygen therapy – the value of saturation (division criterion: saturation <95%). Statistical analysis was performed with use of the Statistica v. 13.3 package (StatSoft Poland, Kraków, Poland).

Results. The study group consisted of 2139 COVID-19 patients – 1076 women (50.30%) and 1063 men (49.70%). The mean age of patients was 63.73 ±15.69 years. Among 2139 COVID-19 patients, 473 (22.11%) suffered from diabetes type 2/prediabetes. Among patients with no documented pre-hospitalization oxygen therapy, the diabetic patients with $spO_2 < 95\%$ showed a significant increase of: D-dimer (95% compared to 60%), procalcitonin (76% compared to 35%), albumin (95% compared to 55%), lymphocytes (52% compared to 32%), RDW-SD ≥ 47 (35% compared to 29%), potassium (40% compared to 20%), creatinine (62% compared to 42%), and troponins (82% compared to 60%) compared to diabetic patients with $spO_2 \geq 95\%$. In the same strata (no pre-hospitalization oxygen therapy), the group with no diabetic disorders and $spO_2 < 95\%$ showed a significant increase in: IL-6 (32% compared to 16%), CRP (90% compared to 65%), albumin (75% compared to 43%), lymphocytes (60% compared to 40%), RDW-SD ≥ 47 (30% compared to 21%), glucose (82% compared to 65%), potassium (22% compared to 10%), sodium (25% compared to 10%), creatinine (30% compared to 20%), and ALAT (35% compared to 20%) compared to patients with no diabetic disorders and with $spO_2 \geq 95\%$.

Conclusions. Lower saturation was associated with an increase in potassium and glucose levels among patients who did not undergo any oxygen therapy before hospitalization due to COVID-19. This may have been caused by damage of pancreatic β-cells by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and alterations within the potassium channel, which determines cell membrane depolarization and insulin secretion.

Key words: type 2 diabetes, COVID-19, glucose, potassium

Tumorigenic potential of electroporation-generated extracellular vesicles

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Abstract

Background. The cell membrane can be permeabilized when subjected to calibrated short electric pulses. This membrane alteration can be reversible, leaving cell viability unaffected. This set of events is called electroporation (EP). Electroporation is used in clinical applications to introduce hydrophilic drugs into the cytoplasm. One of the EP applications is electrochemotherapy (ECT), in which EP is used for the selective delivery of drugs used to treat cancer. The combination of EP with chemotherapy allows for local cancer treatment, lowering the drug dose and reducing the side effects of systemic chemotherapy.

Objectives. Analyzing the EP phenomenon and the objective complexity of the associated effects at the cell level, we came across a problem that still needs to be investigated in increasing the therapeutic effectiveness of ECT. Until now, there has been no available analysis of the profile and kinetics of extracellular vesicles (EVs) released from cells subjected to EP. It also needs to be clarified how the profile of the released EVs depends on the pulse duration.

Materials and methods. The studies analyzed the effect of melanoma EVs on the mediated transformation of normal fibroblasts into tumor-associated fibroblasts. For experiments, various reversible EP parameters were used. The level of expression of the vascular cell adhesion molecule-1 (VCAM-1) and changes in phosphohistone H3, which is specific for cells in mitosis, were assessed. Cell viability and migration capacity were also analyzed. Extracellular vesicles isolated from 2 melanoma cell lines (A375 and Me45) were used in the analyses. The investigations were performed on a primary human gingival fibroblast cell line (HPFs).

Results. The experiments showed that EVs released from cells of a metastatic melanoma line induce a more substantial pro-carcinogenic effect when released from cells without prior EP procedure. In contrast, in the case of a melanoma cell line originating from a primary focus, EVs show a strong tumor-stimulating effect when released from cells after an earlier EP.

Conclusions. The observed changes in the degree of proliferation and migration of fibroblasts, as well as markers related to the induction of the neoplastic process, suggest that further studies on the influence of reversible EP on the properties of EVs released from tumor cells are significant and necessary.

Key words: electroporation, extracellular vesicles, melanoma, fibroblasts, VCAM-1, phosphohistone H3

An in vitro study of the impact of APRF+ on autogenous gingival fibroblasts

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Conflict of interest
None declared

Abstract

The study assessed the impact of the action of advanced platelet-rich fibrin (A-PRF+) on autologous gingival fibroblasts. Fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), transforming growth factor beta 1 and 2 (TGFβ1 and TGFβ2), and soluble collagen released from A-PRF+ conditioned with autogenous fibroblasts levels were evaluated. The A-PRF+ combined with fibroblasts displayed significantly higher values of released VEGF at every time point and, after 7 days, significantly higher values of released TGFβ2. The exposition to the factors released from A-PRF+ combined with fibroblasts activated an increase in proliferation fibroblasts after 72 h and rate of wound closure after 48 h. These results imply that platelet-rich fibrin (PRF) enhanced the regenerative effect of fibroblasts.

Key words: A-PRF+, TGFβ2, VEGF, fibroblast culture, wound healing

Identification of novel disease modulators of Huntington's disease: Application of direct reprogramming-based in vitro model derived from patients

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Abstract

Background. Neurodegenerative diseases (NDDs) are devastating age-related disorders, which despite substantial efforts remain largely incurable. Huntington's disease (HD) is the most common monogenetic NDD, and is caused by the expansion of triplet repeat of CAG within exon 1 of the huntingtin gene (*HTT*). The mutation leads to the polyglutamine (polyQ) expansion within HTT that results in misfolding and aggregation of the protein and subsequent neuronal death. The accumulation of misfolded huntingtin in a cell points to a disruption of a protein quality control (PQC) system in HD.

Objectives. Since PQC plays a key role in the protection against misfolding proteins, enhancing PQC systems could serve as an attractive therapeutic strategy in HD. In line with that, we have previously shown the beneficial effect of boosting PQC in a mouse model of HD.[1] The goal of our study is to identify novel potential disease modifiers of HD within the PQC space.

Materials and methods. We will evaluate the effect of the gain or loss of function of preselected candidates on disease phenotypes in vitro and in vivo. We will implement a model based on direct reprogramming of patient-derived fibroblast into medium spiny neurons (MSNs) that maintain the genetic and epigenetic background of HD. In vitro results will be validated in an organismal context in HD mouse model.

Results. We have established a cell reprogramming protocol and obtained MSNs that express cell type-specific markers. In the following steps, neurons will be produced from HD patients' cells and healthy controls. Next, we will assess the effects of genetic manipulation of selected candidates on disease phenotypes. The most effective modifiers will be studied in vivo.

Conclusions. We expect to reveal novel factors capable of modifying the pathological phenotypes of HD, with a potential to be further explored for therapeutic purposes in HD.

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Key words: Huntington's disease, neurodegeneration, directly reprogrammed neurons, protein quality control

Evaluation of the effect of the antioxidant and anti-inflammatory activity of luteolin on the viability and level of free radicals in normal and cancer cell lines stimulated with PMA

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Abstract

Background. Luteolin, belonging to flavonoids, is widely recognized as a substance with anti-inflammatory and anti-cancer properties; it can also "sweep" free radicals. Disturbance of homeostasis of redox processes causes oxidative stress, which is associated with many diseases, e.g., cardiovascular diseases and rheumatoid arthritis.

Objectives. The study aimed to evaluate the effect of luteolin on survival and to evaluate the antioxidant effect of luteolin on normal and cancerous human cells stimulated by the Phorbol 12-myristate 13-acetate (PMA).

Materials and methods. Human cell lines – fibroblast synoviocytes (SW982), chondrocytes (TC28a2) and an osteosarcoma line (MG-63) – were used in the study. Luteolin (concentrations 5–20 µM) was administered in 3 combinations: 1) luteolin alone; 2) pre-incubation with PMA followed by luteolin; and 3) simultaneous administration of PMA and luteolin. After 6 h, 24 h and 48 h of incubation, MTT tests were performed to assess cell survival. The fluorometric method (DCF-DA test) was used to test the level of reactive oxygen species (ROS), and the Griess test was used to assess the concentration of nitric oxide metabolites.

Results. It was observed that preincubation of cells from all cell lines with PMA inhibited their viability, and the simultaneous administration of PMA and luteolin caused less inhibition of cell survival compared to the preincubation with PMA. Luteolin in each combination reduced ROS proportionally to the concentration used in all tested lines. In the Tc28a2 and SW982 cell lines, a greater reduction in ROS was observed after PMA stimulation compared to the administration of luteolin alone. It was shown that, regardless of the tested cell line and incubation time, there were no differences in the concentration of NO metabolites after treating cells with luteolin (stimulated and unstimulated cells).

Conclusions. The obtained results prove the antioxidant activity of luteolin, and further research will help explain the mechanism of action of luteolin.

Key words: antioxidant activity, luteolin, PMA, cells

Evaluation of microbiological purity of autogenous dental material: A preliminary study

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Conflict of interest
None declared

Abstract

Background. In recent years, there has been an increased interest in alveolar bone regeneration using autogenous ground dental material. Teeth are usually extracted for surgical or orthodontic indications. Autogenous material is believed to contain living cells and valuable proteins, necessary for osteogenesis. Properly prepared hard tissues of the patient's tooth contain a biocompatible dentin matrix characterized by high histological and biochemical similarity to bone tissue. At the same time, the oral cavity contains diverse bacterial flora, which is associated with contamination of teeth intended for autotransplantation. However, appropriate biochemical preparation of the material to be transplanted can contribute to obtaining microbiologically pure autogenous material.

Objectives. The aim of this study was to check the microbiological purity of ground dental hard tissue after chemical processing in DentinCleanser solution.

Materials and methods. Tooth samples were obtained from 22 patients. Each tooth was treated separately – prepared immediately after extraction with aseptic maintenance. Autogenous material was prepared according to the protocol recommended by the manufacturer of The Smart Dentin Grinder (KometaBio, Fort Lee, USA). The extracted teeth were mechanically cleaned and ground in a grinder. The material from each sample was divided into 2 equivalent portions (A and B). Portion A was biochemically processed for 10 min in DentinCleanser solution, while portion B was chemically untreated. Subsequently, both samples were transferred to a previously prepared culture medium (10 mL of Thioglycollate Fluid Medium (Biomaxima, Lublin, Poland)) for microbiological examination. Incubation was carried out for 10 days at 37°C. The samples were then screened onto solid media for aerobic bacteria (Columbia agar supplemented with 5% sheep blood) and anaerobic bacteria (Schedler agar supplemented with 5% sheep blood). The obtained isolates were subjected to identification using the MALDI-TOF MS technique (Bruker Daltonik, Bremen, Germany).

Results. Aerobic bacterial growth was obtained from samples of 22 teeth in 20 cultures. In 9% of the ground tooth preparations prepared for autotransplantation, there was complete microbiological purity in both A and B samples. In 59% of sample A, there was a complete reduction in the presence of microorganisms compared to sample B. Twenty bacterial strains were cultured, belonging to 8 genera: *Enterococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Kocuria*, *Rothia*, *Bacillus*, *Micrococcus*, and 2 fungal strains (*C. albicans* and *C. glabrata*). The bacteria most commonly found in cultures are: *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus epidermidis*, and *Lactobacillus rhamnosus*.

Conclusions. The results of the qualitative microbiological tests show the presence of bacterial strains prior to biochemical processing. Thanks to the processing, a complete reduction of microorganisms was achieved in most samples. The actions at each stage of the procedure to prepare the tooth for autografting – from cleaning the tooth through grinding to biochemical processing – must be very accurate.

Key words: autogenous dentinal granulate, dentinal grinder, augmentation, microbiological purity

Calcium electroporation in pancreatic cancer in vitro

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Conflict of interest
None declared

Abstract

Background. Pancreatic cancer was the 7th leading cause of cancer death in 2020, with a less than 10% 5-year survival rate. It often remains asymptomatic until reaching an advanced, aggressively metastatic stage, and surgical resection is the sole curative option in early stages. Irreversible electroporation (EP) treatment provides new hope for patients with unresectable tumors.

Objectives. Our study aims to determine the efficacy of calcium electroporation (CaEP) in vitro. This innovative method combines a non-pharmacological approach (EP) with calcium ion administration upon EP protocols.

Materials and methods. We used the HPAF II and BxPC-3 cell lines as an in vitro model for pancreatic cancer. The following EP protocols were used: 3.5 kV/cm × 900 ns × 100, 1 kHZ; 5.7 kV/cm × 600 ns × 100, 1 kHz; 5.7 kV/cm × 900 ns × 100, 1 kHz; compared to clinical standard ESOPE (1.3 kV/cm × 100 µs × 8). All protocols were combined with 2.5 mM calcium chloride ($CaCl_2$) in a HEPES-based buffer, and cell viability was determined using the MTT assay after 48 h and 72 h. The cell membrane permeabilization rate was measured with flow cytometry using a cell-impermeant dye Yo-Pro-1. Additionally, pro-inflammatory cytokines (IL-6 – Hs00174131_m1, IL-8 –Hs00174103_m1) and anti-inflammatory cytokine (IL-10 – Hs00174086_m1) gene expression was quantitatively assessed in HPAF II cells, 72 h after EP protocols using real-time reverse transcription polymerase chain reaction (RT-PCR).

Results. The obtained results highlight a significant anticancer effect following calcium EP. Compared to EP alone, the addition of calcium ions led to a notable reduction in cell viability, reaching 20% of control levels after 48 h and approx. 30% after 72 h. The most effective protocol was observed at 5.7 kV/cm × 900 ns × 100, which yielded results comparable to those of ESOPE. As the electric field intensity increased, cell permeabilization also rose. Although we observed changes in cytokine mRNA expression levels, further research is necessary.

Conclusions. Our research indicated an anticancer potential of calcium EP in pancreatic cancer. It represents a groundbreaking contribution in the molecular and cellular mechanisms, as there are limited reports on calcium ion EP ($CaCl_2$) in pancreatic cancer.

Key words: pancreatic cancer, calcium ions, electroporation, cell viability

Recovery from COVID-19 and changes in MDSC subpopulations in children and adolescents with trisomy 21

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Conflict of interest
None declared

Abstract

Background. Trisomy 21 is caused by the presence of an extra chromosome 21, resulting in certain clinical features recognized as Down syndrome (DS). A higher incidence of metabolic syndrome and its complications, including atherosclerosis and hormonal disorders, has been observed in people with this condition. It has also been reported that patients with Down syndrome have an impaired immune system. Disproportions of lymphoid subpopulations isolated from peripheral blood have been observed, with recognized particular cellular dysfunction. Altogether, it can result in a higher incidence of systemic infectious diseases, particularly viral respiratory infections, and increased hospitalisation rates.

Objectives. The aim of the performed study was to investigate the number of immune system cells in children and adolescents with trisomy 21, and to assess the presence of myeloid-derived suppressor cell (MDSC) subpopulations (including granulocyte-like myeloid-derived suppressor cells – $CD11^+CD14^-$ and monocytic myeloid-derived suppressor cells – $CD11^+CD14^+$) depending on whether or not the patient has undergone a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Materials and methods. The study included 42 patients with Down syndrome (mean age: 13 years) and 21 patients in the control group (mean age: 14 years). The analysis of the results was conducted with a history of SARS-CoV-2 infection (24 patients with DS and 6 from the control group) and without it (18 patients with DS and 15 from the control group). Subpopulations of MDSCs from peripheral blood were determined with cell immunophenotyping and analysis using flow cytometry. The study was approved by the Bioethics Committee of Wroclaw Medical University.

Results. Individuals with Down syndrome who have undergone SARS-CoV-2 infection had a higher average amount of granulocyte-like myeloid-derived suppressor cells (G-MDSCs, $CD11^+CD14^-$ in COVID-19 patients; mean 1.831% of all MDSCs in patients with DS and mean 0.940% of all MDSCs in the control group) and monocytic myeloid-derived suppressor cells (M-MDSCs, $CD11^+CD14^+$ in COVID-19 patients; mean 0.113% of all MDSCs in patients with DS and mean 0.043% of all MDSCs in the control group) than those with DS who have not undergone the infection.
In individuals without DS, the inverse relationship could be observed – a higher average amount of G-MDSCs and M-MDSCs occurred in individuals who have not experienced COVID-19 than in those who have undergone SARS-CoV-2 infection (G-MDSCs, $CD11^+CD14^-$ in non-COVID-19 patients – mean 1.697% of all MDSCs in patients with DS and mean 1.325% in the control group; M-MDSCs, $CD11^+CD14^+$ in non-COVID-19 patients – mean 0.087% of all MDSCs in patients with DS and mean 0.073% of all MDSCs in the control group).

Conclusions. The study concluded that there are changes in MDSC subpopulations and that their determination may have clinical significance. However, the impact of SARS-CoV-2 infection on the immune system is not fully understood and requires further study and thorough research.

Key words: Down syndrome, trisomy 21, COVID-19, SARS-CoV-2, viral infection, immune system, MDSC, G-MDSC, M-MDSC, children, adolescence, flow cytometry

Synthesis of new derivatives of pyrazolopyridothiazinylacetohydrazide with potential pharmacological activity

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Abstract

Background. Currently, the trend of hybridization of various pharmacophore systems is of great importance in medicinal chemistry. Combining 2 active structures into 1 molecule can result in their synergistic effect, allowing to obtain a derivative with even higher activity. According to the literature, 1,2,4-triazole-3-thione derivatives have a variety of pharmacological effects, e.g., analgesic, anti-inflammatory, antimicrobial, and anticancer. Also, compounds based on the structure of *N*-Mannich bases are characterized by multidirectional biological activity.

Objectives. Our goal was to synthesize the hybrids containing pyrazolopyrydothiazine core and 2 active pharmacophores: 1,2,4-triazole-3-thione ring and phenylpiperazine fragment.

Materials and methods. The starting material for the synthesis was 2-(7,9-dimethyl-5,5-dioxido-3-pyrazolo[4,3-*c*]pyrido[3,2-*e*][1,2]thiazin-4(2*H*)-yl)acetohydrazide, which was obtained by the general procedure developed in the Department of Medicinal Chemistry of Wroclaw Medical University. Hydrazide was condensed with phenyl isothiocyanate in ethanol and then cyclized to 4-phenyl-1,2,4-triazole-3-thione. The formation of final *N*-Mannich bases was achieved via a convenient and efficient one-step reaction with appropriate phenylpiperazine derivatives and formaldehyde in ethanol. The structure of the new molecules was confirmed with spectral data analysis such as ^{1}H NMR, ^{13}C NMR, FT-IR, and MS.

Results. New derivatives of *N*-Mannich bases containing the 4-phenyl-1,2,4-triazole-3-thione ring in the structure were obtained with good yield. The spectroscopic properties of all newly synthesized derivatives were in good agreement with their predicted structures.

Conclusions. Currently, pharmacological research is underway to determine the activity of new compounds. In addition, we plan to synthesize analogous compounds using other isothiocyanates to replace the phenyl substituent with others in the 1,2,4-triazole-3-thione ring and be able to determine the structure-activity relationship.

Key words: pyrazolopyrydothiazine, 1,2,4-triazole-3-thione, *N*-Mannich base, spectral data

Isolation and biochemical characterization of cysteine protease inhibitor from salsify (*Tragopogon porrifolius*)

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None declared

Abstract

Background. In plants, papain-like proteases are involved in many physiological processes, including seed germination, programmed cell death, leaf senescence, and plant responses to biotic and abiotic stress. Such complex functions require precise regulation of their activity. One way of this regulation is the presence of appropriate protease inhibitors.

