BOOK OF ABSTRACTS

XI. Miniconference of PhD. students of Centre of Experimental Medicine

7TH of JULY 2022

JULY 2022

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XI. Miniconference of PhD. students of Centre of Experimental Medicine

Organized by:

Center of Experimental Medicine, Slovak Academy of Sciences

Institute of Experimental Pharmacology and Toxicology
Institute for Heart Research
Institute of Normal and Pathological Physiology

Organizing committee:

Natália Andelová
Dominika Besterciová
Barbora Botanská
Ezgi Dayar
Samuel Golas
Sonam Kapoor
Mireia Viñas Noguera

PROGRAM FOR MINI-CONFERENCE OF PH.D. STUDENTS 2022

9.15 OPENING OF CONFERENCE

9:15-9:25 **Mičurová A**.: Effects of polyethylene glycol-coated magnetite nanoparticles on molecular and biochemical parameters in the heart and liver of normotensive and hypertensive rats

9:25-9:35 **Lepáček M.**: Novel approaches to monitor the impact of cemtirestat on the gut microbiota, and its relations with type 2 diabetes mellitus

9:35-9:45 **Ballóová A.**: New preclinical findings about potential triple reuptake antidepressant SMe1EC2M3, a compound with a pyridoindole structure

9:45-9:55 **Pôbiš P.**: 3D reconstructed human cornea-like tissue model for in vitro biocompatibility and phototoxicity testing of medical devices

9:55-10:05 **Özbaşak H.**: Investigating the effects and toxicity of novel HE-10 as light sensitive nitric oxide donor on human skin fibroblast cells

10:05-10:15 **Aydemir Gunes B.**: The vasoactive role of sulfide signal pathway in experimental model of metabolic syndrome

10:15-10:45 Break

10:45-10:55 **Khademnematolahi S**.: The anti-arthritic affects of Niosome loaded Melittin on experimental arthritis in rats–a preliminary study

10:55-11:05 **Kissová L**.: Effect of CDN1163, CMTI, OXY-CMTI and newly proposed indole derivates on pancreatic β-cells viability

11:05-11:15 **Chrastina M**.: Biological disease-modifying drugs in the treatment of rheumatoid arthritis

11:15-11:25 **Andelova K.**: Impact of increase thermogenesis on cardiac connexin-43 mediated intercellular communication and extracellular matrix in spontaneously hypertensive rats

11:25-11:35 **Pružinská K.**: The effect of selected carotenoids on progression of experimental arthritis

11:35-11:45 **Boďo P.**: Metabolic transformation of cemtirestat in vivo and in vitro

11:45 CLOSING OF CONFERENCE

7TH OF JULY 2022

BOOK OF ABSTRACTS

THE EFFECT OF SELECTED CAROTENOIDS ON PROGRESSION OF EXPERIMENTAL ARTHRITIS

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Rheumatoid arthritis is a chronic inflammatory disease affecting mainly joints, possibly leading to disability if left untreated [1]. During the onset and progression of the disease, there is a shift in redox balance and an accumulation of reactive oxygen species occurs [2]. With their capacity to alter prooxidant and pro-inflammatory pathways, dietary phenolic substances, flavonoids, carotenoids, and alkaloids have been successful in slowing the progression of arthritic illness [3]. The most favourable dose-dependency for natural astaxanthin was evaluated in a pilot study. In this study we evaluated the effect of potent antioxidants – carotenoids, such as natural astaxanthin produced by *Blakeslea trispora* (AS, dose 20 mg/kg) and synthetic astaxanthin (ASYN, 20 mg/kg), beta-carotene (BEKA, 20 mg/kg), beta-cryptoxanthin (KXAN, 0.10 μg/kg) in monotherapy or in combinational therapy with methotrexate (MTX) on clinical parameters in Lewis rats with adjuvant-induced arthritis. Compounds were administered daily *per os*, except for MTX which was administered twice a week in a subtherapeutic dose 0.3 mg/kg. In monotherapy, all carotenoids improved the loss of the weight on 14th and 28th day. Moreover, they all improved change of hind paw volume (HPV) statistically at day 14. KXAN together with natural AS were the most effective on days 21 and 28. Both AS, synthetic and organic, were more effective in combinational therapy than MTX alone in prevention of weight loss and in amelioration of HPV. Surprisingly in combination therapy, synthetic AS was having even better effect.