Objectives. The purpose of the study was the isolation and biochemical characterization of cysteine protease inhibitor from salsify (*Tragopogon porrifolius*).

Materials and methods. The inhibitor was isolated from salsify roots according to the method based on affinity chromatography with immobilized S-carboxymethylated papain. Protein concentration was estimated using the BCA method. The final preparation was aliquoted and lyophilized for long-term storage. Inhibitor quality and purity were assessed with regard to the antipapain activity against Nα-Benzoyl-DL-arginine β-naphthylamide (BANA) and SDS-PAGE in 12% resolving gel under reducing conditions. Proteins were visualized with the use of colloidal Coomassie Brilliant Blue. Kinetic study was performed with Z-Phe-Arg 7-amido-4-methylcoumarin hydrochloride as a substrate using Barrett method.

Results. The inhibitor was isolated from salsify roots with a yield of 7%. Its basic biochemical properties were determined: molecular weight (MW) = 26 kDa, isoelectric point (pI) = 6.0 and papain inhibition constant Ki = 0.1 nM.

Conclusions. This is the first report of phytocystatin isolated from *Tragopogon porrifolius*. Our findings revealed that it is one of the most potent cysteine protease inhibitors, with Ki value of 0.1 nM.

Key words: phytocystatin, cysteine protease inhibitor, *Tragopogon porrifolius*

Design of dual DNA gyrase/dihydrofolate reductase inhibitors as antibacterial agents: Molecular modeling study

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Conflict of interest
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Abstract

Background. Bacterial resistance to antibiotics has become a very important issue in recent years due to the mutation in microorganisms. Bacterial resistance leads to a reduction of effectiveness of existing drugs and causes a major global public health crisis.[1] One of the most important molecular targets for antibacterial drugs are DNA gyrase and dihydrofolate reductase enzymes. The first one plays an important role in bacterial cell viability, mainly due to its impact on DNA replication and background of negatively supercoiled DNA during this process.[2] Another protein, dihydrofolate reductase, is responsible for the reduction of dihydrofolate to tetrahydrofolate, and is involved in transcription and translation of nucleic acids.[3]

Objectives. The main goal of the present study was to design dual DNA gyrase/dihydrofolate reductase inhibitors as potential antibacterial agents.

Materials and methods. We used the virtual screening strategy, which consists of pharmacophore-based virtual screening and molecular docking, to design a series of small-molecule inhibitors. The pharmacophore was obtained with the use of ZINC database and structures of well-known inhibitors of both DNA gyrase and dihydrofolate reductase. Next, designed compounds were docked to *Staphylococcus aureus* DNA gyrase (pdbID: 6FM4) and dihydrofolate reductase (pdbID: 5JG0). Biovia Discovery Studio Visualizer was used to analyze the docking results. Finally, all compounds were analyzed in terms of their physicochemical properties and ADMET parameters using ADMETlab 2.0.

Results. The designed pharmacophore consists of 8 pharmacophore features such as hydrophobic groups, hydrogen bonds acceptors and donors, which are necessary due to the nature of binding cavity of both enzymes. In the case of inhibition of dihydrofolate reductase, an important role is played by hydrogen bonds between Ile 7, Glu 30, Asn 64, and Arg 70 amino acid residues and potential inhibitors. The DNA gyrase enzyme is able to form 2 hydrogen bonding interactions trough Ser 1084 with reference drugs (ciprofloxacin), which seems to be crucial in inhibition mechanism. As indicated by the results, most of the designed inhibitors can bind to the both protein binding centers via desirable type of interactions. The predicted physicochemical and pharmacokinetic properties of inhibitors show their drug-likeness and allow for considering them as candidates for the discovery or development of new drugs.

Conclusions. We have proposed very promising candidates for antibacterial drugs. However, we are planning further studies based on molecular dynamics that provide information on the dynamics of interactions between the proposed inhibitors and the molecular targets considered in this project.

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Key words: computer-aided drug design, antibacterial agents, antibiotic-resistant *Staphylococcus aureus*

PEF application enables extraction of phytochemicals from *Scutellaria baicalensis* roots to a DES medium

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Abstract

Background. Exposing biological tissue to pulsed electric field (PEF), after properly adjusting its parameters, leads to cell electroporation (EP). Increased porosity of the membrane results in increased exchange of intra- and extracellular compounds, enabling extraction of compounds from plant tissue.

Objectives. Our aim is to successfully combine PEF and deep eutectic solvents (DES) in extraction of phytochemicals and optimize the treatment parameters in order to ensure plant survival and process repeatability.

Materials and methods. Aeroponically cultivated 3-months-old *Scutellaria baicalensis* (Sb) was used as a model plant in this study. Two variants of PEF were applied: E = 0.5 kV/cm, N = 100, f = 1, Hz t = 100 µs (A); and E = 0.5 kV/cm, N = 200, f = 10 Hz, t = 50 µs (B), where E – electrical field strength, N – number of pulses f – frequency, t – pulse length. The following solvents were used: choline chloride:glucose (1:2) + 30% water; choline chloride:ethylene glycol (1:2); choline chloride:fructose (1:2) + 30% water; choline chloride:saccharose (1:2) + 40% water; tap water. After pulse administration to the plants' roots, the medium was collected from the cuvette and DPPH tests were conducted, using baicalin as reference. The change in electrical conductivity of solvents was noted and membrane disintegration index Z was calculated.

Results. There are statistically significant differences in medium conductivity and antioxidant activity between samples, being generally higher for PEF treatment B. The highest disintegration index Z (40%) was noted for a DES mixture of choline chloride:glucose (1:2) + 30% distilled water, and the lowest when using tap water (11%). None of the treated specimens survived the process.

Conclusions. The PEF-assisted extraction of Sb flavonoids was successful and its efficiency varies depending on DES composition and treatment parameters.

Key words: pulsed electric field, electroporation, deep eutectic solvents, root phytochemicals extraction, *Scutellaria baicalensis*, flavonoids, baicalin

Evaluation of the antitumor activity of liposomal formulations of lysosomotropic surfactants

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Abstract

Background. Both melanoma and human colorectal adenocarcinoma are cancers with high mortality rates and low efficacy of current therapy.

Objectives. The aim of this study was to investigate the anticancer activity of selected lysosomotropic surfactants and their liposome forms against cancer cell lines.

Materials and methods. Three lysosomotropic surfactants, namely DMM-11, DMPM-11 and DMGM-14, were used in this study. Melanoma A375, adenocarcinoma HT-29, and Normal Human Dermis Fibroblast (NHDF) cells were exposed to lysosomotropic surfactants, and cytotoxic effect was evaluated with an MTT assay. On the other hand, the wound healing assay was used to evaluate cell migration ability and the qRT-PCR assay was used to assess the ability to induce apoptosis.

Results. The MTT assay showed significant inhibition of the viability of cancer cell lines. The IC_{50} doses for A375 were determined to be 0.01875 mg/mL (DMM-11), 0.0156 mg/mL (DMPM-11), 0.00195 mg/mL (DMGM-14) and 0.35 mg/mL (DMM-11), 0.425 mg/mL (DMPM-11) and 0.4375 mg/mL (DMGM-14) for those encapsulated in liposomes. For HT-29, the IC_{50} doses were determined to be 0.125 mg/mL (DMM-11), 0.09 mg/mL (DMPM-11), 0.0156 mg/mL (DMGM-14) and 0.125 mg/mL (DMM-11), 0.125 mg/mL (DMPM-11) and 0.0156 mg/mL (DMGM-14) for those encapsulated in liposomes. In the case of NHDF lines, the use of liposome forms significantly reduces their mortality. For NHDF, the IC_{50} values were 0.09 mg/mL (DMM-11), 0.1 mg/mL (DMPM-11) and 0.0078 mg/mL (DMGM-14), and for the encapsulated form they were 0.350 mg/mL (DMM-11), 0.6 mg/mL (DMPM-11) and 0.2375 mg/mL (DMGM-14). A qRT-PCR assay showed at least a two-fold increase in the characteristic apoptotic protein Bax compared to the untreated control. The migration assay showed significantly decreased migration at 24 h and 48 h after lysosomotropic surfactant treatment for both A375 and HT-29 cell lines.

Conclusions. Novel liposomes composed of phosphatidylcholine (PC) and lysosomotropic surfactants (DMM-11, DMPM-11 and DMGM-14) were produced, and inhibitory effects of surfactants on the growth of melanoma A375 and adenocarcinoma HT-29 cells leading to apoptosis were obtained. Moreover, encapsulation decreased their toxicity to NHDF cells.

Key words: lysosomotropic agents, surfactants, MTT, apoptosis, Bax and Bcl-2 proteins, cell migration

Physicochemical properties and biological activity of dendrimer complex with fluorouracil

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Conflict of interest
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Abstract

Background. Novel therapies rely on reliable drug delivery, and drug delivery systems (DDS) are gaining interest for targeted, effective and low-side-effect treatments. In biomedicine, molecular research on hybrid systems is limited. The PAMAM dendrimers, well-studied for biomedical applications, offer controlled synthesis and pH-dependent drug release. Designed with water-soluble terminal groups and hydrophobic interiors, they encapsulate hydrophobic drugs for improved solubility.

Objectives. Our work aimed to investigate the correlation between the physicochemical properties of the carrier and active substance and the system efficiency and optimizing conditions for the formation of an efficient system based on poly(amidoamine) (PAMAM) dendrimers and 5-FU. An additional aspect was the verification of the biological activity of the obtained formulations.

Materials and methods. Experiments were conducted on 4th and 6th generation PAMAM dendrimers with ethylenediamine-based core and terminal NH_2-groups. The effectiveness of dendimer–drug complex formation (DLS, LDV, NMR, FTIR, QCM-D) and the loading efficiency (UV-Vis) was analyzed. The anticancer potential of PAMAM-5-FU was verified with MTS assay on 4 malignant cell lines: A375, SNB-19, Du-145, and HT-29. Additionally, L929 cells were examined using the AlamarBlue® test and visualized with live/death fluorescent assay using calcein-AM/PI staining.

Results. The complex formation between the ligand and dendrimer was studied under various conditions using UV-Vis spectroscopy. Higher efficiency in binding was achieved with an excess of the drug relative to the dendrimer, lower ionic strength and higher pH levels. Zeta potential measurements confirmed the presence of attached 5-fluorouracil (5FU) molecules on G4 PAMAM dendrimers, indicating both internal and external drug binding. Optimal complex formation conditions were confirmed using NMR, FTIR and QCM-D methods.
Cytotoxicity tests showed no toxicity of dendrimers to L929 cells. A significant decrease in cell viability was observed in melanoma, glioblastoma, prostate cancer, and colon adenocarcinoma, when incubated with PAMAM/5FU complexes, suggesting their potential efficacy in targeting and reducing the viability.

Conclusions. The effectiveness of ligand binding to dendrimer structures depends on complex formation conditions, including molar ratio, ionic strength, pH, and dendrimer generation. These studies have shown that the system can attach approx. 20 5FU molecules to 4th-generation dendrimers and about 25 molecules to 6th-generation dendrimers. The G4PAMAM and G6PAMAM exhibit no toxicity to normal cells and demonstrate the activity of 5-FU/PAMAM complexes in 4 cancer cell lines, reducing 5-FU's IC50 dose by up to 30%. This drug conjugation with dendrimers holds promise for enhancing drug stability and efficacy while overcoming drug resistance during transportation.

Key words: PAMAM dendrimers, 5-fluorouracil, drug delivery systems (DDS), dendrimer cytotoxicity, nanomedicine, nanomaterials, dendimer–drug complex formation

Theranostic nanocarriers of doxorubicin based on dendrimer

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Abstract

Background. Drug delivery systems (DDS) have gained increasing attention due to their potential to enhance drug efficacy while minimizing side effects. Nanocarriers, with their unique physicochemical properties, play a crucial role in developing effective therapeutic systems.

Objectives. This study aimed to explore the relationship between carrier and active substance properties and system efficiency, optimize conditions for poly(amidoamine) (PAMAM) dendrimer-based doxorubicin delivery, and assess the biological activity of the formulations.

Materials and methods. In this study, 4th-generation PAMAM dendrimers with ethylenediamine-based cores and terminal NH_2-groups were used, along with the anticancer drug doxorubicin hydrochloride (DOX). Various analytical techniques, including DLS, LDV, NMR, FTIR, QCM-D, CD, and UV-Vis, were employed to evaluate the formation of dendrimer–drug complexes. The anticancer potential of PAMAM-DOX was tested on several cancer cell lines and normal keratinocytes, with intracellular doxorubicin distribution visualized through CLSM study.

Results. The results demonstrated the effectiveness of G4.0 PAMAM-DOX complexes on different cell types, sometimes enhancing the therapeutic effect of DOX. Cellular localization studies revealed that free doxorubicin was localized in nuclei, while G4.0 PAMAM-DOX was distributed in the cytoplasm and nuclei.

Conclusions. From the physicochemical point of view, we have developed the conditions for the DOX loading strategy and the consequences of drug release. From a biological point of view, we verified in vitro tumor imaging efficiency, drug delivery and anti-cancer efficacy. The G4PAMAM can be a promising carrier for doxorubicin and constitute an essential part of biomedical applications and cancer therapy.

Key words: PAMAM dendrimers, doxorubicin, drug delivery systems (DDS), dendrimer cytotoxicity, nanomedicine, nanomaterials, dendimer–drug complex formation,

Viscosinamide production by *Pseudomonas fluorescens* DR54 and its interaction with *Candida rugosa* lipase

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Conflict of interest
None declared

Abstract

Background. Lipopeptides, derived from microorganisms, constitute a promising category of surface-active compounds known as biosurfactants. Despite the significant progress in biosurfactant research, several challenges still need to be resolved. These include optimizing biosurfactant production processes to achieve cost-effectiveness at an industrial scale.

Objectives. The aim of the study was to optimize the production process and characterize a lipopeptide-type biosurfactant, namely viscosinamide. Furthermore, the interactions between the viscosinamide and lipase from *Candida rugosa* (CRL) were investigated.

Materials and methods. The production of viscosinamide by *Pseudomonas fluorescens* DR54 cultivated on waste glycerol was optimized using response surface methodology, following a Box–Behnken design. The purification of biosurfactant was carried out using solid phase extraction (SPE). The pendant drop method was used to determine the surface tension of lipopeptide solutions. The effect of viscosinamide on lipase activity was evaluated using colorimetric methods and circular dichroism (CD) spectra were recorded on a spectropolarimeter.

Results. The optimal conditions for viscosinamide production, such as glycerol 70.8 g/L, leucine 2.7 g/L, phosphate 3.7 g/L, and urea 9.3 g/L were determined. Through optimization, the biosurfactant production was increased to 7.3 g/L. Preliminary characterization of lipopeptide involved the measurement of surface tension. The critical micelle concentration (CMC) of lipopeptide was determined to be 5 mg/L. Furthermore, measuring circular dichroism (CD) shows that the α-Helicity of CRL increases with an increase in the concentration of viscosinamide, while random coil structure decreases.

Conclusions. The results of this study showed that *P. fluorescens* DR54 can grow in an MSM supplemented with waste glycerol obtained from various industrial processes, serving as a carbon source. The preliminary characterization of the new lipopeptide revealed its capability to reduce surface tension. Additionally, it was observed that the presence of the viscosinamide led to an increase in CRL activity and influenced its structure.

Key words: *Pseudomonas fluorescens*, biosurfactant, lipopeptide, viscosinamide, crude glycerol

Expression of genes encoding key proteins involved in COX2/PGE2 pathway in colorectal cancer (CRC)

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Abstract

Background. Cyclooxygenase-2 (COX2/*PTGS2*) and its product – prostaglandin E2 (PGE2) – stimulate colorectal cancer (CRC) development. However, less is known about other pathway players, such as PGE2-degrading enzyme HPGD, influx and efflux transporters – PGT and MRP4, and PGE2 receptors – PTGER2 and PTGER4.

Objectives. To determine the expression status of key pathway players in colorectal tumors as compared to patient-matched resection margins and assess whether/how cancer histopathology affects tumor-to-margin expression ratios.

Materials and methods. A pairwise analysis of *PTGS2*, *HPGD*, *PGT*, *MRP4*, *PTGER2*, and *PTGER4* expression, using cDNA of tumors and macroscopically and histologically normal resection margins from 53 CRC patients, was conducted using quantitative polymerase chain reaction (PCR). It was followed by data character and distribution-adjusted statistical analysis of potential differences in tumor–margin ratios in patients stratified by cancer pathological (TNM) and histological (G) advancement.

Results. Except for unaltered *PTGS2*, the gene expression was significantly ($p < 0.05$) decreased in tumors as compared to patient-matched resection margins: *HPGD* by 6-fold, *MRP4* by 1.6-fold, *PGT* by 2.9-fold, *PTGER2* by 1.7-fold, and *PTGER4* by 2.1-fold. Tumor-to-margin expression ratios were independent from local advancement (T). However, tumor *PTGS2* expression was lower than in margins in N0 (3.2-fold) and N1 patients (3.6-fold) but significantly ($p = 0.014$) higher in N2 patients (5.6-fold). The *HPGD* was more downregulated in tumors from patients without lymph node involvement (10.3-fold against 3.7-fold, $p = 0.035$) and its tumor-to-margin ratio positively correlated with cancer cell dedifferentiation ($\rho = 0.33$, $p = 0.23$). In turn, tumor PGT expression was more downregulated in M1 than M0 patients (12.3-fold against 2.3-fold, $p = 0.008$).

Conclusions. Downregulated tumor expression of PGE2's degrading enzyme – hydroxyprostaglandin dehydrogenase-15 – is responsible for, as reported elsewhere, PGE2 elevation in tumor microenvironment of non-metastatic colorectal cancers, while upregulated expression of *PTGS2* is a main contributor to PGE2 elevation in advanced metastatic cancers. Therefore, patients in early stages of the disease might benefit from boosting HPGD expression/activity, while targeting COX2/*PTGS2* would be more effective for those with metastasizing cancers.

Key words: cyclooxygenase (COX)-2, hydroxyprostaglandin dehydrogenase-15 (HPGD), colorectal cancer (CRC), lymph node metastasis, molecular-based anticancer therapies

Studies of the anti-inflammatory properties and interactions with human serum albumin of pyrimidine derivatives

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Abstract

Background. The COX-1 and COX-2 are enzymes with cyclooxygenase and catalase activities. The COX-1 is constitutively expressed in many normal types of tissue, while COX-2 is usually undetectable in most tissues under normal conditions, and its concentration increases rapidly during inflammation. In addition, COX-2 has been suspected as being involved in cancer development and progression. Human serum albumin (HSA) is the main transport protein in the body. The mode of binding of drugs to albumin is central to understanding their pharmacokinetic profiles and has a major influence on their in vivo efficacy.

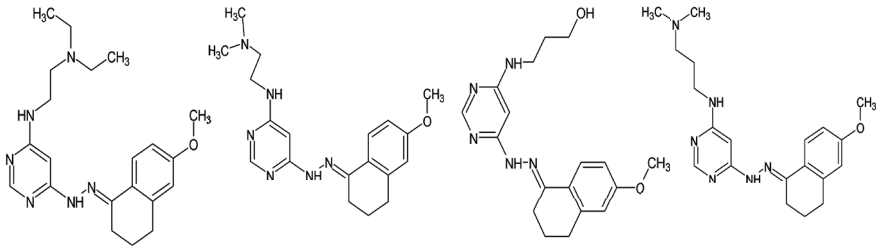

Fig. 1. *N*-{6-[2-(6-Methoxy-3,4-dihydronaphthalen-1(2*H*)-ylidene)hydrazinyl]pyrimidin-4-yl}-*N*′*N*′-diethylethane-1,2-diamine – L1, *N*-{6-[2-(6-Methoxy-3,4-dihydronaphthalen-1(2*H*)-ylidene)hydrazinyl]pyrimidin-4-yl}-*N*′*N*′-dimethylethane-1,2-diamine- L2, 3-({6-[2-(6-Methoxy-3,4-dihydronaphthalen-1(2*H*)-ylidene)hydrazinyl] pyrimidin-4-yl}amino)propan-1-ol- L3 and *N*-{6-[2-(6-Methoxy-3,4-dihydronaphthalen-1(2*H*)-ylidene)hydrazinyl] pyrimidin-4-yl}-*N*′*N*′-dimethylpropane-1,3-diamine- L4

Objectives. While 4 pyrimidine derivatives (Fig. 1) were previously studied toward antitumor activity, their inhibition of COX activity and the interaction with human serum albumin were unknown.

Materials and methods. Activity through COX inhibition was checked for all compounds via the COX Calorimetric Inhibitor Screening Assay Kit. For L1 and L2, the molecular docking was performed. Binding effect and interaction of all mentioned ligands with HSA were investigated using the fluorescence spectroscopy, circular dichroism (CD) spectroscopy and isothermal titration calorimetry (ITC).

Results. According to the molecular modelling, similar to in vitro study compounds, L1 and L2 bind more strongly to the active center of COX-2. In both cases, the potency of binding was almost the same as meloxicam. It was also observed that the binding manner is dependent on the structural properties of docked ligand. Based on thermodynamic and spectroscopic results, it can be concluded that all 4 examined derivatives are able to interact with human albumin with different affinity.

Conclusions. Previously published studies of L1–4 ligand sand the expansion of the research indicate their antitumor nature. In addition, L1–4 ligands are able to interact with blood-transporting proteins, so they are promising structures for the development of new drugs with a wide range of applications.

Key words: COX, pyrimidine derivatives, molecular docking, HSA/drugs interactions

Studies of stem cell lysates in potential use as a factor regenerating nerve cells

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Conflict of interest
None declared

Abstract

Background. New factors regenerating the cells of the nervous system are constantly being sought. The use of stem cells, as already shown in various studies, can lead to the activation of the carcinogenesis process. For this reason, the idea arose to investigate stem cell lysates as potentially inducing the regeneration of nerve cells and at the same time being a safer alternative to stem cells.

Objectives. The aim of the study was to evaluate the effect of MIC-1 stem cell lysate on neuron-like cells.

Materials and methods. Studies were performed using the differentiated cell line PC12 (medium containing 100 ng/mL nerve growth factor (NGF) for 72 h), which was treated with MIC-1 cell lysate for 24 h and then viability (MTT and LDH assays) and cell morphology (length and density of neurites) were assessed.

Results. Cell lysates in the range up to 250,000 homogenized cells per mL presented no cytotoxic effect on PC12 cells in both the MTT and LDH assays. In the mean concentration range of 50,000–150,000 homogenized cells per mL, an increased effect on length and density was observed.

Conclusions. Stem cell lysates from MIC-1 have a positive effect on neuron-like cells (they do not indicate cytotoxic activity and at the same time they extend the length of the neurite).

Key words: stem cells, neurons, PC12

Novel antimetastatic agents based on curcuminoid-porphyrin conjugates

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Conflict of interest
None declared

Abstract

Designing optimal (neo)adjuvant therapy is a crucial aspect of the treatment of oncological diseases. Standard methods of chemotherapy, radiotherapy and immunotherapy represent effective strategies for cancer treatment. However, in some cases with high metastatic activity and high levels of circulating tumor cells, the efficacy of standard treatment methods is insufficient and results in treatment failure and reduced patient survival. It is well known that the majority of deaths of oncology patients are not caused by the primary tumor but by metastasis. Classical neoadjuvant therapeutic regimens aim to shrink tumors using drugs that target cancer cells (mainly cytostatic drugs) and only indirectly affect metastasis-initiating cells.
Nevertheless, some polyphenol compounds, such as curcuminoids, display strong potential for the repression of metastatic spread by various independent mechanisms. The combination of their structural motif with anticancer agents, which display high activity against cancer cells could lead to the development of a new type of agents for shrinking of primary tumor and repressing metastatic activity. Inspired by this idea, we prepared a series of porphyrin derivatives substituted by curcuminoid functional groups. Biological studies showed that their application led to a decreased mobility of cancer cells, and anticancer efficiency of porphyrin was significantly improved by curcuminoid substitution.

Key words: porphyrin, curcumin, antimetastatic agents

Cyclic and open form of pentamethinium salts for the mitochondrial staining and targeting

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Conflict of interest
None declared

Abstract

Background. Mitochondria serve as a critical cellular energetic and signaling hub. Their disturbance plays a significant part in the oncological diseases. Strongly fluorescent small molecules targeting mitochondria have a high potential for diagnostic applications; they can also play a therapeutic role. Polymethines have a high potential for use in fluorescence microscopy and medicinal chemistry. A combination of the pentamethinium structure motif with perspective pharmacophore such as quinoxaline could lead to the development of promising new types of biological agents and mitochondrial fluorescent probes. Compounds containing quinoxaline units displayed promising anticancer effects. In addition, many quinoxaline derivatives have been reported to represent compounds with an even wider range of biological activity and interesting photophysical properties.

Objectives. The presented work is focused on the preparation and study of novel mitochondrial targeting agents based on the pentamethinium conjugates with quinoxaline cyclic form with quinoxaline directly incorporated in the pentamethinium chain and an open form with quinoxaline substitution in the γ-position.

Materials and methods. We have observed the interaction of tested salts with mitochondrial phospholipids (cardiolipin and phosphatidylcholine) by spectroscopic methods (UV-Vis, fluorescence and nuclear magnetic resonance (NMR) spectroscopy) and molecular docking. Their mitochondrial distribution and cytotoxicity were studied by fluorescence microscopy and cytotoxicity assay.

Results. We observed that the prepared salts display different spectral behavior in the presence of mitochondrial phospholipids. Both of them have strong fluorescence response for the cardiolipin, but only cyclic form for phosphatidylcholine. More importantly, prepared compounds, especially cyclic form, display high usability in mitochondrial imaging and cytotoxicity for pancreatic cancer cells.

Conclusions. Tested salts, mainly cyclic form, represent a promising structural motif for mitochondrial probes and mitochondrial targeting agents.

Key words: pentamethinium salt, quinoxaline, fluorescence mitochondrial probe

Effect of sphingomyelin consumption on the efficacy of colon cancer treatment

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Conflict of interest
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Abstract

Colon cancer is one of the most common types of cancer. It is also considered one of the leading causes of death worldwide. In the vast majority of cases, the main cause of colon cancer is a poor diet. The currently popular Western diet, characterized by increased meat, processed foods and reduced fiber consumption, contributes to the increasing number of reported cases of colorectal cancer.[1]-Sphingomyelin is a component of biological membranes. It is involved in many important biochemical processes, most notably cell signaling and lipid and protein sorting.[2] Lipid and protein sorting in cellular transport pathways is a key process for maintaining compartmentalization in eukaryotic cells. It may also inhibit the proliferation of cancer cells, particularly colon cancer cells.[3] The ubiquity of this substance in foods, such as milk, may allow it to be rapidly implemented to support anti-cancer therapy, which may speed up the treatment process for patients with colorectal cancer.[4] Electronic databases were searched using PubMed and Scopus. Search terms included the following keyword combinations: "colorectal cancer" and/or "sphingomyelin". In mice fed sphingomyelin, the incidence of colon cancer was 20%, while in the control group (without sphingomyelin supplementation) it was 47%.[4] Furthermore, sphingomyelin supplementation can increase chemotherapy sensitivity by approx. 100–300%. Reduced chemotherapy sensitivity due to sphingomyelin deficiency may be due to impaired cell signaling.[5] Sphingolipids (which include sphingomyelins), through their metabolism, which is the hydrolysis of sphingomyelin to ceramide, cause the regulation of signal transduction.[6]

The number of studies relating to the effect of sphingomyelin on the course of colon cancer is not yet enough to unanimously confirm the hypothesis on the use of this component in the treatment of patients. However, it is known that sphingomyelin has the potential to limit the growth of colon cancer cell populations. More studies on the effect of increased intake of sphingomyelin and the inhibition of colon cancer development need to be performed in order to conclusively establish the validity of its supplementation in patients with colon cancer. Sphingomyelin may be a new helpful substance for the treatment of colon cancer.

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Key words: colon cancer, sphingomyelin

The effect of the Mediterranean diet on insulin concentration

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Conflict of interest
None declared

Abstract

Diet plays an important role in human life. Due to the development of research methods, the modern world is faced with extremely promising opportunities in the field of healthy eating and optimal healthcare. Scientific research and advances in technology allow us to better understand how various nutrients affect our body. One of the more popular diets is the Mediterranean diet (MedDiet), which can have a positive effect on human health.[1,2]

The aim of the study was to discuss the Mediterranean diet and its impact on insulin concentrations in various disease states (insulin resistance, type 1 diabetes, type 2 diabetes). For this purpose, an in-depth review of the latest literature was performed.

Research indicates that the MedDiet is associated with a greater improvement in insulin resistance in obese people compared to other nutritional interventions.[3] In addition, the implementation of this diet may result in a reduction in body weight, homeostatic model assessment of insulin resistance (HOMA-IR) and lipid concentrations.[4] In the case of type 2 diabetes, the positive effect of the MedDiet has also been demonstrated.[5] Studies conducted so far suggest that the MedDiet can help improve the sensitivity of cells to insulin, as well as stabilize glycemia. Despite this, the pharmacological properties of individual dietary components and their clinical use should be further investigated.

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Key words: mediterranean diet, insulin resistance, obesity, diabetes mellitus

Choosing the right diet in the prevention and treatment of cancer

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Conflict of interest
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Abstract

Despite the achievements of medicine and major advances in treatment, cancer remains one of the leading causes of death worldwide. A proper diet plays an important role in the patient's health and wellbeing. It is suspected that just as a proper diet can affect the risk of cancer, it can also affect therapy.[1] Recently, the ketogenic diet, which consists in a significant reduction in carbohydrate consumption and an increase in the share of fats, as well as moderate protein intake, has been very popular in the context of cancer treatment.[2]

The aim of this study was to discuss the impact of proper nutrition on cancer prevention and therapy, with a particular emphasis on the ketogenic diet. For this purpose, an extensive review of the latest scientific literature was carried out, which largely included clinical trials.

The ketogenic diet can be considered a metabolic therapy in the treatment of cancer, which is based on reducing the energy supply to the cells, thereby inhibiting tumor growth. Thanks to this, the ketogenic diet creates an unfavorable metabolic environment for cancer cells.[3] One of the studies showed that breast cancer patients who were also supported by a ketogenic diet, had a longer survival time and better biochemical results.[4] Other studies indicate that the ketogenic diet may also contribute to reducing the mass and volume of the tumor.[5]

In conclusion, properly adjusted nutrition can be an effective adjuvant in cancer therapy. The ketogenic diet is of particular interest here, as its implementation can contribute to a significant improvement in patient's health.

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Key words: ketogenic diet, cancer treatment, nutrition therapy

Atomic force microscopy study on morphology of amyloid beta aggregates in the presence of chicken cystatin

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Conflict of interest
None declared

Abstract

Background. Amyloid beta (Aβ) aggregation is one of the neurodegenerative pathomechanisms observed in Alzheimer's disease. Ovocystatin was shown to affect fibrilization of Aβ peptides in vitro and protect the neural crest-derived cells from amyloid toxicity, but the detailed effect of ovocystatin on Aβ aggregates remains unknown.

Objectives. This study aimed to evaluate the morphology of Aβ aggregates in the presence of ovocystatin. Atomic force microscopy (AFM) as high-resolution scanning probe microscopy was used to assess density and complexity of the populations of Aβ fibrils.

Materials and methods. Amyloid β42 peptide was verified for aggregation properties using ThT assay. Briefly: Aβ (10 μM in phosphate-buffered saline (PBS)) was incubated with increased concentration of ovocystatin in 37°C for 24 h in the presence of 25 μM ThT. The fibrilization was monitored with in-time fluorescence measurement. The samples for AFM were prepared by mixing 50 μM of Aβ with various concentration of ovocystatin in PBS and incubated in 37°C for 72 h. Every 24 h, aliquots of 3 μL of samples were dispersed onto V-1 grade mica discs (Ø15 mm; NanoAndMore GmbH, Wetzlar, Germany) and examined using AFM system Drobnowidz (10.24425/mms.2021.137698) with a resolution of 0.1 nm. Areas of 1×1 μm were acquired using MountainsLab® Premium 9.3.10281 software.

Results. Ovocystatin impaired fibrilization of Aβ by inhibiting the aggregation kinetics in a concentration-dependent manner. The half-life of amyloid fibrils was 2.21 h and was noticeably reduced in the presence of ovocystatin (1.496 h for 1 μM of ovocystatin). The AFM images of Aβ showed dominant population of branched structures with the height of 90–110 nm, accompanied by globular forms. Instead, AFM images of Aβ for 3 days in the presence of ovocystatin revealed only small spheroidal structures of height up to 40 nm.

Conclusions. Ovocystatin inhibits the formation of Aβ aggregates and can disintegrate those already present.

Key words: ovocystatin, amyloid beta, AFM, Alzheimer's disease

Screening and characterization of biological properties of betulin derivatives

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Abstract

Background. Betulin and its derivatives are characterized by a wide spectrum of biological activity at low concentrations. We present preparations of betulin derivative with biological activity. Betulin 1 is a natural pentacyclic triterpenoid that has a wide range of pharmacological activities. In chemical classification, betulin is a pentacyclic triterpenoid from the lupane family, one of the most abundant groups of triterpenes found in nature.

Objectives. Synthesis of betulin 1 and obtaining new derivatives. Examination of the obtained derivatives for anticancer and antibacterial activity.

Materials and methods.
General. NMR analysis was performed on a Bruker Avance DRX 600.
Anticancer activity. It is found in significant amounts in white birch bark cell lines: 1) Me45 human malignant melanoma – a line that was derived at the Radiobiology Department of the Oncology Center Maria Skłodowska-Curie in Gliwice, 2) human cells derived from Lu1205 – lung metastases of melanoma (ATCC).
Antibacterial activity evaluation. Bacterial strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145 and *Staphylococcus aureus* ATCC 25923) were obtained from the Polish Academy of Sciences (Wrocław, Poland).

Results. Betulin 1 esterification is not carried out with acids, as they catalyze the formation of an ether bond between the hydroxymethylene group (C-17) and the isopropenyl group (C-19). The above results provide evidence that the 9 derivative of the invention has the ability to inhibit the growth of melanoma cells of both lung and lymph node metastases. Moreover, in antibacterial assays, Gram-positive *Staphylococcus aureus* strain was susceptible to one of the tested derivatives. The lowest concentration of bromo derivative of betulin 9 that visibly limited the growth of *S. aureus* was 100 μg mL^{-1}.

Conclusions. In nature, betulin can be easily acquired and transformed chemically or biotechnologically, in order to obtain new compounds with potential biological activity.

Key words: botulin, anticancer activity, antibacterial activity

The analysis of serum clusterin glycosylation in rheumatoid arthritis and psoriatic arthritis: The search for new diagnostic glycomarkers

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Conflict of interest
None declared

Abstract

Background. Rheumatoid arthritis (RA) and psoriasis of the joints (psoriatic arthritis (PsA)) are chronic autoimmune inflammatory diseases.[1,2] However, in the course of the latter, rheumatoid factor does not appear in the blood.[3] An accurate and fast diagnosis in the early stages of the disease progression allows for appropriate therapy. However, differentiating between RA and PsA can be challenging for clinicians. Therefore, new biomarkers are being sought to facilitate and accelerate the diagnosis and differentiation of PsA and RA. Clusterin (CLU, apolipoprotein J), a chaperone N-glycoprotein, is characterized by various activities and a propensity to interact with a wide spectrum of molecules. Changes in CLU glycosylation are closely related to its function as a chaperone protein.[4] Clusterin is also one of the protein biomarkers with the potential to predict the response to biological therapy in PsA.[5]

Objectives. In the present study, we analyzed the profile and degree of serum CLU glycosylation in the context of the utility of glycomarkers to differentiate RA patients, PsA patients and healthy subjects.

Materials and methods. Using a modified lectin-ELISA method with specific biotinylated lectins, CLU N-glycosylation was analyzed in sera of patients with RA (n = 34) and PsA (n = 37) and of healthy volunteers (n = 21). Sialic acid (SA) expression was examined using *Sambucus nigra* agglutinin (SNA, specific to α2,6-linked SA) and *Maackia amurensis* agglutinin (MAA, specific to α2,3-linked SA). For the determination of fucose expression, *Lotus tetragonolobus* agglutinin (LTA, recognize antennary fucose 1,3-linked), *Ulex europaeus* agglutinin (UEA, recognize antennary fucose 1,3- and 1,2-linked) and *Lens culinaris* agglutinin (LCA, recognize core fucose) were used. The results were analyzed using Statistica software v. 13.3 PL (StatSoft Polska, Kraków, Poland).

Results. The obtained results are presented in Table 1.

Table 1. Comparison of the concentration/relative reactivity of the analyzed parameters between the groups

Parameter	Group		
	C (n = 21)	RA (n = 34)	PsA (n = 37)
	mean ±SD	mean ±SD	mean ±SD
CLU [µg/mL]	30.62 ±37.30	24.58 ±32.36 pPsA = 0.000246	21.36 ±9.51
SNA [AU]	0.420 ±0.316	0.624 ±0.278 pC = 0.003711	0.837 ±0.412 pC = 0.000186
MAA [AU]	0.064 ±0.140	0.020 ±0.013	0.017 ±0.013
LTA [AU]	0.201 ±0.101	0.269 ±0.150	0.205 ±0.071
UEA [AU]	0.037 ±0.016	0.049 ±0.028	0.043 ±0.012
LCA [AU]	0.091 ±0.059	0.110 ±0.057	0.128 ±0.093

C – control (healthy subjects); RA – rheumatoid arthritis; PsA – psoriatic arthritis; SD – standard deviation; CLU – clusterin concentrations; SNA – relative reactivity with *Sambucus nigra* agglutinin; MAA – relative reactivity with *Maackia amurensis* agglutinin; LTA – relative reactivity with *Lotus tetragonolobus* agglutinin; UEA – relative reactivity with *Ulex europaeus* agglutinin; LCA – relative reactivity with *Lens culinaris* agglutinin. The p-values of less than 0.05 were considered significant.