To sum up, AS showed evident beneficial effect, but our data suggest KXAN to be even more effective in these clinical parameters and could be used for future research. Carotenoids may be a prospective addition to RA therapy. To confirm this hypothesis, biochemical and immunological analysis will be performed in plasma and tissue samples.

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The Anti-Arthritic Effects of Niosome loaded Melittin on Experimental Arthritis in Rats – a preliminary study.

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Background

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease, affecting the joints with varying severity among patients. The risk factors include age, gender, genetics, and environmental exposure. The therapeutic goal for RA is to alleviate symptoms, prevent joint destruction, and preserve joint function (1). Melittin from Bee Venom therapy has been utilized to relieve pain and cure inflammatory diseases, such as RA in humans. Niosomal drug delivery is potentially applicable to many pharmacological agents with the aim to improve their effect in various diseases (2).

Objectives:

- Physicochemical evaluation of niosome in-loaded melittin.
- Assessment of arthritis clinical signs by the measurement of hind paw swelling every week for one month.
- Analysis of biochemical and immunological factors such as C-reactive protein, IL-17, IL-6, IL-1beta, MMP-9 and MCP-1 in plasma.
- Compare the effectivity of pure melittin and melittin loaded in niosomes on the development of rat adjuvant arthritis (AA).

Methods: Melittin will be obtained from Serva Electrophoresis, Santa Cruz Biotechnology, and Bio-Connect Life Sciences, and loaded in niosomes in Kerman Medical University. The prepared formulations will then be transported to Centre of Experimental Medicine, Slovak Academy of Science in Bratislava, where these formulations will be studied in rats with adjuvant arthritis. The injection will be performed near the tail base. The experiments will include healthy animals, arthritic animals not treated, and arthritic animals treated with melittin and melittin loaded in niosomes in intraperitoneal or subcutaneous dose of 10 µg/kg/day. In each experimental group, 8 – 10 animals will be used. The duration of the experiment will be 28 days. Plasma will be stored at – 70 °C until biochemical and immunological factors such as CRP, IL-17, IL-6, IL-1beta, MMP-9, MCP-1 will be analysed. **Outcomes**: According to these advances, it is expected that the main results from the biochemical and immunological analysis of the present study will be useful for the treatment of RA patients and developing new drug delivery systems based on niosomes will provide a scientific background for further research.

Acknowledgment: This study was supported by VEGA 2/0136/20

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EFFECTS OF POLYETHYLENE GLYCOL-COATED MAGNETITE NANOPARTICLES ON MOLECULAR AND BIOCHEMICAL PARAMETERS IN THE HEART AND LIVER OF NORMOTENSIVE AND HYPERTENSIVE RATS

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This study investigated the biological effects of polyethylene glycol (PEG)-coated magnetite nanoparticles (~30 nm core size, ~51 nm hydrodynamic size, 1 mg Fe/kg/day, administered intravenously) in the left heart ventricle (LHV) and liver of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) 90 min post infusion. Iron oxide nanoparticles (IONs) had no effect on the blood pressure and heart rate of ION-treated rats, however there was significant decrease of plasma corticosterone in ION-treated SHR (SHR_{ION}) vs. control SHR ($SHR_{C)}$ and ION-treated WKY (WKY_{ION}). Nitric oxide synthase activity was unaltered in the LHV, but significantly decreased in the liver of SHR_{ION} vs. SHR_C. For superoxide productions, there were significantly higher levels found in WKY_{ION} compared to WKY_C in both the LHV and liver. No such changes in superoxide production were found in SHR.
Saturation magnetization (a parameter associated with content of natural iron-containing compounds) of the liver of SHR_C was significantly higher than in WKY_C, however, in LHV we found the reduction in saturation magnetization of SHR_C compared to WKY_C. ION-originated iron content was significantly lower in the liver of SHR_{ION} vs. $WKY_{ION.}$ In the LHV we found no significant change, however there was a decreasing tendency in nanoparticles-derived iron content in SHR_{ION} compared to WKY_{ION}. There were no ION-dependent changes in *iNOS*, *eNOS*, *NRF2* and *PPARγ* gene expressions vs. the respective control group in both tissues investigated. In the liver, IONs significantly reduced the expressions of *SOD1, SOD2* and *FTH1* in SHR_{ION} vs. SHR_C. In the LHV, IONs elevated *SOD1* gene expression in WKY and *TFR1* in SHR vs. the respective controls. In conclusion, the results showed reduced incorporation of IONs and lower superoxide production in the liver and LHV of SHR compared to WKY suggesting considerable influence of high blood pressure on pharmacodynamics and kinetics of PEG-coated iron oxide nanoparticles.