Conclusions. The results of the receiver operating characteristic (ROC) curve analysis have shown that the only parameter with average clinical utility to differentiate RA and PsA groups is CLU concentration (area under the ROC curve (AUC) = 0.754); however, RA and PsA patients can be differentiated from healthy subjects based on the expression of SNA-reactive SA α2,6-linked (AUC = 0.735 and AUC = 0.798, respectively). These 3 biomarkers are worth of future study in the context of their utility in RA and PsA routine diagnostics.

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Key words: blood serum, clusterin glycosylation, rheumatoid arthritis, psoriatic arthritis, diagnostic glycomarkers

Polymorphism studies of fenamic acid complexes

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Abstract

Background. Continuation of research on cocrystals and salts of fenamic acids belonging to the group of non-steroidal anti-inflammatory drugs (NSAIDs) revealed that these systems exhibit polymorphism. Depending on the crystallization conditions, specific crystal forms can be obtained. Polymorphism is common in medicinal substances and can have a significant impact on their physicochemical and pharmacodynamic properties.[1] One of the promising methods of obtaining new forms of drugs with improved properties is the preparation of cocrystals and salts.[2] Further studies on the complexes of fenamic acids with acridine were undertaken,[3] revealing the formation of new polymorphs.

Objectives. The objectives are as follows: studying fenamic acid complexes using X-ray powder diffraction method and thermal analysis, as well as investigation of crystal structures of new polymorphs.

Materials and methods. Powder diffraction data for all cocrystals were collected on Brucker D2 Phaser instrument. Data for new polymorphs were collected on XtaLAB Synergy R (DW system, HyPix-Arc 150). Thermal analysis was carried out using differential scanning calorimeter DSC 214 Polyma instrument (Netzsch, Selb, Germany) equipped with an Intracooler IC70 (Netzsch).

Results. Cocrystals of fenamic acids with acridine were analyzed with X-ray powder diffracion method (XRPD), and thermal analysis was performed. Crystal structures of new polymorphic forms have been investigated with single-crystal X-ray diffraction.

Conclusions. As a result of the continuation of studies on cocrystals of fenamic acids using X-ray and thermal methods, it was found that new polymorphic forms can be obtained.

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Key words: fenamic acids, cocrystals, polymorphism

The diagnostic utility of LECT2 in metabolic and renal diseases in geriatric patients

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Abstract

Background. Leukocyte cell-derived chemotaxin 2 (LECT2) is a hepatokine with pro-inflammatory and chemotactic function involved in the pathogenesis of metabolic diseases.

Objectives. The aim of the study was to verify the diagnostic utility of LECT2 in metabolic and renal diseases in geriatric population. The relationship between this protein and selected parameters of metabolism, such as scavenger receptors type A and B (SR-A and SR-B) and glomerular filtration rate (GFR), were analyzed. Additionally, we investigated LECT2 as an atherosclerosis exponent, and more precisely – whether LECT2 is expressed inside the arterial walls, where the atherosclerotic plaque develops.

Materials and methods. The investigated material comprised the sera of 79 patients and fragments of the human thoracic or abdominal aorta from 22 patients who died a sudden death. The concentration of LECT2 in serum samples was performed using human enzyme-linked immunosorbent assay (ELISA) kits from ELK Biotechnology (Wuhan, China). Immunohistochemistry analysis of LECT2 protein content in tissue was performed using primary anti-LECT2 antibody (Abcam, Cambridge, UK).

Results. The mean serum concentration of LECT2 in geriatric patients was 63.1 ng/mL, standard deviation (SD) of 50.2, indicating the high variability of this protein content in the investigated group of individuals over 65 years old. A positive correlation between SR-A and LECT2 ($r \sim 0.8$, $p < 0.05$) and a negative correlation between GFR and LECT2 ($r \sim -0.4$, $p < 0.05$) were observed.

Conclusions. The association of LECT2 with SR-A, a receptor that binds LDL and advanced glycation end products (AGEs), considered as a common point of lipid and sugar metabolism, suggests a complicity of LECT2 in atherogenesis and/or diabetic pathology. The negative LECT2–GFR correlation presents an association of this protein with loss of normal renal function. An interesting observation is also the expression of LECT2 in macrophages, myocytes and other aortic cells, with a tendency to be overexpressed in highly developed atherosclerotic plaques.

Key words: LECT2, atherosclerotic plaque, renal diseases

The role of angiotensin converting enzyme polymorphisms in the development of diabetic nephropathy

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Abstract

Background. Angiotensin converting enzyme (ACE) is responsible for the production of angiotensin II, a key component of the renin-angiotensin system.[1] An increased production of angiotensin II is observed in diabetes, which in turn is accompanied by an increase in oxidative stress.[2] Studies conducted so far suggest that *ACE* polymorphisms may play a role in the development of diabetic nephropathy.[3,4] It has been observed that the D allele (rs4646994 polymorphism) may be associated with an increased likelihood of developing diabetic nephropathy in patients with diabetes.[5]

Objectives. The aim of the study was to assess the role of selected *ACE* polymorphisms (rs4343 and rs4646994) in the risk of development and progression of diabetic nephropathy, and to check whether these polymorphisms may contribute to the increased likelihood of renal replacement therapy.

Materials and methods. The *ACE* polymorphisms were analyzed in a group of 225 patients who were divided into 3 subgroups: the diabetic nephropathy group, the kidney transplant diabetic group and the control group. The rs4343 polymorphism was determined using the polymerase chain reaction and restriction fragment length polymorphism analysis (PCR-RFLP), and the rs4646994 polymorphism (insertion/deletion) was determined using the polymerase chain reaction.

Results. It has been observed that the G/G genotype (rs4343 polymorphism) is associated with an over 2.5-fold increased odds of developing diabetic nephropathy. In addition, the G allele is also associated with a 2.5-fold higher risk of this disease. Similar results were obtained in patients who had already had a kidney transplant as a result of diabetic nephropathy – the G/G and G/A genotypes (rs4343 polymorphism) were associated with an increased likelihood of renal replacement therapy. Likewise, the G allele was associated with a 1.73-fold increased likelihood of renal replacement therapy.

Conclusions. The G allele of rs4343 polymorphism seems to be associated with an increased likelihood of diabetes complications in the form of diabetic nephropathy, and may also be a risk factor for renal replacement therapy. In turn, the rs4646994 polymorphism turned out to have no effect on the development of diabetic nephropathy.

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Key words: angiotensin converting enzyme, polymorphism, diabetes, diabetic nephropathy, kidney transplantation

Electrotransfer of plasmid DNA by unipolar and bipolar asymmetric electric pulses in rat cardiomyocytes

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Abstract

Background. Electroporation, a technique involving the application of brief electric pulses, offers a versatile and efficient means of introducing exogenous genetic material into cells, holding significant promise for diverse applications in genetic engineering and biomedicine. The effect of nanosecond electric pulses of various shapes has not been demonstrated in genetic engineering. Mostly millisecond pulses are used, and recently also microseconds. Using a shorter pulse in the range of nanoseconds, coupled with gene delivery, can be a promising tool for excitable cells, e.g., cardiac or skeletal muscles.

Objectives. The aim of our study was to investigate how electroporation with nanosecond pulses with respect to different pulse shapes (unipolar, bipolar and asymmetric) influences cardiomyocyte permeabilization and gene transfer.

Materials and methods. Rat cardiomyocytes (H9c2) were used for the study. The BTX ECM 820 (Harvard Apparatus, Holliston, USA) electric pulse generator was used to deliver ESOPE and millisecond protocols, and a High-Frequency Bipolar Electroporator was used to deliver unipolar and asymmetrical bipolar pulse sequences. The efficacy of the pulsed electric field protocols was assessed with flow cytometry using Yo-Pro-1 membrane permeability marker. The MTT assay determined cell viability (24 h and 48 h), cytoskeleton organization was visualized with F-actin distribution (fluorescent microscopy), and muscle atrophy F-box (MAFbx) marker was detected using confocal microscopy. Gene electrotransfer (GET) was performed with DNA plasmid vector pEGFP-C1 (4731 bp long); DNA coding enhanced green fluorescent protein was propagated in DH5α *E. coli* strain and analyzed using fluorescent and holotomographic microscopy.

Results. Our results showed that optimized pulsed electric field is not cytotoxic for cardiac muscle cells, and can be efficiently used for gene transfection. Additionally, unipolar and asymmetric nanosecond pulses are similarly effective in cell permeabilization as a standard ESOPE protocol. The sequence with 700-ns pulses resulted in the highest GET efficiency. In the case of other protocols, GET efficacy was on the control level; only a slight increase in the fluorescent signal was observed for the ESOPE protocol and standard GET protocol using milliseconds.

Conclusions. Our results show that nanosecond pulses can be used for electroporation, thereby facilitating gene delivery within cardiomyocytes. Notably, the investigation revealed that nanosecond unipolar and asymmetric protocols exhibit a remarkable absence of cytotoxicity while concurrently abstaining from eliciting cellular atrophy within the scrutinized cell population. These discerning observations collectively contribute to a pioneering advancement in our comprehension of gene transfer methodologies.

Key words: electrotransfer, unipolar and asymmetric pulses, cardiomyocytes

Comparison of commercial and non-commercial immunoenzymatic assays and trouble-shooting in optimization, using ELISA on SR-AI as an example

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Abstract

Background. Scavenger receptors are an interesting research object in the context of diabetes and atherosclerosis since their ligands are both modified lipoproteins and advanced glycation products. We have already tested one of the scavenger receptors: SR-AI/MSR1/CD204 on the sera of diabetic patients and others where we expected the intensification of glycation. Our research methodology is based on commercially available kits and more economical enzyme-linked immunosorbent assay (ELISA) tests, which we develop ourselves.

Objectives. The aim of our work is to present a comparison between commercial tests and self-developed tests and to present ways to overcome methodological and technical problems in optimizing ELISA tests.

Materials and methods. An enzyme-linked immunosorbent method was used. Commercial human ELISA kits were obtained from ELK Biotechnology and BioAssay Technology Laboratory. The manufacturer's instructions were followed. Self-optimized tests used: standard proteins: (1) Recombinant Human SR-AI/MSR Protein (R&D, 2708-MS-050); (2) SR-AI/MSR Recombinant Protein Antigen (Novus Biologicals, NBP1-88125PEP); primary antibodies: (1) goat polyclonal primary anti-SR-AI antibodies IgG (R&D, AF2708), (2) rabbit polyclonal anti-CD204 antibody (Abcam, ab123946), (3) rabbit polyclonal SR-AI/MSR antibody (Novus Biologicals, NBP1-88125); secondary antibodies: (1) donkey anti-goat secondary antibodies IgG (Abcam, ab6885), (2) goat anti-rabbit IgG H&L (HRP) Abcam, ab672, (3) goat anti-rabbit Peroxidase AffiniPure IgG (H+L) (Jackson ImmunoResearch; 111-035-003).

Results. The results for one of the kits (BioAssay Technology Laboratory) were completely unreliable because non-specific effects were found (the products were subject to a complaint procedure). The ELK Biotechnology kits were the most convenient compared to all other options, although using Abcam antibodies (ab217318) and the Novus Biologicals standard (H00000949-P01) also seem reliable.

Conclusions. The ELISA kits are a more expensive option because they do not require validation and are usually shorter. Unfortunately, some manufacturers sell tests with non-specifically interacting antibodies, which requires verification. Self-optimized ELISAs are, however, a much cheaper and a more reliable option if the validation is carried out correctly. The most common problem, associated with minimizing the background effect, is solved by lowering the concentration of secondary antibodies and selecting the lowest serum concentration that will allow to obtain the absorbance within the standard curve.

Key words: enzyme immunoassay methods, ELISA, scavenger receptors, SR-A, diabetes, diabetology

Fluorescence studies of the interaction of hemiaminal moiety with ubiquitin

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Abstract

Background. The fluorescence quenching is a method of studying the interaction (i.e., the binding mechanisms) of small molecules with proteins. The intrinsic fluorescence of protein originates from the tryptophan, tyrosine and phenylalanine. Ubiquitin (Ub) is a highly conserved small cytoplasmic globular protein, containing a single tyrosine (Tyr-59) located within the helix and 2 phenylalanines (Phe-4 and Phe-45) located within the β-sheets.
Hemiaminals are intermediates in the reaction between carbonyl compounds and primary amines. These species are generally regarded as unstable and thus possible to be investigated only in special conditions with highly sophisticated methods. A group of stable hemiaminals has been obtained in a reaction between 4-amino-1,2,4-triazole and nitro- or cyano-substituted benzaldehydes.

Objectives. Hemiaminals may interact with protein targets, but this suggestion has not yet been tested. The main aims of this work were to examine the possibility of hemiaminal interaction with a protein of biological importance such as ubiquitin.

Materials and methods. All reagents were of analytical grade and purchased from Sigma-Aldrich, ubiquitin from bovine erythrocytes (U6253). Fluorescence measurements were performed on a Jasco 8200 spectrofluorimeter with a Xenon lamp. The excitation wavelength was set at 280 nm, and the emission wavelength was recorded between 300 nm and 500 nm. A 2 mg/mL aqueous solution of ubiquitin was prepared. Native protein solutions were prepared by 20-fold dilution of the initial mixture directly in the 1.0-cm quartz cell and further titrated with 10 portions (10 µL) of hemiaminal (2,4-dinitrophenyl)(4*H*-1,2,4-triazol-4-ylamino) methanol (24dnbATR) solution.

Results. The fluorescence intensity of ubiquitin decreases remarkably upon increasing the concentration of 24dnbATR, and the emission maximum is slightly shifted from 350 nm to 354 nm at 298 K. This shift of the maximum emission wavelength was due to the interaction of the protein with the ligand and formation of Ub-24dnbATR complexes.

Conclusions. Ubiquitin forms noncovalent complexes with hemiaminal molecules.

Key words: hemiaminal, hemiaminal–protein interaction, fluorescence quenching

Interaction of promising bioactive Babylon snail peptides with ctDNA

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Abstract

Background. Interest in natural peptide compounds as potential medicinal substances has significantly increased. One of the advantages of peptides is their possibility of anti-cancer action which can be carried out using various mechanisms such as induction cell apoptosis or necrosis, inhibition of angiogenesis, and activation or inhibition of proteins.[1] So far, many anti-cancer drugs have been discovered, including cytostatics, whose mechanism is associated with the interaction of DNA. However, the research for new substances that could be used in anti-cancer therapy is still ongoing. One of the strategies is searching for bioactive peptides from plants, fungi or animals. The new compounds could be obtained from snails, e.g., *Babylonia areolate*, *Pomacea canaliculata* and *Helix aspersa maxima*.[2–4]

Objectives. The aim of the research was to characterize the interaction between bioactive peptides and ctDNA on the molecular level. For specific purposes, proposing the type of binding of the tested compounds to the DNA molecule. The bonding strength was described by determining the apparent association constants K_{app}. Moreover, the influence of the tested peptides on the DNA structure was verified.

Materials and methods. Two peptides were found and identified in spotted Babylon snail hydrolysates. The octa- and nanopeptides with the following sequences: HTYHEVTKH and WPVLAYHF, are responsible for the antioxidant properties of hydrolytes. These compounds reduce the concentration of oxygen and nitric oxide-free radical species. Moreover, the hydroxyl radical-induced DNA damage assay was evaluated in vitro in the human adenocarcinoma colon (Caco-2) cell line.[2]
The study of the interaction of bioactive compounds with biomolecules could explain the mechanism of their action. In this work, multi-spectroscopic methods such as ultraviolet absorption spectra, circular dichroism and fluorescence were used to characterize the interaction between promising bioactive Babylon snail peptides and ctDNA.

Results. The study showed that the peptides form complexes with the ctDNA molecule, and this interaction is more robust for HTYHEVTKH, for which K_{app} was determined to be higher. At the same time, they do slightly decompose the structure of ctDNA.

Conclusions. The investigated Babylon snail peptides bind to ctDNA; therefore, they may interfere with the process of translation and replication, and thus require further research in the direction of anticancer activity.

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Key words: Babilon snail peptides, ctDNA interaction, the apparent association constants

Synthesis of new meloxicam derivatives and their interaction with the artificial models of biological membranes

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Abstract

Background. The interaction of the drugs with the phospholipid bilayer is crucial in many biochemical processes. Model membranes are often used to assess such interactions. Meloxicam belongs to a class of medicines known as non-steroidal anti-inflammatory drugs (NSAIDs). Their biological target is cyclooxygenase (COX) – a membrane protein associated with the phospholipid bilayer surrounding the endoplasmic reticulum as well as the cell nucleus. Drugs in this group are mainly taken orally; therefore, the drug–membrane interaction is a preliminary stage in the body, as drugs must cross biological membranes in order to be absorbed and distributed.

Objectives. The purpose of the study was to assess the ability of 8 newly designed and synthesized meloxicam analogues to interact with phospholipid bilayers.

Materials and methods. In the present work, we describe the synthesis and results of calorimetric studies of 8 new analogues of meloxicam on the phase behavior of phospholipid bilayers. In new meloxicam derivatives, the substituents in position 2 and 3 of the 1,2-benzothiazine scaffold have been modified in order to obtain better pharmacological parameters and reduced toxicity.

Results. All examined compounds decreased the main transition temperature of DPPC in a concentration-dependent manner. The addition of studied compounds to DPPC also resulted in broadening of the transition peaks. Moreover, all examined compounds decreased the enthalpy of the DPPC main phase transition. In case of all DPPC gel-liquid crystalline phase transition parameters, the most pronounced effects were found for compounds ZW2, ZW1 and ZW8.

Conclusions. In the present work, we have synthesized 8 new meloxicam analogues and used differential scanning calorimetry to study their interactions with DPPC phospholipid bilayers. We have shown that these interactions depend on the chemical structure of individual 1,2-bezothiazine derivatives. We may conclude that all studied compounds alter phospholipid bilayer properties.

Key words: model membranes, drug–membrane interaction, DPPC, DSC, 1,2-benzothiazine derivatives, synthesis, oxicams, meloxicam analogues

The effect of calibration technique on the stability of liposomes in formulation against the infection progress of SARS-CoV-2

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Conflict of interest
None declared

Abstract

Background. Applying liposomes as drug delivery platforms remains challenging, as formulations must be characterized by high homogeneity and excellent long-term stability. In the current study, we use 2 liposome calibration techniques to synthesize a liposome-based drug inhibiting the infection progress of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Objectives. The study aimed to investigate whether the high-throughput homogenization technique enables the acquisition of homogeneous liposomal suspensions with stability comparable to liposomes obtained with the extrusion method.

Materials and methods. Liposomes were composed of the following lipids: hydro-soy PC, cholesterol, DSPC, various PGs, DSPE-$PEG_{1000,}$ and DSPE-PEG_{2000}-Maleimide. After initial hydration, liposomes were calibrated via homogenization with LM20 Microfluidizer (Microfluidics) or pressure extrusion (patent pending). Further, the peptide sequence directed against SARS-CoV-2 was conjugated to liposomes. The excess peptide was separated from preparation via dialysis. Each preparation was characterized in terms of lipid and peptide content. Stability and homogeneity were assessed by changes in particle size distribution and zeta potential, measured with ZetaSizer Nano (Malvern). Measurements continued for 6 months or until the homogeneity was lost. Accelerated aging tests were also performed in samples incubated with a culture medium at 37°C.

Results. Liposomes subjected to homogenization were less stable than those obtained with pressure extrusion. The type of PG exerted the greatest influence on long-term stability. Extruded liposomes with DPPG were stable for at least 6 months, whereas homogenized liposomes lost their stability after 30–45 days. Liposomes with DOPG lost their stability within a month, even when subjected to extrusion. Finally, POPG liposomes showed high stability regardless of the calibration method, which was confirmed with the accelerated aging tests.

Conclusions. We demonstrated that liposome stability strongly depends on their composition and the calibration technique. The pressure extrusion supports the long-term stability; however, with a thorough optimization of the lipid composition, it is possible to obtain a stable, highly-homogeneous formulation based on the high-throughput homogenization technique.

Key words: liposomes, extrusion, microfluidization, SARS-CoV-2

Antioxidant properties of selected ingredients in the diet for skin anti-aging

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Abstract

A healthy, well-balanced diet and adherence to the principles of rational nutrition not only provide the body with the right amount of nutrients and vitamins, but also improve the skin appearance. Nutritional deficiencies adversely affect the condition of the skin, hair and nails. The aging process of the skin is associated with a decrease in the activity of fibroblasts and in the production of collagen and elastin fibers, and thus loss of skin firmness and the appearance of wrinkles. The most common anti-aging dietary supplements include vitamin A, vitamin E, coenzyme Q10, and phytoestrogens.

The purpose of the study was to track signs regarding the connections between diet quality and the impact on skin quality and health.

Studies conducted by research institutes provide convincing evidence that nutrition is a risk factor for skin aging. The exact extent to which diet contributes to skin aging is currently unknown. It is certain that dietary interventions that prolong life and positively affect health have been confirmed by scientific research. Data from recent studies clearly indicate that moderate caloric restrictions while maintaining the proper nutritional status of the body can have a beneficial effect on health. This is done by reducing many important metabolic factors associated with the development of the most common chronic diseases.

For the sake of body condition and inhibition of skin aging processes, it is worth using a varied diet, based on the principles of proper nutrition – natural, unprocessed products, vegetables and fruits. It is also worth introducing a diet with a low GI and lower content of carbohydrates, fat in the form of saturated fatty acids, and rich in fiber.

Key words: natural products, anti-aging, diet

The investigation of photosensitizing efficacy of aloe emodin-encapsulated liposome following photodynamic therapy on melanoma cells

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Conflict of interest
None declared

Abstract

Background. In the present study, the potential of aloe emodin encapsulated in liposomes as a photosensitizer in photodynamic therapy (PDT) has been investigated. Encapsulation in liposomes is a versatile drug delivery system. It is not toxic and exhibits longer circulation time among all drug carriers. The conducted research presents the phototoxic and anti-cancerous effects of aloe emodin encapsulated in liposomes on melanoma cell lines.

Objectives. The aim of the study was to measure the effectiveness of aloe emodin encapsulated in liposome in the photokilling of melanoma cell lines after blue light irradiation. Additionally, we measured the ability to accumulate natural substance in cell lines, and evaluated the amount of apoptotic proteins in cell lines before and after treatment.

Materials and methods. Experiments were carried out on the 3 melanoma cell lines – MUG-Mel2, Buf1286 and FM6 – with the use of aloe emodin encapsulated in liposomes as a photosensitizer and photodynamic therapy. We performed the MTT assay, measuring the cytotoxicity effects of the natural compound. Additionally, we performed the cellular uptake with flow cytometry to measure the ability to accumulate aloe emodin encapsulated in liposomes, and immunocytochemical staining, which allows examining whether the proposed therapy appears apoptosis-related proteins in cancer cell lines.

Results. In MTT assay, we observed a significant difference in cell viability after irradiation. The proposed therapy was not phototoxic against normal skin cells in comparison to cancer cells. In cellular uptake, we observed that the cells did not display a significant difference in the rate of uptake of a natural substance, so we incubated them for the same duration. Additionally, in immunocytochemical staining, an increase of apoptotic proteins in melanoma cell lines was observed after therapy.

Conclusions. Our results showed that liposome with aloe emodin has a cytotoxic effect on melanoma cell lines. We need more research to confirm the anticancer potential of aloe emodin encapsulated in liposome as a photosensitizing agent.

Key words: photodynamic therapy, melanoma, cancer, plant substance, liposomes

Lipid-indomethacin conjugates from anti-inflammatory to anticancer agent

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Abstract

Background. Colon cancer (CRC) is known as the third most common cause of cancer death in both women and men. One of the main risk factors of CRC is inflammation. Thus, numerous studies have been undertaken to introduce an alternative against CRC with the use of non-steroidal anti-inflammatory drugs (NSAIDs). Indomethacin (IND), non-selective cyclooxygenase enzyme inhibitor, is a member of NSAIDs. Anticancer activity of IND was confirmed in many studies. Also, IND may enhance the activity of chemotherapeutic agents, which can be eliminated from the cell by MDR transporters. Unfortunately, due to the high risk of side effects which may be related to lipid membrane perturbations, the use of IND is limited.

Objectives. The aim of our work was to synthesize lipid-IND conjugates and to study their activity against CRC cells, both sensitive and resistant to chemotherapy. Also, we checked their ability to influence the lipid environment of model membrane.

Materials and methods. The activity of derivatives containing lysophosphatidylcholine (1-IND-LPC) or oleic acid (1-OA-2-IND-PC) was studied against colon cancer cells HT29 and its resistance to doxorubicin subline HT29/Dx. The cytotoxic activity was estimated using the SRB method. Cell cycle analysis, induction of apoptosis and the level of reactive oxygen species (ROS) was examined with flow cytometry. The lipid-conjugate interaction was studied using differential scanning calorimetry (DSC).

Results. We did not observe any difference between the activity of the compounds in HT29 as compared to HT29/Dx. However, 1-IND-LPC derivative exhibited the highest anticancer potency and the ability to enhance doxorubicin action in HT29/Dx cells. This compound induced apoptosis which was accompanied by an increased ROS level. Also, 1-IND-LPC showed a weak effect on thermotropic properties of model membrane compared to other compounds.

Conclusions. The 1-IND-LPC may be a good candidate for adjuvant therapy of CRC.

Key words: anticancer activity, lipid conjugates, CRC

Determination of eosinophil-derived neurotoxin (EDN) in stool as a non-invasive method in the diagnosis of gastrointestinal diseases in children

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Abstract

Inflammatory bowel diseases (Crohn's disease, ulcerative colitis) are a group of chronic diseases of the gastrointestinal tract with not fully understood multifactorial etiology. The increasing number of cases of children with cow's milk allergy whose main symptom is lower gastrointestinal bleeding makes it necessary to search for new diagnostic methods and criteria, including new serous and fecal biomarkers.

The activity and diagnosis of non-specific inflammatory and allergic diseases and the effectiveness of the therapy are assessed primarily using invasive endoscopic examinations with the collection of sections for histopathological examination. The gold standard for the diagnosis of IgE-independent allergy is the oral challenge test, which is time-consuming and fraught with numerous limitations. In addition, the concentrations of C-reactive protein (CRP) in blood serum and calprotectin in feces are determined.

A new marker that can be used in the diagnosis of inflammatory bowel diseases, chronic diarrhea and bleeding from the lower gastrointestinal tract may be eosinophil-derived neurotoxin (EDN), determined in the feces. It is a quantitative test which detects clinical or subclinical chronic intestinal inflammation, assesses disease activity and predicts its recurrence.

Changes in EDN concentration in allergic and foodborne diseases have been demonstrated. Therefore, finding a correlation between EDN values and current routine diagnostic methods will enable the use of EDN to determine the severity of the development of inflammatory bowel diseases. The new marker will improve early non-invasive diagnostics and monitoring of the disease, and thus affect the quality of life of pediatric patients and their parents.

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Key words: eosinophil-derived neurotoxin (EDN), Crohn's disease, ulcerative colitis, allergic diseases

Design of selective metalloproteinase 13 inhibitors based on pyrimidine structure

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Abstract

Background. Recently, there has been increasing interest in proteins from the matrix metalloproteinase family. Metalloproteinase 13 (MPP-13) and its biological properties seem to be of particular interest.[1] The MPP-13 is considered a key factor in the degradation of type II collagen in cartilage tissue. In various types of conditions, such as osteoarthritis, rheumatoid arthritis (RA) is responsible for the damage and progression of the diseases. Therefore, further studies are needed to verify it as a molecular target in the treatment of RA.[2] To date, many different potential inhibitors of MMP-13 have been described. Unfortunately, most of them are characterized by low efficacy and low selectivity towards the MPP-13 protein, which makes it necessary to develop new, more effective and selective compounds.

Objectives. The main aim of the project was to design small-molecule compounds with potential MMP-13 inhibitory properties. Selective pyrimidine derivatives, which have optimal ADMET parameters, were proposed to bond with the active site of the MPP.

Materials and methods. We used modern software for modeling and predicting the properties of macromolecular protein systems. Based on the structure of known, active ligands, we designed a pharmacophore and proposed the lead structure using a pyrimidine scaffold. In the next stage, several dozen different compounds were selected. Using quantum-chemical and QSAR methods, we determined the physico-chemical properties of the analyzed compounds, and their pharmacokinetic and ADMET parameters. Taking into account the obtained results, we selected the most promising candidates. For them, the molecular docking was performed in order to predict their binding ability and selectivity to proteins of the MPP family.

Results. All designed compounds are able to bind to the binding pocket of MPP-13 and most of them interact stronger than the reference compound. In addition, all of the proposed compounds are characterized by high selectivity, binding constant with MMP-13 significantly higher than the one obtained for other considered proteins (MMP-1, MPP-4 and MPP-8). The origin of the protein-inhibitor complex stability included van der Waals forces and numerous π-type interactions. It is related to the structure of designed inhibitors and the nature of the binding site of enzyme. A detailed analysis of the binding mode of all proposed compounds showed that the interactions with Lys140, His222, Leu218, Thr245, and Thr247 amino acid residues seem to be crucial from the point of view of inhibition of MMP-13 activity.

Conclusions. The proposed compounds compounds could be used in the treatment of RA. Verification by the molecular dynamics simulation is mandatory before the synthesis of proposed compounds. In the near future, more complex studies are going to be performed to select the most promising ligand.

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Key words: computer-aided drug design, rheumatoid arthritis, metalloproteinase inhibitors

The change in blood lipid profile as a response to the oral glucose tolerance test in young people with metabolic syndrome: Primary results

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Conflict of interest
None declared

Abstract

Background. A significant factor for the development of lipid metabolism disorders is the dynamics and direction of changes in the levels and the residence time of individual lipid fractions in the bloodstream.[1]

Objectives. The study aims to search for characteristic profiles of changes in the concentrations of blood lipid fractions during the standard oral glucose tolerance test (OGTT) in young people with metabolic syndrome.

Materials and methods. The study involved 91 volunteers (aged 18–35) who underwent OGTT, with blood samples taken at 0 min, 30 min, 60 min, and 120 min. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), non-esterified fatty acid (NEFA), glucose, non-HDL, arterial blood pressure, body mass index (BMI), and waist circumference were measured. Metabolic syndrome (MS) was diagnosed based on 2022 criteria.[2] For statistical analysis, the analysis of variance (ANOVA) and the linear mixed models for repeated measurements were used.

Results. Metabolic syndrome was found in 9.9% of the study group. Lower values of total cholesterol and NEFA at subsequent measurement points were found in both analyzed groups compared to the fasting values. The LDL and triglycerides revealed lower concentrations in subsequent points of OGTT than in the fasting condition in the group without MS. Lower triglycerides for participants without MS at any time point and lower NEFA levels for subjects with MS under fasting conditions were found. For the MS group, in opposite to the time point 0', in subsequent time points, NEFA were consequently higher than in subjects without MS.

Conclusions. Differences in the blood lipid profile during OGTT were found between participants with and without MS. The longer and higher concentrations of triglycerides and NEFA in the bloodstream after glucose loading are associated with an unfavorable effect of increasing atherogenic particles in the bloodstream and the lipotoxic effect leading to the development of insulin resistance in MS participants.

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Key words: OGTT, metabolic syndrome, young people, lipid profile

Exosomes as components of the immune system

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Abstract

The history of exosome research dates back to 1983, but has only recently become the center of deeper scientific interest. Exosomes comprise a 30–150-µm-sized subpopulation of extracellular vesicles (EVs), which are lipid bilayer-bound structures released from basically all cell types into the extracellular space. Exosomes can be passed to other local or distant cells as intercellular communication mediators, and are able to alter behavior and functions of recipient cells due to the unique composition of bioactive molecules enclosed inside the vesicle, composed of proteins, RNA, DNA, lipids, and metabolites. Since exosomes are key players in cell-to-cell communication, they participate in both physiological and pathological processes.[1,2]
In this work, we focus on reviewing the role of exosomes in the context of the immune system function.
Among the roles of exosomes produced and released by immune cells, activation or inhibition of these cells, modulation of immune responses and antigen-presenting cell functions can be listed. They also regulate the transcription of numerous genes and cytokine expression, but the exact functions of immune cell-derived exosomes depend on the type of producing cell, dictated by its metabolic state. Since many types of immune cells activate specific responses in order to combat pathogens, exosomes released by these can be involved in the pathogenesis of inflammatory diseases, autoimmunity or allergies, but on the other hand may also act towards immune response suppression. Immune cell-derived exosomes have been shown to play a role in infectious diseases, transplant rejection and cancer development.[3] In addition, accumulating evidence demonstrates that tumor-derived exosomes are able to affect immune cell function.[4]
Owing to the fact that exosomes released from different cells of the immune system have distinct biomolecular contents under certain conditions, their protein contents might be useful as diagnostic markers. There are also methods to isolate exosomes from patient's immune cells to be injected as an autologous graft. It is suggested that immune-derived exosomes can find therapeutic application in a number of pathologies, including autoimmune diseases and cancer, but more research is needed in this field.[3]

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Key words: exosomes, immune system, intercellular communication

Metabolomics as a potential tool in the diagnostics of leukemia in children

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Abstract

Background. Metabolomics, which is profiling of metabolites formed in living cells, has become routinely applied as a tool for biomarker discovery. Metabolomics is used in many areas of medicine. The variability of biochemical processes in cancer cells in relation to healthy cells is essential for this type of research.

Objectives. Leukemia is the most common group of childhood cancers, classified into 4 main types: acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myeloid leukemia (CML). However, children are dominated by ALL, of which up to 85% are of B-cell lineage. Because of its asymptomatic nature and rapid progression, acute leukemia is usually diagnosed at active disease stage. A high tendency to metastasize makes CNS involvement at diagnosis a major clinical concern which requires immediate prophylactic CNS-targeted intrathecal chemotherapy, but with side effects.

Materials and methods. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) are the main analytical platforms used in metabolomics, often in combination with gas chromatography (GC-MS) or liquid chromatography (LC-MS). The bioinformatics phase involves data processing to extract biologically relevant information from the obtained data. The statistical analysis uses univariate and multivariate methods to the categorization and prediction of sample properties. Metabolomics method uses biological tissue, e.g., blood plasma, serum and cerebrospinal fluid.

Results. The known oncometabolites are usually involved in glucose, amino acids, lipid, and nucleotide metabolisms. Nineteen significant CSF metabolite–brain phenotype associations have been indentified.

Conclusions. The abnormal metabolite composition may reflect their significance in tumorigenesis and metastasis. Moreover, they have the potential to be biomarkers for future personalized cancer therapy. Our review provides insight into already known childhood leukemia metabolites, which might be a promising tool for diagnostic applications.

Key words: metabolomics, childhood leukemia, cerebrospinal fluid, metabolites, review

3D printed personalized molds for suppositories

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Abstract

Background. Personalized medicine enables to tailor treatments to the needs, characteristics and preferences of individual patients. The use of additive manufacturing can make it practical and affordable to prepare different solid pharmaceutical formulations for specific categories of patients (pediatric, geriatric and patients with certain needs). The subject of this work is the manufacture of molds for intraurethral, rectal and vaginal applications. In cases where specific molds are not available, there are difficulties in preparing the magistral preparations using indicated pharmacopoelial methods. A suppository may be only prepared by hand if maintaining the recipe conditions is to be ensured. However, this can result in problems regarding the required microbiological purity as well as in uneven distribution of active substances in each unit forms. It is important to note that people have varying needs and a universal approach may not work for every patient, potentially leading to reduced effectiveness of medication and unpleasant or ineffective applications. However, with 3D printing technology, personalized shapes can be produced, ensuring the criteria for suppositories are met.

Objectives. The aim of our research is to enhance the production of personalized suppositories through the use of designed 3D printed molds. The mold for the suppository is designed through 3D computer modelling and printed using a 3D printer. With a personalized mold, the production of suppositories can be done in a consistent and sanitary manner, allowing for their easy application without causing harm to the patient.

Materials and methods. Lipophilic and hydrophilic media were used as model substrates for testing the molds. The molds were produced using 3D printing from polymeric materials.

Results. Molds have been designed for intraurethral, rectal and vaginal applications. In addition, 2 urethral suppository formulations based on hydrophilic and lipophilic matrix were used to test the molds. Before pouring, the molds were coated with a substance to facilitate pulling them out.

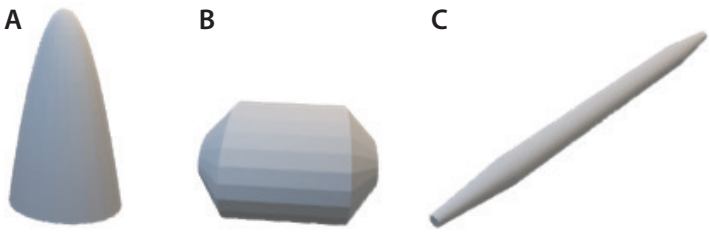

Fig. 1. A – computer-aided design (CAD) project of rectal suppositories; B – vaginal suppositories; C – urethral stick

Conclusions. The use of 3D printing makes it possible to produce molds that would provide an adequate manufacturing of suppositories granting individual application needs, which are not fulfilled by the currently available molds.

Key words: 3DP, personalized medicine, FDM, additive manufacturing

An experimental and theoretical study of 5-butyl-4,6-dimethyl-2-{[4-(*o*-fluorophenyl)-1-piperazinyl]-2-oxoethyl}-pyrrolo[3,4-*c*] pyrrole-1,3(2*H*,5*H*)-dione with anti-inflammatory activity

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None declared

Abstract

Background. Effective treatment of inflammation is one of the many challenges of modern medicine. The inhibition of constitutive cyclooxygenase-1 leads to numerous side effects, i.e., gastrotoxicity and nephrotoxicity. Therefore, new molecules that act selectively against cyclooxygenase-2 are constantly being sought because they would be a safer solution for patients.

Objectives. Our goals were the synthesis of 5-butyl-4,6-dimethyl-2-{[4-(*o*-fluorophenyl)-1-piperazinyl]-2-oxoethyl}-pyrrolo[3,4-*c*]pyrrole-1,3(2*H*,5*H*)-dione, evaluation of the biological properties, and theoretical study of the effect of the structure on anti-inflammatory activity.