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EFFECT OF CDN1163, CMTI, OXY-CMTI AND NEWLY PROPOSED INDOLE DERIVATES ON PANCREATIC β-CELLS VIABILITY

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In the framework of the study of possible therapeutic intervention in diabetic complications, we examined the effects of CDN1163, newly proposed indole derivatives, CMTI and oxy-CMTI compounds on pancreatic β-cell viability.

We performed the experiment on the pancreatic cell line INS-1E. As an indicator of damage we used the MTT reduction assay [1] (3- (4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2H-tetrazolium bromide; Sigma Aldrich). From experiments, we found that the highest concentration of substances used (100 μM) slightly reduced cell viability. On the other hand, for indole derivates and CDN1163, we also observed mildly potentiating effects of all substances except In-4. CMTI and oxy-CMTI had a mild enhancing effect on pancreatic β-cell viability even at the lowest concentrations used.

In conclusion, the newly proposed indole derivatives, CDN1163, CMTI and oxy-CMTI, did not show negative effects on pancreatic β-cells in the concentration range used during treatment.

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Biological disease-modifying drugs in the treatment of rheumatoid arthritis

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Rheumatoid arthritis (RA) is described as an autoimmunity disease that occurs in around 0.5% of the population with no known etiology [1]. Throughout history (the last 100 years) different treatments were developed to reduce symptoms that come with RA [2]. But not every patient responds well to treatment with synthetic disease-modifying drugs (sDMARDs) as expected or at all. That fact creates a need for an improvement in RA therapy with the usage of biological DMARDs (bDMARDs) such as antibody-based drug systems. bDMARDs is a new approach in many diseases RA included. In the last three decades were developed numerous biological treatments some of which are still in the testing phase and many others are on the rise. We focused on bDMARDs which are used for the treatment of RA. We also described some treatments which were intended for RA but came out as non-affective in this indication. The pros and cons of bDMARDs result from a different mechanism of action of these drugs. bDMARDs for RA have shown themselves as effective but expensive. That is the reason why it is still the second choice after the conventional treatment with sDMARDs, which include methotrexate and other disease-modifying drugs from the non-biological group, such as corticosteroids, non-steroid anti-inflammatory drugs, and others. As the price for bDMARDs will be dropping down due to a wide range of diseases that can be treated by bDMARDs, potentially it will become the first choice in RA patients.

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IMPACT OF INCREASE THERMOGENESIS ON CARDIAC CONNEXIN-43 MEDIATED INTERCELLULAR COMMUNICATION AND EXTRACELLULAR MATRIX IN SPONTANEOUSLY HYPERTENSIVE RATS

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We have previously shown that down-regulation of myocardial gap junction channel protein, connexin-43 (Cx43) and up-regulation of extracellular matrix (ECM) proteins in hearts of spontaneously hypertensive rats (SHR) promote development of lethal arrhythmia. Cold acclimation has a potential for reducing cardiovascular disease risk, but it is not known whether cold adaptation affects Cx43 and ECM, that are crucial factors in modulation the propensity of the heart to malignant arrhythmias.

To explore these issues, we used adult hairless males and females SHR^M strain and wild type SHR. Animals were housed at standard 22 °C (that is below thermo-neutrality for these animals) and 12:12 hour light:dark cycle. WKY control rats were used as reference normotensive strain. Heart samples of the left ventricular tissue from euthanatized rats were used for the proteomics of Cx43, protein kinases, which phosphorylates Cx43, and for ECM markers: TGF-β, SMAD2/3, MMP-2 and collagen-1. Immunofluorescence detection of Cx43 was performed to examine its myocardial topology.