Materials and methods. Based on the previously obtained series of compounds, we synthesized a new structure with a pharmacophore fragment proposed by D.S. Dogruer. The compound was obtained through direct alkylation of 3,4-pyrrolodicarboximide with (1-chloroacetyl-4-(*o*-fluorophenyl)piperazine) in DMF. The structure of the new molecule was confirmed with spectral data analysis such as ^{1}H NMR, ^{13}C NMR, FT-IR, MS, and crystallographic studies. A colorimetric inhibitor screening assay was used to determine its inhibitory potency towards COX-1 and COX-2. Molecular docking was performed to estimate the binding affinity of new compound and reference drugs for COX-1/COX-2 receptors. The QTAIM and the RDG methods were employed to reveal non-covalent interactions. Born–Oppenheimer molecular dynamics (BOMD) simulations were carried out for the complexes to obtain structures for further quantum-chemical analyses.

Results. The synthesized derivative shows a selective inhibition against COX-2 stronger than the Meloxicam reference. The application of RDG and QTAIM methods revealed the presence of intramolecular non-covalent interactions in the studied ligands. The BOMD allowed for the description of the dynamical nature of the binding site.

Conclusions. The titled compound, the new pyrrolo[3,4-*c*]pyrrole derivative after the Background of an oxoethyl fragment as a linker, shows selective inhibitory activity against COX-2. On the basis of in silico methods, it was shown that the most important type of non-covalent interactions responsible for ligand binding to the COX-2 human receptor were the weak hydrogen bonds as well as Met522, Leu352 and Val523 amino acids.

Key words: anti-inflammatory activity, COX-1/COX-2 activity, cyclic imides, pyrrolopyrroles, non-covalent interactions, molecular docking, Born–Oppenheimer molecular dynamics, DFT, QTAIM, RDG

The role of iron ions in cardiac muscle protection

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Conflict of interest
None declared

Abstract

Background. Cardiovascular diseases are one of the main causes of death in Poland, not only due to stress factors, low physical activity and poor diet. The most common cause of death is ischemic heart disease, which is responsible for the occurrence of episodes of ventricular fibrillation.

Objectives. The aim of the proposed research is to answer the question whether iron compounds, i.e., ethylenediaminetetraacetic acid (EDTA), sodium iron (III) salt and iron (III) citrate, have a protective effect in an in vitro setting after simulated damage to rat cardiomyoblasts (H9C2) and skeletal muscle cells (L6).

Materials and methods. Two cell lines – rat cardiomyoblasts (H9C2 line) and rat muscle cells (L6) – will be the model for research. The 1st stage of the research includes the evaluation of the cytotoxicity of iron compounds: iron (III) sodium salt and iron (III) citrate, with the following concentration range: 1–1000 μM. The next stage of the research will involve the use of microsecond high-amplitude electrical impulses to simulate myocardial damage/ablation by membrane electroporation (EP). The sensitivity of cardiomyoblasts exposed to EP-induced ablation will be assessed. Then, the protective effect of iron compounds after simulated damage to cardiomyocytes will be determined with MTT and clonogenic assay.

Results. As the concentration of iron compounds increased, the viability of H9C2 cells decreased. Mitochondrial activity of H9C2 cells was lower with prolonged incubation time with tested compound. Additionally, when the intensity of electric pulses increased, the viability of cells of tested lines decreased. The concentration of iron ions and the parameters used during EP determined the protective effect of iron ions on cardiac cells.

Conclusions. Our results revealed that iron compounds may contribute to improving function or minimizing damage to heart cells after they have been damaged. These findings open opportunities for further investigation into the specific mechanisms through which these compounds operate and how they can potentially be translated into therapeutic strategies to improve cardiac health and function.

Key words: myocardial cell damage, iron ions, myocardial protection, EDTA, iron (III) sodium salt, iron (III) citrate, H9C2

Quantitative analysis of meldonium using electrochemical methods

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Abstract

Background. Meldonium is commonly used as an anti-ischemic drug in Eastern European countries. In the sports environment, the drug is taken to enhance an athlete's performance and improve post-exercise recovery. Due to numerous reports of its performance-enhancing effects, meldonium was classified as a prohibited substance by the World Anti-Doping Agency (WADA) in 2016.

Objectives. The aim of the study was to investigate the electrochemical behavior of meldonium (3-(1,1,1-trimetylohydrazyn-1-ium-2-ylo)propanian) and develop a method for determining this substance, using voltammetry, ultimately in a synthetic urine solution.

Materials and methods. The adaptation of the experimental conditions involved determining the optimal pH for the measurements, selecting an appropriate working electrode, and defining the voltammetric parameters. Initial tests were conducted to observe the behavior of meldonium with a platinum electrode, glassy carbon electrode and gold electrode, but none of them provided a suitable voltammetric signal.

Results. The remaining studies were carried out using a gold electrode modified with poly-acetanilide (pNAANI) with adsorbed eriochrome black T (EBT) or methyl orange in a Britton–Robinson buffer solution. The detection threshold for the described modified electrodes was set at 6.141 µg/mL. The range of quantitation has been estimated to be about 300 µg/mL. The exact values depend on the method and time of preparation of the modified electrode.

Conclusions. Due to its structure, meldonium is difficult to determine with electrochemical methods, in particular voltammetric methods. For the determination of this substance, it is necessary to use modified electrodes. The developed method for determining meldonium requires further refinement, but it can be used for preliminary detection of meldonium in body fluids.

Key words: mildronate, meldonium, voltamperometry, doping in sport

Synthesis, structural studies, and molecular docking of isoxazole and oxazole derivatives with potential immunomodulatory and anti-inflammatory activities

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None declared

Abstract

Background. Immunomodulation is a therapeutic method used to modify the response of the immune system by increasing or decreasing the production of serum antibodies. It is focused on the manipulation of the processes of autoregulation of the immune system. This strategy is currently being applied in transplantology, oncology and the treatment of inflammatory, autoimmune and infectious diseases.[1] However, available immunomodulatory drugs show serious adverse effects of treatment. Therefore, the design of novel and improved alternative therapeutic agents is of extreme importance. Small molecule heterocyclic compounds containing isoxazole or oxazole rings exhibit a broad spectrum of biological activity, including immunomodulatory, antiviral, antibacterial, anti-inflammatory, and anticancer effects.[2,3]

Objectives. This study aimed to obtain low-molecular-weight compounds containing isoxazole or oxazole moieties due to the biological properties of their known heterocyclic derivatives. It is well known that they have great potential in drug discovery. The main goals were to propose synthesis pathway in order to prepare novel derivatives of isoxazole and oxazole with potential immunomodulatory and anti-inflammatory activities, and then to perform structural and in silico studies.

Materials and methods. First, the synthesis of the designed compounds was carried out using commercially available reagents. In the next step, the purification of all obtained compounds was performed using crystallization. The structural studies used MS, MS/MS, IR, and NMR (1D and 2D variants) methods. The physicochemical and drug-like properties of proposed derivatives were obtained using a well-known 3D QSAR platform for the prediction of features of potential drugs. Finally, molecular docking to tumor necrosis factor alpha (TNF-α) was performed to predict the possibility of binding. It is a known molecular target for immunomodulatory compounds released by monocytes and macrophages in response to immunological reactions that provides immunity to the body.

Results. The novel 4-substituted 5-amino-3-methylisoxazole and 2-substitued 5-amino-4-cyanooxazoles were obtained and purified. The chemical homogeneity of obtained compounds was determined with the TLC method. The analysis of data obtained with spectral methods confirmed their structures. According to the QSAR study, all compounds exhibit satisfactory ADMET properties. Additionally, they were found with high binding potential to TNF-α. They can interact with the receptor binding surface, which is composed mainly of aromatic tyrosine residues.

Conclusions. In this work, we have successfully designed, synthesized and characterized the new isoxazole and oxazole derivatives. These compounds were obtained with good yields. The results of molecular docking show that they may be interesting as potential immunomodulatory and anti-inflammatory agents. The evaluation of the biological effect of the obtained compounds will be the subject of further research.

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Key words: isoxazole derivative, oxazole derivative, structural studies, molecular docking

Clusterin glycosylation changes in the course of COVID-19

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Abstract

Background. Coronavirus disease 2019 (COVID-19), an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), still remains a global health challenge. Clusterin (CLU) is responsible for supporting the folding of secreted proteins and is involved in pro- or anti-apoptotic processes. It has been shown to be involved in many disease processes related to oxidative stress, including neurodegenerative diseases, cancer, inflammatory diseases, and aging.

Objectives. The aim of our study was the analysis of serum CLU glycosylation in the context of SARS-CoV-2 infection.

Materials and methods. The blood sera were obtained from COVID-19 patients (n = 87), convalescents (n = 50) and healthy subjects (control; n = 65). The profile and degree of CLU N-glycosylation was determined using a modified lectin-ELISA test with biotinylated lectins: MAA – detecting α2,3-linked sialic acid (SA), SNA – specific to sialic acid α2,6-linked, LCA – specific to sequences containing fucosylated tri-mannose N-glycan core sites, and lectins specific to antennary fucoses: LTA – specifically reacting with fucose α1,3-linked to GlcNAc, characteristic for $Lewis^x$ oligosaccharide structures, UEA – specific to antennary fucoses α1,2-linked to Gal and α1,3-linked to GlcNAc ($Lewis^y$ sugar structures).

Results. The obtained results are presented in Table 1.
The results of statistical analysis have shown significant differences in the CLU concentrations and in the relative reactivities of CLU N-glycans with lectins used between COVID-19 patients, convalescents and healthy subjects, with one exception – the expression of UEA-reactive fucose.

Table 1. Serum clusterin concentrations and relative reactivities of serum clusterin glycans with specific lectins

Groups	CLU [μg/mL]	Relative reactivity with lectins (AU)				
		SNA	MAA	LCA	LTA	UEA
COVID-19 n = 87	26.26 ±10.08 24.99 (18.20–31.88)	1.203 ±0.302 1.261 (1.003–1.446)	0.273 ±0.183 0.212 (0.139–0.366)	0.175 ±0.093 0.153 (0.112–0.214)	0.166 ±0.103 0.145 (0.109–0.198)	0.092 ±0.043 0.082 (0.067–0.112)
Convalescents n = 50	32.36 ±11.85 29.83 (22.13–38.15) $p^1 = 0.007441$	1.018 ±0.203 1.029 (0.849–1.113) $p^1 = 0.000150$	0.119 ±0.060 0.103 (0.083–0.143) $p^1 = 0.000001$	0.119 ±0.038 0.108 (0.091–0.141) $p^1 = 0.003390$	0.138 ±0.066 0.114 (0.094–0.190)	0.078 ±0.015 0.077 (0.071–0.087)
Controls n = 65	31.76 ±6.20 30.69 (27.37–35.12) $p^1 = 0.000100$	1.090 ±0.282 1.072 (0.862–1.328) $p^1 = 0.025120$	0.106 ±0.198 0.076 (0.055–0.108) $p^1 = 0.000000$ $p^2 = 0.005094$	0.088 ±0.044 0.082 (0.063–0.098) $p^1 = 0.000000$ $p^2 = 0.000453$	0.120 ±0.055 0.114 (0.082–0.145) $p^1 = 0.000996$	0.106 ±0.158 0.072 (0.063–0.101)

The relative reactivities of clusterin N-glycans with lectins were expressed in absorbance units (AU) as mean values ±SD, median and range (in brackets). Significant differences versus groups: [1] with COVID-19, [2] convalescents, and control (healthy subjects). SNA – *Sambucus nigra* agglutinin; MAA – *Maackia amurensis* agglutinin; LCA – *Lens culinaris* agglutinin; LTA – *Lotus tetragonolobus* agglutinin; UEA – *Ulex europaeus* agglutinin. Significant differences were accepted for a p-value of less than 0.05.

Conclusions. Our previous studies indicate the presence of changes in CLU glycosylation in diseases of various origins. In the present study, we observed that CLU concentrations, similarly like the expression in CLU of α2,3- and α2,6-linked SA and core fucose, differentiate COVID-19 patients from convalescents and healthy subjects. What is important, the expression of MAA-reactive SA and LCA-reactive core fucose in CLU glycans also significantly differ between control group of healthy subjects and convalescents, which makes these glycomarkers a promising diagnostic tool, the usefulness of which in routine diagnostics to differentiate COVID-19 patients, convalescents and healthy subjects should be the subject of future research.

Key words: COVID-19, serum clusterin, glycosylation, inflammation

The participation of carbamazepine-10,11-epoxide and 10-hydroxycarbazepine in the generation of oxidative stress in the course of epilepsy treatment

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Conflict of interest
None declared

Abstract

Background. The results of numerous studies suggest that long-term use of some antiepileptic drugs intensifies the formation of free radicals and oxidative stress (OS) in neurons. It is believed that some reactive metabolites of antiepileptic drugs may covalently bind to cell macromolecules and thus generate OS. Carbamazepine (CBZ) and oxcarbazepine (OXC) are antiepileptic drugs commonly used in neurological disorders. However, the role of both of these drugs in generating free radicals and consequently damaging neurons is not so obvious.

Objectives. The scientific aim of the study was to assess the participation of active metabolites, carbamazepine-10,11-epoxide (CBZE) and 10-hydroxycarbazepine (MHD) of 2 antiepileptic drugs – CBZ and OXC – in generating OS. The main assumption was to monitor the concentrations of active metabolites during therapy, which was to clarify whether and, if so, to what extent they are responsible for changes in the efficiency of the antioxidant potential. Biomarkers reflecting the generated OS in the course of treatment included the level of HMGB1 protein, the activity of myeloperoxidase (MPO) and the concentration of total glutathione (tGSH) and 8-isoprostaglandin F2α (8-isoPG).

Materials and methods. Seventy-one patients were qualified, including 21 treated with OXC for more than 6 months and 50 treated with CBZ. Determination of concentrations of active metabolites and selected markers was carried out in the secured blood, which allowed to confirm the relationship between the effects of CBZ-E and MHD and the generation of OS, based on the markers such as HMGB1, MPO, tGSH, and 8-isoPG.

Results. Mean HMGB1 levels of 5.7 µg/L (4.0–9.8 µg/L) for the group of patients treated with OXC and 15.1 µg/L (8.2–19.2 µg/L) for those treated with CBZ were determined. Mean MPO activities were 15.1 U/mL (7.3–23.4 U/mL) in the OXC and 35.7 µg/L (28.2–49.8 µg/L) in the CBZ group. The mean concentration of tGSH was 35.1 µmol/L (23.7–43.4 µmol/L) for the OXC and 19.9 µmol/L (8.2–23.3 µmol/L) for the CBZ group. Mean 8-isoPG levels of 255.1 pg/mL (212.0–276.8 pg/mL) in the OXC and 355.9 pg/mL (112.9–456.2 pg/mL) in the CBZ group were determined.

Conclusions. Significant correlations were found in all measurements of OS in the group of patients using CBZ, showing that the higher the concentration of CBZ, the stronger the generated oxidative stress observed in the measured levels of HMGB1, MPO, tGSH, and 8-isoPG. The data obtained in the group of patients treated with OXC were different. In this group, a significant dependence on the concentration of MHD was found only in relation to the level of HMGB1. Interestingly, an inverse relationship was found when measuring the level of 8-isoPG. The higher the MHD concentration, the lower the observed values of 8-isoPG. The results obtained in the group of patients treated with CBZ indicated the pro-oxidative nature of the drug. The results obtained in the group of patients treated with OXC no longer fully support this assumption, which could speak for its antioxidant nature.

Key words: oxidative stress, epilepsy, reactive metabolites, AED

Assessment of biocompatibility of modified poly(glycerol adipate) biomaterials with human fibroblasts in vitro

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Conflict of interest
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Abstract

Background. Poly(adipinyl)glycerol (PGA) is a biopolymer that can be used as scaffolding for regenerating tissues.

Objectives. The aim of this study was to assess the in vitro biocompatibility of modified biomaterials constructed from PGA towards human fibroblasts.

Materials and methods. The study was conducted on a primary cell line derived from a human gingival tissue fragment. The biomaterials used in the study were constructed from PGA, PGA with bioactive glass, and PGA with bioactive glass and L-lysine. The growth rate and cytotoxicity were determined using the PrestoBlue assay. The level of methylated histone H3 was examined through immunofluorescent staining. The migration speed of fibroblasts in the presence of biomaterials was assessed using the wound healing assay.

Results. Biomaterials made of PGA, PGA with bioactive glass, and PGA with bioactive glass and L-lysine showed no cytotoxicity towards human fibroblasts. In the PrestoBlue assay, the biomaterials exhibited a stimulating effect on fibroblast proliferation.

Conclusions. The biomaterials did not significantly reduce the migration rate of fibroblasts.

Key words: biomaterials, biocompatibility, poly(adipinyl)glycerol, bioactive glass, fibroblasts

Can electroporation enable the "training" of immune system cells for targeting cancer cells?

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Abstract

Background. Immunooncology is the latest field at the intersection of medicine and cell biology, focusing on stimulating the immune system (IS) to combat cancer cells. A healthy IS recognizes and destroys cancers, but unfortunately, these cells have developed mechanisms that make them resemble normal human cells to avoid detection by the IS.

Objectives. The main goal of this research is the application of electroporation (EP), which involves short electrical impulses capable of rearranging the cell membrane structure to stimulate the IS to fight cancer cells. The hypothesis assumes that EP will stimulate the activation of molecules appearing on the surface of IS cells and/or enhance the secretion of factors by IS cells into the cancer environment. As a result, IS cells will more effectively eliminate cancer cells.

Materials and methods. The research model consisted of murine colon cancer cells CT26Wt and murine macrophages P388. Nanosecond electrical impulses (5–9 kV/cm, 10 ns, 200; $\pm Ca2^{+}$) were used to stimulate P388. Observations of the interactions between different cells were conducted using specialized equipment, including a 3D Cell Explorer Fluo holotomographic microscope (Nanolive, Tolochenaz, Switzerland) and a confocal microscope Mica (Leica, Wetzlar, Germany). Activation markers of IS were determined using immunofluorescence. The expression level of these markers, such as surface factors (IL-10, IFN-γ, etc.), corresponds to the readiness of IS cells to combat pathogens.

Results. The analysis revealed changes in marker expression on P388 cells due to EP. Preliminary observations also suggest greater cytotoxicity of macrophages after EP than after CaEP. However, further research and testing of a broader range of parameters are necessary.

Conclusions. Immunooncology is a relatively new field, but it already shows promising results in cancer treatment. Further research and technological advancements may help develop even more effective treatment methods. In the future, immunooncology may become a key element in the fight against cancer and many other diseases.

Key words: macrophages, cancer cells, electroporation, stimulation, cell death, immunooncology

Effects of alternating magnetic field on the expression of activation markers CD154 and CD69 in Jurkat cell line

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Abstract

Background. In the realm of oncology, the exploration of physical methods of modifying membrane antigens attracts considerable interest. A relatively novel approach involves the utilization of magnetic fields in cancer therapy, particularly in conjunction with ferromagnetic compounds. These compounds serve as magnetosensitizers, enhancing therapeutic effects. Additionally, magnetic fields influence metalloenzymes, potentially offering a means of cell modulation. This investigation seeks to assess the viability of utilizing alternating magnetic fields (AMF) to activate the Jurkat cell line.