The expression level of Cx43 protein and its phosphorylated status was reduced in both males and females SHR^M and SHR vs WKY. Increase thermogenesis in SHR^M enhanced both parameters compared to wild type SHR regardless the sex. Moreover, pathological enhanced localisation of Cx43 at lateral sides of cardiomyocytes in SHR was attenuated in SHR^M. We also observed gentle differences in protein levels of PKA, PKG, PKCε, MAPK, Akt between SHR and SHR^M. Protein level of TGF-β and SMAD 2/3 were increased in SHR compared to WKY rat hearts but decreased in SHR^M, unlike collagen-1 that was not altered in SHR^M compared to SHR.

Findings revealed benefit of cold acclimation in hairless SHR^M due to up-regulation of myocardial Cx43 along with down-regulation of extracellular matrix proteins. We assume that cold acclimation may improve myocardial Cx43 mediated intercellular communication and hamper cardiac arrhythmia occurrence.

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THE VASOACTIVE ROLE OF SULFIDE SIGNAL PATHWAY in EXPERIMENTAL MODEL OF METABOLIC SYNDROME

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Hydrogen sulfide (H_2S) represents an important gaseous transmitter which could interfere with etiopathogenesis of different cardiovascular and metabolic diseases. However, H_2S in interaction with nitric oxide (NO) may also trigger compensatory vasoactive effects to counterbalance pathologically increased vascular tone in both arterial hypertension and metabolic syndrome. We aimed to study the role of sulfide signal pathway in vasoactive responses of mesenteric artery (MA) in spontaneously hypertensive rats (SHR) fed with fructose.

12 weeks-old SHR were divided into three groups: control rats, rats treated with 10% fructose in drinking water for 8 weeks and rats treated with fructose and during last three weeks with H_2S donor, GYY-4137 (266 µg/kg/day, i.p.). Vasoactivity of MA was recorded as changes of isometric tension. H_2S inhibition was performed by acute incubation with DL-propargylglycine (PPG, 10 mmol/l). Acute incubation with N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-5} mol/l) was used for inhibiting of NO production.

The chronic fructose intake significantly increased plasma level of triacylglycerols (TAG) and the body adiposity, expressed as retroperitoneal fat weight to tibia length ratio whereas the treatment with H_2S donor partially attenuated. The SBP was increased in fructose-fed rats, however 3-week-long treatment with GYY-4137 decreased the SBP, in both control and fructose-fed rats. We observed that fructose intake enhanced endothelium-dependent vasorelaxation and decreased adrenergic contraction of MA, along with the sensitivity to noradrenaline remained unchanged. While GYY-4137 administration did not significantly affect vasorelaxant responses, it partially restored reduced contractility in fructose-fed rats. There was no difference in participation of endogenous H_2S in vasoactive responses among groups. While a significantly decreased participation of NO in contractile response was demonstrated in GYY4137 only, the acute pretreatment with L-NAME, inhibited the vasorelaxant response significantly more in fructose and GYY-4137 treated rats.

Our results suggest that fructose intake triggered compensatory vasoactive responses of mesenteric artery which included action of NO signal pathway. Moreover, slow H_2S releasing donor could partially amend metabolic-related changes and ameliorate impaired mesenteric contractility.

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METABOLIC TRANSFORMATION OF CEMTIRESTAT *IN VIVO* AND *IN VITRO*

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Cemtirestat, a derivative of indol-1-yl acetic acid, is the low molecular weight compound that is effective inhibitor of an aldose reductase (ALR2). ALR2, from group of oxidoreductases, is the first enzyme of polyol pathway and its contributes to the development of diabetic complications (macro- and microangiopathy, neuropathy, nephropathy, cataract, retinopathy). Activity of ALR2 in these diseases is also associated with a reduced concentration of intracellular NADPH which serves as a cofactor for ALR2 in reducing glucose to sorbitol. In diabetic patients, the increased flow of glucose through the polyol pathway may contribute to oxidative stress. Inhibition of ALR2 can prevent diabetic complications and improve the quality of life of patients with *diabetes mellitus*.

Cemtirestat is currently undergoing a comprehensive preclinical evaluation. The results obtained so far from *in vitro* and *in vivo* experiments point to cemtirestat as an effective bifunctional agent combining the ability to inhibit ALR2 with antioxidant activity. At the same time no toxic effects of cemtirestat have been reported, which may indicate the pharmacological use of cemtirestat in the prevention and treatment of diabetic complications.

An important part of the development of a new drug is the elucidation of its pharmacokinetics. The research is aimed at studies of bioavailability, metabolic changes and pharmacokinetic behavior of cemtirestat *in vitro* and *in vivo*. Cemtirestat contains two functional groups in its structure, thiol and carboxyl, which are expected to be reactive centers. Attention will be paid to possible chemical changes in these functional groups (most likely the oxidation of the thiol group and in the case of the carboxyl functional group the formation of conjugates).

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New preclinical findings about potential triple reuptake antidepressant SMe1EC2M3, a compound with a pyridoindole structure

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Major depression is becoming the most common psychiatric disorder worldwide. The monoamine theory is supported by antidepressant mechanism of action that modulates monoaminergic systems. Available antidepressants, have various limitations and side effects, which calls for the search of new therapeutics. In our previous study, an antidepressant effect was revealed after acute administration of SMe1EC2M3 [1].

We studied the effect of chronic administration of the SMe1EC2M3 under stressed conditions induced by chronic mild stress (CMS) procedure in Sprague-Dawley male rats (n=72). From Day 8 of the CMS, we intraperitoneally treated the animals by 5 or 25 mg/kg/day dose. We evaluated changes in behavior in sucrose preference test (SPT), open field test (OF) and forced swim test (FST). By fluorescent immunohistochemistry we analyzed the neuronal progenitor cells proliferation in the *dentatus gyrus* (DG), CA1 and CA3 hippocampal regions using molecular marker SOX2 and the specific marker of mature astrocytes GFAP (n=6 animals/group). Also, the potential neurotoxicity was investigated using primary hippocampal neurons cultures from Wistar neonates. Cells were treated without or with SMe1EC2M3 (0.25; 0.50; 1.00; 1.50 µM) or all-trans retinoic acid (ATRA). We evaluated 3 coverslips/group and 7 areas of interest/coverslip. Using Sholl analysis, we counted dendrites intersection by concentric circles from the soma to 200 µm and the length of the longest neurite from the nucleus to the apical end.

Higher immobility in FST, lower consumption in SPT and shorter distance traveled in OF confirmed the depression-like behavior. Both doses reversed the effect of CMS by reducing immobility and prolonging the swimming. Analysis of progenitor's formation revealed a significant reduction of SOX2+ cells in the untreated stress group (SV). The administration of 25 mg/kg dose increased SOX2+ cells. In the CA1 region we found decrease of the SOX2+ cells in 5 mg/kg treated non-stress group, however in CA3 region, a significant decrease after 5 mg/kg dose was observed in the stress group. In DG significant reduction of GFAP+ cells in the SV group were observed. Administration of 5 mg/kg decreased GFAP+ cells in non-stress and stress group. Also, increase of the GFAP+ cells in 25 mg/kg group were detected. No neurotoxicity of the SMe1EC2M3 was observed. In group 1.50 µM SMe1EC2M3, neurites length was stimulated, and we found more neurons with the longest neurite over 200 µm. No significant changes in the number of neurite branches were found between groups.

Together with our previous preclinical findings, the pyridoindole derivative SMe1EC2M3 may be a good candidate for future studies of treatment options for MDD.

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Novel approaches to monitor the impact of cemtirestat on the gut microbiota, and its relations with type 2 diabetes mellitus

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The gut microbiome is made up of as many as 4 billion microbes which may metabolize pharmaceutical compounds into active, inactive or even toxic metabolites. On the other hand, pharmaceuticals can affect the gut microbiome. At the present time, it is still relatively little known about how medications interact with microbiome [1,2]. Cell culture viability tests performed recently on six different cell lines revealed remarkably low cytotoxicity of cemtirestat, a novel inhibitor of aldose reductase, projected as a drug to treat diabetic complications [3]. The aim of the present study is to investigate the effect of cemtirestat on gut microbiome in ZDF "lean" rats.