Objectives. This study aims to evaluate the potential of AMF in activating the Jurkat cell line. It seeks to establish the optimal conditions for AMF treatment and subsequently examine the impact of such treatment on Jurkat lymphocytes, focusing on MMP-2 activity, immunophenotype, cellular viability, and proliferation.

Materials and methods. The experimental approach involves exposing Jurkat lymphocytes to 10 mT AMF. The study begins by determining the appropriate duration of irradiation. Subsequent steps include assessing MMP-2 activity using enzymatic assays, analyzing cell immunophenotype through flow cytometry, conducting viability tests, and simulating magnetic field treatment conditions to ensure minimal impact on cells.

Results. The flow cytometry analysis encompassed activation markers CD69 and CD154, as well as other indicators such as CD7, CD25, CD95, CD61, and CD162. Alongside this, cell proliferation, ATP content and MMP-2 activity were evaluated. Prior to experimentation, careful optimization of AMF treatment conditions was undertaken to ensure the influence of alternating electric fields without heat-induced effects. Optimal irradiation time was established at 60 min, correlating with decreased MMP-2 activity and diminished fluorescence signal. Findings suggest that Jurkat cells can be activated via magnetic fields, leading to the overexpression of CD69 and CD154. Elevated CD95 and CD25 expressions were attributed to MMP-2 deactivation, whereas CD61 integrin expression decreased. The CD162 and CD7 molecules exhibited no notablealterations in expression.

Conclusions. A hypothesized mechanism involves AMF interference with metalloproteinases, thereby influencing the expression of membrane antigens regulated by these enzymes. The technique of AMF treatment for lymphocyte activation displays promise, warranting further exploration through additional research endeavors in this domain.

Key words: alternating magnetic field, activation markers, Jurkat cell line

Evaluation of antitumor activity of lipopeptide biosurfactant with resveratrol

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None declared

Abstract

Background. Cancer, especially malignant melanoma and colorectal variants, continues to challenge global health due to rising morbidity and limited treatments, often accompanied by severe side effects. With an urgency to discover effective therapies, research highlights the potential of natural compounds, notably lipopeptide biosurfactants such as surfactin. Surfactin's unique properties make it a potential therapeutic agent with lesser side effects than synthetic counterparts.

Objectives. This study explores the potential of biosurfactant and polyphenol, specifically surfactin and trans-resveratrol, in the context of cancer cell viability, migration and apoptosis. The research focuses on 2 cancer cell lines, melanoma A375 and human colorectal adenocarcinoma HT-29, and normal human dermal fibroblasts (NHDF) cell line.

Materials and methods. Investigating the therapeutic potential of both compounds, we assessed their cytotoxic effects utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The influence on cellular migration was evaluated through the scratch healing assay. Further insight into the induction of apoptosis in treated compared to untreated cells was garnered using qRT-PCR.

Results. The combined application of surfactin and trans-resveratrol in both A375 and HT-29 cell lines resulted in a significant reduction in cell viability compared to the controls. However, the decrease in viability of cancer cell lines was similar to that observed when surfactin and trans-resveratrol were used alone. In the treatment of NHDF, using a combination of these compounds in lower concentrations as well as the half maximal inhibitory concentration (IC_{50}) values that were determined for cancer cell lines, a lesser impact on viability was observed.

Conclusions. The study concluded that surfactin and trans-resveratrol could potentially be used as therapeutic agents in cancer treatment due to their ability to induce apoptosis in cancer cells and inhibit cell migration. The research also highlighted the importance of further studies to fully understand the mechanisms of action of these compounds.

Key words: biosurfactants, polyphenols, surfactin, trans-resveratrol, cell viability, cell migration, apoptosis, cancer cell lines, A375, HT-29, MTT viability assay, scratch/wound healing assay, NHDF cell line, Bax and Bcl-2 proteins, qRT-PCR analysis

Spectroscopic and molecular docking study of the simultaneous binding of doxorubicin and cyclophosphamide to human serum albumin

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Conflict of interest
None declared

Abstract

Background. Human serum albumin (HSA) is the main carrier of free drugs and the most abundant protein in human blood plasma. It plays a crucial role in drug transport and pharmacological efficacy as well improves solubility of bounded hydrophobic drugs. Ligand binding may lead to protein structural alterations and modify its biological functions; therefore, studies of the interactions between anticancer drugs and HSA are necessary from the point of view of pharmacology and clinical practice.

Objectives. In this work, we investigate the interaction of HSA with doxorubicin in the presence of cyclophosphamide. Both drugs are the base of many anticancer therapies and the effect of their simultaneous binding to HSA has not been studied so far.

Materials and methods. The interaction of HSA with doxorubicin and cyclophosphamide was determined under pseudophysiological conditions using fluorescence and circular dichroism spectroscopy, electrophoretic light scattering, and molecular modelling.

Results. Fluorescence analysis proved that the HSA-Cyp-Dox complex was formed. The K_{sv} and K_a values for the system are $1.60 \cdot 10^4 M^{-1}$ and $4.57 \cdot 10^4 M^{-1}$, respectively. Probably only 1 binding site exists for the drugs, and hydrophobic or ionic interactions are predominant in the interaction. The CD results showed that the binding of the drugs do not significantly affect the protein secondary structure. The distance between donor and acceptor in the HSA-Cyp-Dox system was found to be 3.67 nm. Zeta potential analysis confirmed the existence of the hydrophobic interaction and some instability of the protein–drug complex. According to molecular docking study, in both cases, doxorubicin binds to the same site of albumin with very similar free energy of biding. However, the origins of stabilization and the binding modes of Dox in the Sudlow site I of HSA are different.

Conclusions. Analyzed drugs can be stored and carried by the HSA in human body. Simultaneous administration of cyclophosphamide and doxorubicin can affect their LADME, which is of great importance during multidrug anticancer therapy.

Key words: anticancer drugs, doxorubicin, cyclophosphamide, human serum albumin, protein–drug interactions, spectroscopic and molecular analysis

Evaluation of the expression of folic acid receptors in ovarian cancer after chemotherapy: In vitro research

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Conflict of interest
None declared

Abstract

Background. Ovarian cancer has the highest mortality malignancy in female gynecological tumors. It is a considerable challenge for doctors due to the late diagnosis of the disease and increasing resistance to standard treatment. The overexpression of folic acid receptors (FRα), found in 90% of ovarian cancer cells, is essential in the search for new diagnostic and treatment methods targeting cells with high expression of this receptor. Previous studies confirm the high affinity of folic acid-bound substances to FRα.

Objectives. The study aimed to evaluate the impact of chemotherapy using cisplatin on folic acid expression in ovarian cancer cells.

Materials and methods. The studies were performed on the hamster ovarian cell line (CHO-K1) and 2 human ovarian cancer cell lines (OVBH-1 and SKOV-3). For various concentrations of cisplatin, cell viability was determined with the MTT test. The expression of the folic acid receptor after experiments was assessed with the immunocytochemical test.

Results. As the concentration of cisplatin increased, the viability of cells of all tested lines decreased. After 24 h, the IC50 for the CHO-K1, OVBH-1 and SKOV-3 cell lines was 30.44 μM, 26.66 μM and 51.85 μM, respectively, while after 72 h, they achieved 10.67 μM, 10.08 μM and 23.79 μM. The CHO-K1 cell line showed slight expression of the FRα receptor with low intensity of staining. The SKOV-3 and OVBH-1 cell lines overexpressed the FRα receptor. The effect of cisplatin on all the tested cell lines increased the expression of the folic acid receptor. The longer the incubation time in the test compound, the more significant the impact.

Conclusions. The effect of cisplatin on ovarian cancer cell lines increased the expression of the FR-α. The impact was more pronounced after the longer incubation time with the drug. More research is needed in this field.

Key words: ovarian cancer, folic acid receptors, cisplatin

Zoonotic and anthropozoonotic diseases in Poland: A concurrent occurrence study

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Abstract

The pronounced surge in interest surrounding zoonotic infectious diseases can be attributed to humanity's historical encounters with cyclically recurring pandemics. This engenders a multifaceted concern that intertwines animal and human health, propelled by an intricate web of pathogen dissemination through vectors comprising viruses, bacteria and parasites. The interconnectedness of the animal–human interface has delineated 2 critical terminological distinctions: zoonoses and anthropozoonoses. This nuanced division is at the nexus of investigation for both veterinary practitioners and clinicians specializing in human infectious maladies.

Recent years have seen an intensified contemplation of pathogenic dispersion within the environmental milieu, as well as a more discerning analysis of the underlying predisposing factors. These deliberations are amplifying the momentum within the clinical research domain, fostering a burgeoning landscape for therapeutic interventions tailored to these intricate entities.

Harnessing an extensive array of scholarly sources, this discourse orchestrates visual narratives that encapsulate the cardinal symptomatic presentations of zoonotic afflictions across both human and animal populations. Moreover, the narrative pivots to underscore seminal revelations in the identification of specific infectious disease occurrences that are contextualized within the Polish milieu. This scholarly exposition strategically employs scientific illustrations as a didactic tool to project the salient insights distilled from meticulous analysis.

The purview of human health resonates beyond the medical sphere, reverberating in societal and political echelons. This resonance is especially poignant amid the contemporaneous backdrop of the coronavirus disease 2019 (COVID-19) pandemic and the geopolitical tensions tracing Poland's eastern border.

The presented corpus of data proffers ramifications not solely confined to the theoretical realm but also permeating the realm of clinical praxis. Delivered in the form of a didactic visual exhibit, these insights command attention to ailments meriting consideration in the differential diagnostic schema, pertinent to both the veterinary and medical cadres. In the context of veterinary practitioners, who operate in close contact with animal custodians, human symptoms stand as sentinel signposts guiding accurate diagnosis in treating animals. Furthermore, these findings assume a pivotal role in galvanizing a synergistic dialogue between veterinary practitioners and primary care physicians, serving as informational nuclei in scenarios shadowed by suspected infectious disease emergence.

Key words: zoonosis, anthroponosis, infectious diseases, veterinary science

Methods for determining the concentrations of neutrophil elastase and S100B protein: Potential predictors of neurological disorders in the course of COVID-19

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Conflict of interest
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Abstract

Background. In the course of coronavirus disease 2019 (COVID-19), inflammatory changes in the lungs and a wide spectrum of organ complications, including cardiovascular events, are common, and appear to be caused by a "cytokine storm", with an increased inflammatory and immune response. The increased incidence of thromboembolic events, myocardial damage and acute coronary syndromes, acute heart failure, acute kidney and liver damage, and blood pressure fluctuations complicate the course of COVID-19. Pilot studies show that potential predictors of neurological disorders in the course of COVID-19 may include neutrophil elastase and S100B protein.

Objectives. The aim of the study was to perform a quantitative analysis to determine the concentration of neutrophil elastase and S100B protein in the blood sera of patients suffering from COVID-19. To make this possible, it was necessary to select methods that would allow the determination of these proteins in the tested biological material. The effectiveness of available ready-made kits was checked and the results obtained in the enzyme-linked immunosorbent assay (ELISA) test developed by our team were compared.

Materials and methods. The study analyzed the medical documentation from the COronavirus in the LOwer Silesia (COLOS) registry including data from 187 patients admitted and treated at the university and temporary COVID-19 hospital organized by the University Medical Hospital in Wrocław (Poland). Concentrations of neutrophil elastase and S100B protein were determined using an enzyme-linked immunosorbent method. Commercial human immunoassay ELISA kits were obtained from Sunlongbiotech: human neutrophil elastase ELISA Kit, (SL1269 Hu) and human protein S100-B ELISA Kit (E2200 Hu). The manufacturer's instructions were followed. Self-optimized tests used: standard proteins – human neutrophil elastase (324681-50UG, Merck), primary antibodies – goat anti neutrophil elastase monoclonal antibody (ELA10-101.5, No. MA1-10608; Thermo Fisher Scientific, Waltham, USA) and secondary antibodies – goat anti-mouse IgG1 Secondary Antibody(HRP, 1 mL, No. # PA1-74421; Thermo Fisher Scientific).

Results. The results obtained for the determination of neutrophil elastase using a ready-made kit were completely unreliable, as non-specific effects were found (the products were subject to a complaint procedure). Therefore, an attempt was made to develop and optimize the method using the self-ELISA test. The obtained results were promising and the work is currently underway to optimize the method. The concentration results obtained using the S100B protein assay kit (SL1269 Hu) were reproducible and this method was considered reliable.

Conclusions. Determining the concentrations of various compounds using ready-made ELISA kits is a convenient, although often more expensive, enzyme-linked immunosorbent method. They do not require validation and the obtained are reliable. However, it is better to check each test before using it for planned research, as they sometimes show non-specific effects. The test used to determine the S100B protein showed that the concentration of this protein is significantly higher in some patients with COVID-19. Self-developed ELISA test allows not only to significantly reduce the costs of experiments, but also to control each stage of the test. However, it requires optimization of incubation times and concentrations of all analytes and tested biological material. The test for the determination of neutrophil elastase developed by our team allows for the determination of the concentration of this protein in the tested material. The next stage of the research will be statistical analysis.

Key words: COVID-19, neutrophil elastase, ELISA, S100B

Antitumor activity and mechanisms of resistance to olivacine

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Abstract

Background. One of the compounds known for its anticancer activity (discovered in the 1960s) is ellipticine. It occurs naturally in several trees in the genera Ochrosia, Rauvolfia, Aspidosperma, and Apocynaceae. Despite ellipticin's anticancer properties, it has low bioavailability and solubility, which limits its use. Olivacine (1,5-dimethyl-6*H*-pyrido[4,3-b]carbazole) (Fig. 1) is a natural alkaloid, an isomer of ellipticine, first isolated in 1958 from the bark of *Aspidosperma olivaceum Müll Arg*., an evergreen tree native to Brazil. Alkaloids present in plants of the Aspidosperma genus exhibit antipyretic, analgesic and antimicrobial properties. Aspidosperma bark extracts are used in traditional medicine to treat malaria.

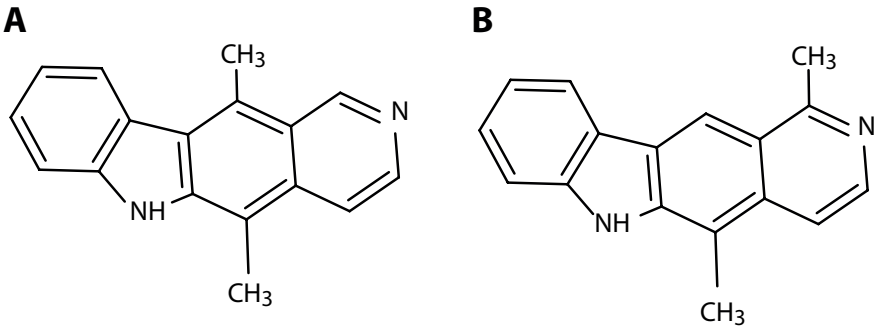

Fig. 1. A – ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-b]carbazole); B – olivacine (1,5-dimethyl-6*H*-pyrido[4,3-b]carbazole)

Objectives. The purpose of this study was to determine the antitumor activity of olivacine and to investigate the mechanisms of resistance to this drug in a cell culture model.

Materials and methods. The study was conducted on a panel of tumor lines: A549, MCF7, LoVo, and LoVo DX. The study used SRB and MTT assays to examine the in vitro anticancer activity of olivacine and ellipticine. Staining of intracellular vesicles was also performed.

Results. Both compounds showed cytotoxic effects on various cell lines, particularly on the doxorubicin-resistant LoVo/DX cell model, with olivacin's cytotoxicity being about 3 times higher than that of doxorubicin.

Conclusions. Olivacine showed differential cytotoxicity against a panel of cancer cells. The analysis of the exocytosis process of the tested compounds indicates the need to search for derivatives with comparable anticancer activity, but ones that will not be subject to removal from cells by exocytosis.

Key words: olivacine, cell cultures, intracellular vesicles

Influence of microsecond pulsed electric field on exosome production and their immune activity

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Conflict of interest
None declared

Abstract

Background. Exosomes are extracellular vesicles secreted ubiquitously by every cell type, which mediate cell communication and shuttle their cargo which can influence target cell function. They are crucial to cancer development as they mediate the establishment of the tumor microenvironment (TME) . Tumor-derived exosomes (TEx) are produced in high amounts in response to hypoxia. The microsecond pulsed electric field (μsPEF) is also a stressor that could influence TEx production. However, there is no knowledge about what the immune activity of μsPEF-induced TEx is.

Objectives. The study is aimed to identify the immune activity of the exosomes produced by MC38 cells cultured in normoxia and hypoxia conditions following different parameters of μsPEF.

Materials and methods. The MC38 cells were electroporated in 4 different parameters delivering 8 pulses with 1-second interval; 1200 V 0.1 ms (I), 1200 V 0.5 ms (II), 600 V 0.1 ms (III), and 600 V 0.01 ms (IV) before being cultured in hypoxia and normoxia for 48 h. The supernatant was then collected for exosome isolation using size exclusion chromatography (SEC). Finally, TEx sample protein concentration, TEx size profile and TEx influence on the activity of dendritic cells were analyzed.

Results. The MC38 cells treated with II parameter of μsPEF were characterized by the highest propidium iodide internalization and the lowest viability among all tested groups. However, the rate of exosome production was the greatest in this group. These exosomes also activated DCs better than control exosomes produced by unelectroporated cells both in hypoxia and normoxia conditions. Cells cultured in normoxia after application of III and IV parameters produced exosomes with higher potential to induce DC maturity compared to controls. Nonetheless, exosomes from the same groups produced in hypoxia condition caused the reduction of costimulatory antigens on DC surface.

Conclusions. The microsecond pulsed electric field is efficient in inducing the production of TEx with immunostimulatory activity. However, the final effect depends on the cell culture conditions.

Key words: microsecond pulsed electric field, MC38 murine colon carcinoma, tumor-derived exosomes, hypoxia

Properties of materials used for the fabrication of occlusal splints: Comparison of various technologies and the effect of artificial aging

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Conflict of interest
None declared

Abstract

Background. Occlusal splints are intraoral devices used as an important component of the noninvasive management of bruxism and temporomandibular disorders (TMDs). They can be applied for occlusal positioning or stabilization, as well as for the prevention of tooth wear. The growing popularity of occlusal splints creates a need of searching for new, convenient methods of manufacturing, which will provide mechanically stable and biologically safe splints, resistant to the influence of the long-term usage.

Objectives. The aim of this in vitro study was to compare the mechanical behavior of specimens manufactured via 3 techniques used for the fabrication of occlusal splints. The changes in the materials' properties upon the artificial aging were also analyzed.

Materials and methods. Three types of materials were tested: (a) a conventional heat-curing acrylic resin (Villacryl H Plus), (b) a material processed using thermoforming (Duran) combined with a light-curing resin (Durasplint LC), and (c) a photopolymer resin for 3D printing using stereolitography method (Dental LT Clear). Disc-shaped specimens were fabricated for a Shore D hardness evaluation, and bar-shaped specimens – for a flexural properties evaluation ($n \geq 15$ for each group). The comparison of the properties of the non-aged and artificially aged materials was performed.

Results. The resin for 3D printing had the highest Shore D hardness among all non-aged materials, while the conventional heat-curing acrylic resin had the highest flexural strength and modulus. Properties of all tested materials were significantly deteriorated due to the artificial aging. The heat-curing acrylic was most resistant to changes due to a prolonged water storage.

Conclusions. The mechanical properties of the compared materials differ significantly. Currently, conventional heat-curing material for the manual method of occlusal splint manufacturing still seems to provide the best clinical performance with the lower risk of splint deformation or fracture. The development of novel materials, less susceptible to aging, is still necessary.

Key words: dental materials, biomaterials, oral splint, polymer, 3D printing, thermoforming

3D-smart printing of hydrogels with itraconazole

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Conflict of interest
None declared

Abstract

Background. Treatment of fungal infections in patients is problematic due to predispositions related to the weakening of the body's immunity and defense mechanisms, as well as deterioration of general health in the course of chronic diseases. Itraconazole belongs to the pharmacotherapeutic group of third-generation antifungal drugs with an azole structure (the chemical name: 2-butan-2-yl-4-[4-[4-[4-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one).

Objectives. Itraconazole (67 μg/g) was imprinted into a hydrogel matrix containing Pluronic-127 polymer and additionally enriched with nanohydroxyapatite. The wet and elastic structure of 3D printing hydrogel was maintained by the presence of strongly hydrophilic polyethylene glycol 400.

Materials and methods. The ability to release itraconazole from the hydrogel 3D printing and the effectiveness of the action on selected fungal strains were assessed with inhibition zone measurement.

Results. The experiment has confirmed the antifungal effect on *Candida albicans* and *Candida tropicalis*. The release of itraconazole depended on the presence of nanohydroxyapatite, which affected the physical parameters (increasing the viscosity of the hydrogel material) and the porous structure of the 3D print (based on SEM images).

Conclusions. The 3D printing design strategies for hydrogel materials with antifungal drugs may be a good approach for their application in the external formulations.

Key words: itraconazole, 3D-smart printing, hydrogel

Interaction of trimetazidine dihydrochloride with human serum albumin by spectroscopic methods

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Conflict of interest
None declared

Abstract

Background. Trimetazidine (1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride) is an antianginal drug that has been used in treatment since the 1960s. Since this drug is often recommended to patients with various diseases, it is worth considering what effect it may have on biodistribution in the blood, considering 2 forms of albumin: native and modified by the glycation process.

Objectives. The aim of the study was to determine the affinity of trimetazidine to native human albumin and glycated form of albumin, and to investigate the thermodynamics of their interaction.

Materials and methods. The interaction of trimetazidine with human albumin was investigated using the UV-pH titration method combined with the UV-Vis absorption, the circular dichroism and fluorescence spectroscopy.

Results. The hydrophobic interactions and the electrostatic interactions are the main forces for the binding of trimetazidine to albumin with the static quenching mechanism. Trimetazidine bounds reversibly and displays moderate affinities for the protein. The binding constants are 3.00×10^4 M^{-1} for native albumin and 1.05×10^4 M^{-1} for glycated albumin, respectively. The secondary structure of albumin after incubation with trimetazidine showed only slight changes on the secondary structure of the protein.

Conclusions. Experimental results indicate that the biodistribution of the trimetazidine may be affected by differences in protein affinity.

Key words: albumin, trimetazidine dihydrochloride, spectroscopic methods

Selected inhibitors of carbonic anhydrase: Investigations of non-covalent interactions based on Car–Parrinello and density functional theory

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Abstract

Background. Carbonic anhydrases (CA) can be considered one of the most important types of metalloenzymes found in nature.[1,2] Their main role is the reversible hydration reaction of carbon dioxide with water, which leads to the formation of hydronium ion and bicarbonate.[3] Finding an inhibitor for this group of enzymes could result in elucidating many of the harmful processes and diseases currently plaguing humanity, such as obesity, hypertension, cancer, antibiotic-resistant infections, and glaucoma.[4] Computer-aided drug design methods are helpful in design of new molecules with desired properties.

Objectives. Four potential inhibitors were chosen – acetazolamide, dichlorophenamide, methazolamide, 2-benzisoxazol-3-ylhnethanesulfonamide – to study non-covalent interactions in vacuo, with solvent reaction field and in the crystalline phase. The obtained results will allow us to understand the nature of the interactions between inhibitor molecules, as well as their strength.

Materials and methods. Quantum-chemical simulations were performed using Car–Parrinello molecular dynamics (CPMD) and density functional theory.[5,6] The topology of the electron density of studied inhibitors was analyzed on the basis of Quantum Theory of Atoms in Molecules (QTAIM).[7] The symmetry-adapted perturbation theory (SAPT) was applied for the energy decomposition in the studied dimers.[8]

Results. It was found that in the all studied CA inhibitors, the bridged proton in the hydrogen bond is localized at the donor side. Results from CPMD showed differences in the hydrogen bond strength and dynamics, which is of importance in the modulation of intermolecular properties. The spectroscopic analysis based on power spectra of atomic velocity indicated regions involved in the N-H stretching. The QTAIM results confirmed the presence of intermolecular hydrogen bonds. The energy decomposition results revealed that electrostatics and dispersion contributions to the interaction energy play a crucial role in the dimers of the investigated CA inhibitors.

Conclusions. Quantum-chemical investigations in different states of aggregation are important in the accurate understanding of intermolecular interactions. The use of, e.g., CPMD in combination with static methods allow a broader view of the aspect, which is of great importance in rational design of new compounds.

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Key words: CA inhibitors, crystalline phase, AIMD, CPCM, QTAIM, SAPT

Binding interaction of new N-Substituted 1*H*-Isoindole-1,3(2*H*)-Dione derivatives with ctDNA

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Abstract

Background. Heterocyclic compounds play an important role in many biological processes of the human body. Due to its reactivity, indoles can be easily modified to prepare new compounds with potential drug applications. DNA is one of the main biological macromolecules in organisms and the target of many drugs. After entering the human body, the drug can interact with DNA. Many diseases such as cancer, leukemia and some nerve diseases are closely related to the genetic inheritance and damage of DNA.[1]

Objectives. Isoindole-1,3-dione derivatives show various types of biological activities such as anti-inflammatory, analgesics, cholinesterase inhibitors, or anticancer properties.[2] The aim of this work is to investigate whether 6 new isoindole-1,3-dione derivatives interact with DNA and ctDNA, and also to determine the mechanism of this interaction.

Materials and methods. Spectroscopic methods such as UV-VIS, fluorescence spectroscopy and circular dichroism were applied. Markers were used to determine the mechanism of interaction, i.e., intercalation or groove binding. Molecular docking modelling was also performed.

Results. The results showed that the studied compounds probably interact with DNA through a mixed mechanism: the main part of the molecule could interact in a groove, and planar moiety in an appropriate position, after rotation, could intercalate between base pairs.

Conclusions. N-Substituted 1*H*-Isoindole-1,3(2*H*)-dione derivatives may be prospective anticancer compounds, but further in vitro study is required.

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Key words: isoindole-1,3-dione derivatives, ctDNA, spectroscopic methods, molecular docking

Vitamin D receptor gene polymorphism and the risk of metabolic syndrome in patients with ALL treated in childhood and adolescence

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Abstract

Background. Acute lymphoblastic leukemia (ALL) treatment might adversely affect the development of body weight – both carbohydrate and lipid metabolism. Risk factors for the development of metabolic syndrome (MetS) may be deficiency of vitamin D and polymorphisms of the gene of the receptor for vitamin D (VDR). The study aimed to assess the impact of *VDR* gene polymorphisms (BsmI (rs1544410), Fok1 (rs2228570), ApaI (rs7975232), TaqI (rs731236)) on the incidence of metabolic disorders in ALL survivors.

Materials and methods. The study group: 92 patients – ALL survivors (S-ALL) aged 5–30 years. The control group: 89 healthy children of the same age. The ALL patients were treated with protocols ALL IC-BFM 2002 or 2009.

Results. The MetS occurred significantly more frequently in S-ALL than in the control group (15.15% compared to 4.05%). It was diagnosed only in male S-ALL (10 of 44), but in the control group in only 3 out of 74 individuals. The S-ALL had higher HOMA-IR index (4.51 compared to 3.30), fasting insulinemia (19.9 µIU/mL compared to 14.9 µIU/mL) and vitamin D concentration (27.5 ng/mL compared to 25.3 ng/mL) compared to the control group. The S-ALL with MetS had significantly lower vitamin D concentration than S-ALL without MetS. The MetS was diagnosed significantly more often in the individuals within the S-ALL group, characterized by the following genotype: TT polymorphism TaqI ($p = 0.033$), GG polymorphism BsmI ($p = 0.020$) and GG polymorphism ApaI ($p = 0.047$) compared to the control group. A lower concentration of HDL, which is defined as abnormal, was detected significantly more frequently in the individuals exhibiting GG polymorphism in the S-ALL group in comparison to the individuals exhibiting GG polymorphism in the control group ($p = 0.042$).

Conclusions. Patients who were treated for ALL during childhood and adolescence have an increased risk of developing MetS. Male sex predisposes to MetS. Vitamin D concentration appears to be a predisposing factor for the development of MetS in patients treated for ALL (S-ALL). The presence of *VDR* gene polymorphisms such as TaqI [rs731236], BsmI [rs1544410] and ApaI [rs7975232] might be a predisposing factor for the development of MetS in patients with ALL. Early identification of people with predisposing metabolic disorders *VDR* gene polymorphism should be the basis for taking preventive measures – implementing an appropriate lifestyle and more intensive physical activity – to minimize the effects of those disorders in patients treated for ALL, and also to contribute to the initiation of personalized therapy.

Key words: vitamin D receptor gene polymorphism, metabolic syndrome, ALL

Fullerenes as a pro-/antioxidants in breast cancer chemotherapy: In vitro studies

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Abstract

Background. Chemotherapy is one of the main approaches in cancer treatment. However, its effects on both cancerous and normal cells continue to challenge clinical practice. Nanomedicine is seeking a solution to the targeted delivery of anti-cancer drugs. Nanoformulations containing fullerenes C_{60} have dual characteristics – they can be both pro- and antioxidants, thus yielding a range of medical applications.

Objectives. The purpose of this study is to demonstrate the dual role of fullerenes in chemotherapy – protective against non-cancerous cells and toxic against cancer cells.

Materials and methods. The MCF-10a non-cancerous and MCF-7 breast cancer cells were treated with C_{60}, doxorubicin (DOX) and C_{60}-DOX. The C_{60} and C_{60}-DOX were subjected to size and zeta potential measurements. Proliferation of both cell lines and toxicity of treating agents were observed on a real-time cell analyzer for a minimum of 72 h. The MCF-10a and MCF-7 treated with C_{60}, DOX and C_{60}-DOX were lysed after 72 h, and catalase (CAT) activity and total antioxidant capacity (TAC) were determined.

Results. The C_{60}-DOX treatment of MCF-7 resulted in significantly lower cell proliferation compared to the cells treated with DOX alone, significantly higher toxicity compared to the cells treated with DOX alone, significantly higher CAT activity than the cells treated with DOX alone, and no significant change in TAC levels. The different behavior was observed for MCF-10a cells, whose C_{60}-DOX treatment resulted in significantly higher proliferation compared to the cells treated with DOX alone, significantly lower toxicity than the cells treated with DOX alone, no significant changes in CAT activity, and significantly higher TAC levels than those in the cells treated with DOX alone. Interestingly, a significantly higher proliferation rate of cells treated with C_{60} alone compared to untreated cells was noted for both lines.

Conclusions. This report, combined with our previously published results, indicates that C_{60} may behave in 2 ways – reducing DOX toxicity to non-cancerous cells and promoting their antioxidant defense mechanisms, at the same time increasing DOX toxicity to cancer cells, leading to their increased death during DOX chemotherapy.

Key words: fullerenes, doxorubicin, nanocarriers, breast cancer, oxidative stress

The study of the interaction between pyridazinone derivatives and DNA

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Abstract

Background. Pyridazinone derivatives show several desirable pharmacological properties, such as cytotoxic, analgesic, anti-inflammatory, or anticancer effects, which makes them the subject of analysis by many research groups. New analogues are still being sought. One of the most sought-after drugs today is a group of anti-cancer compounds. The therapeutic response of such molecules is very often related to their interaction with DNA.[2] Therefore, the study of the mechanism of interaction of compounds with potential use of this macromolecule in medicine seems to be an extremely important aspect in the search for new drugs.

Objectives. As previously described, pyridazinone molecules bind to DNA in a non-covalent manner.[1] Nevertheless, different types of this interaction are distinguished, such as intercalation, minor or major groove binding and electrostatic interaction. Occassionally, the mixed manner occurs. The goal of our studies is to verify which type of interaction occurs between the new class of 5 pridazons and ctDNA, and determine and compare the equilibrium binding constants.

Materials and methods. We will present the results describing the interaction of 5 non-toxic pyridazinone derivatives with ctDNA. In order to describe the binding mechanism of the analyzed compounds with the macromolecule, we used a number of analytical methods: fluorescence spectroscopy, UV-Vis spectroscopy and circular dichroism spectroscopy. In addition, all studied interactions will be visualized using molecular modeling.

Results. The spectroscopic studies and molecular modeling confirmed the non-covalent type of binding of pyridazones to ctDNA. The binding reactions are spontaneous processes. Moreover, the binding constant values are similar and consistent with previous literature data. The interaction with tested molecules does not destroy the double-stranded helix of ctDNA; however, the small perturbation is noticeable.

Conclusions. In conclusion, compounds with a pyridazinone skeleton interact with ctDNA and may be promising anti-cancer substances, but further biological evaluation is required.

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Key words: pyridazinone derivatives, ctDNA, spectroscopic methods, molecular modeling

L-arginine and its metabolites in predicting 5-year mortality in patients with cardiovascular disease

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Abstract

Background. Despite constant progress of therapeutic and diagnostic strategies, myocardial infarction (MI) remains the leading cause of death worldwide. The impaired nitric oxide (NO) signaling is suggested as one of the possible mechanisms involved in myocardial damage. L-arginine (L-Arg) is an important player in NO production, being the main substrate for nitric oxide synthase, and L-citrulline (L-Cit) is the by-product of this reaction. However, it may also enter other enzymatic pathways, leading to the formation of metabolites that act as NOS inhibitors, e.g., asymmetric dimethylarginine (ADMA).

Objectives. To determine serum concentrations of L-Arg, L-Cit and ADMA and evaluate their association with 5-year mortality in patients with cardiovascular disease, depending on the incidence of MI.

Materials and methods. Eight hundred and sixty patients with cardiovascular manifestations, hospitalized in the Clinic of Cardiology at Wroclaw Medical University, were included in the study. The L-Arg, L-Cit and ADMA were simultaneously measured in serum samples using validated LC-MS method. Data on 5-year mortality were obtained from the registry of the Polish National Health Fund. Clinical diagnosis was the basis for classifying patients according to the presence of MI.

Results. In the study population, the observed 5-year mortality rate was approx. 13%, while in the group of patients with MI, it increased up to 19.3%. Serum ADMA concentration significantly differed between survivors and non-survivors (0.63 compared to 0.78, $p < 0.001$). Detailed analysis showed that this trend was still observed in the subgroup of patients without MI (0.61 compared to 0.76, $p < 0.001$), while in the subgroup with MI, a significant difference in L-Cit concentration was revealed (24.79 compared to 19.63, $p < 0.009$).

Conclusions. While elevated serum ADMA concentration is a well-recognized independent predictor of all-cause mortality in cardiovascular patients, it seems that decreased L-Cit concentration may be associated with 5-year mortality risk post-MI. The L-Arg concentration was of no clinical value in differentiating patients into deceased and survivors in this study.

Key words: myocardial infarction, L-arginine, L-citrulline, asymmetric dimethylarginine

VITAVE: Unique HV pulse generators in food technology and medical applications

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Abstract

Background. Pulsed electric field (PEF) technology has its roots in the latter half of the 20th century. The specific application of short, high-voltage pulses (PEF) to permeabilize cell membranes started gaining significant attention in the 1960s and 1970s, followed by first applications in the food industry in the 1990s. At its core, PEF technology employs short bursts of high-voltage electric pulses to permeabilize cell membranes. This non-thermal method offers a novel approach to processing, preserving and modifying both biological and food materials without significantly altering their inherent properties.

Objectives. In our research, we introduce state-of-the-art HV Pulse Generators and illustrate their practical applications across diverse fields.

Materials and methods. This study delves into the innovative possibilities these generators offer, with specific cases demonstrating their effectiveness.

Results. The presented cases span multiple application areas, such as: (1) food technology: We demonstrate the HV Pulse Generators' efficacy in reducing mycotoxin levels in malting barley (case 1) and microbial decontamination of red wine (case 2); (2) Medicine: We explore the potential applications of our cutting-edge pulse generators in the field of medicine. Notably, we delve into their role in facilitating gene or drug delivery into living cells through electroporation. This opens up new avenues for medical research and therapies.

Conclusions. Our research findings shed light on the versatile and impactful nature of HV Pulse Generators, offering valuable insights and solutions for various industries and scientific endeavors.

Key words: pulsed electric field, food processing, medical therapies

MicroRNA in pancreatic cancer

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Abstract

MicroRNAs (miRNAs, miRs) represent a biologically important class of small non-coding RNAs closely associated with the post-transcriptional control of gene expression. The miRNAs are single-stranded molecules containing approx. 18–24 nucleotides, and are responsible for regulating the expression of nearly 60% of all protein-coding genes. Pancreatic cancer is one of the most aggressive cancers. The number of new pancreatic cancer cases in the European Union (including the UK) was 59,000 cases in 1990 and 109,000 cases in 2019, and is projected to be 147,000 in 2039. Approximately 90% of pancreatic cancers are PDAC (pancreatic ductal adenocarcinoma), classified as exocrine tumors. It is characterized by high invasive and metastases to lymph nodes, liver, lungs, and intestines. The 5-year survival of patients with PDAC is 3–6%.

The aim of this study is to demonstrate microRNAs as potential biomarkers for the early diagnosis of pancreatic cancer and to profile microRNAs in cell lines PNAC-1 and 1.2B4, representing the most common cancer, which is PDAC, and in tumor tissues of patients obtained intraoperatively; additionally, the expression of microRNAs in the plasma of patients with pancreatic cancer will be studied. It will also be determined how a cancer risk factor such as diabetes affects the microRNA profile. Once the microRNA profile has been established, bioinformatics analysis will be performed to predict the mRNAs that the selected microRNAs target, investigating which signaling pathways become activated in pancreatic cancer.

Gene expression profiling of miRNAs has resulted in a developing understanding of cancer biology and cancer-related signaling pathways. Currently, most clinical trials use miRNAs as diagnostic biomarkers to assess tumor stage and predictive markers of overall survival time. This is especially important in pancreatic cancer where the only currently used marker is CA 19-9, which has low sensitivity and specificity. Combined with the therapeutic potential of miR, it is a promising path forward in the diagnosis and treatment of pancreatic cancer. In conclusion, pancreatic cancer has a small number of specific diagnostic biomarkers and the effectiveness of cancer therapy is limited.

Key words: microRNA, pancreas, PDAC, biomarker

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