Male ZDF Lean rats were divided in two experimental groups, control and treated with cemtirestat in dosage 7.7 mg/kg/day for 2 months. Fecal samples were collected at beginning of the experiment, after 2 weeks, one month and in the end of the treatment and were analyzed for microbiome composition by Oxford Nanopore technology. Blood was collected for biochemical and hematological analyses. Preliminary data suggest a significant microbiome augmentation after treatment with cemtirestat, comprising mainly bacteria of the Lactobacillus genus. No significant changes in the organ weight between treated and untreated groups were observed. Biochemical analyses, including 20 parameters showed only slight increase ($p < 0.5$) in ALT enzyme activity after 2 months of treatment with cemtirestat. More complex genomic assays are in progress.

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3D RECONSTRUCTED HUMAN CORNEA-LIKE TISSUE MODEL FOR IN VITRO BIOCOMPATIBILITY AND PHOTOTOXICITY TESTING OF MEDICAL DEVICES

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Over the past 20 years, researchers have collected solid evidence showing that rapidly evolving reconstructed 3D human tissue models have the potential to replace in vivo experiments required for hazard and safety testing of chemicals and cosmetic products. There is an increasing trend in the implementation of these technologies into the regulations. Only last year, ISO 10993-23 was implemented to assess the intracutaneous irritation of extracts from medical devices (MD) using a 3D reconstructed skin model and OECD adopted a new 3D tissue model test for the phototoxicity testing of dermally applied formulations (OECD TG 498) [1,2,3].

We have utilized the acquired knowledge from the development and validation of ISO as well as OECD protocols and developed an in vitro protocol for the assessment of ocular (photo)irritation of MDs. The protocol uses 3D reconstructed human cornea-like (3D RhC) tissues, that can at the same time serve as a universal tissue model of non-keratinising epithelia (e.g., oral cavity). The 3D model was assessed for its ability to withstand long exposure times and correctly predict phototoxicity. The protocol for biocompatibility testing was evaluated using extracts from medical devices (biopolymers) and also with formulated ophthalmologic drugs and moistening eyedrops classified as MDs. To increase the applicability domain of the protocol and the 3D model, under the same conditions, we further assessed the biocompatibility of materials intended for the oral cavity. The results obtained in these pilot studies indicate high sensitivity and predictive ability towards potentially irritating materials. Our current work focuses on increasing the portfolio of tested materials and submitting a proposal for funding that would be leading to a validation project.

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Investigating the effects and toxicity of novel HE-10 as light sensitive nitric oxide donor on human skin fibroblast cells

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Nitric oxide (NO) is a very important molecule for human physiology and pathophysiology, playing a wide range of roles. At present time, NO donors can cause uncontrolled release of NO by affecting the whole body once applied systemically. Light-activated NO donors can overcome this challenge by using light as a localized stimulant.[1] In recent years, Ruthenium nitrosyl complexes are known to be light-activated to release NO and are potential candidates for photodynamic therapy.[2] HE-10, a ruthenium (II) nitrosyl complex containing newly synthesized 4′-phenyl-terpyridine and benzoquinone diimine ($[Ru(ptp)(o\text{-}bqdi)NO](PF_6)_3$), is a potential candidate molecule that activates with light to release NO. In this study, the effect of NO released from HE-10, induced by white LED light, on cytotoxicity and oxidative stress-related pathways in VH-10 fibroblast cells is investigated.

Cytotoxic and non-cytotoxic concentrations of HE-10 in VH-10 fibroblast cells were determined by MTT assay. The Griess assay was used to measure the major metabolites of NO released from HE-10, namely Nitrite and Nitrate, in cell medium. The AO/EBr staining method was used to morphologically observe the occurrence of apoptosis type cell death in cells. To assess levels of ROS/RNS and endogenous NO production, H_2DCFDA and DAF-2 DA probe were used respectively. Lastly, to measure phase distribution of cell cycle conditions in the cells propidium iodide were used and determined by flow cytometry.

While the IC_{50} value is 9.2 ± 0.397 μM in cells exposed to light for 1 hour, the IC_{50} value is 11.12 ± 0.496 μM in the dark group. A dose-dependent increase in NO metabolites was detected in the cell media. Dose and light-dependent cytotoxicity was supported by increased intracellular ROS/RNS production. When the cells were compared morphologically, a higher frequency of apoptotic cells was observed in the light-induced group. On the other hand, light exposure was not found to increase intracellular NO levels.

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