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CONFERENCE ABSTRACTS

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New medicines

Phytopharmacological investigation of Cissampelos pareira Linn. for anti-inflammatory potential

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Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Cissampelos pareira Linn. (CPL) is a well-known perennial climbing shrub in Ayurveda. In ethno-medicinal practices, CPL has shown wide range of therapeutic activities viz., anti-bacterial, anti-pyretic, anti-tumor, etc. Although in the past decade, CPL has received much attention, however, its pharmacognostic value and pharmacological properties remains unexplored.

Aim: The present study deciphers the pharmacognostic value and certain pharmacological properties of ethanolic extract of CPL leaves.

Method: Pharmacognostical studies involved processing and extraction of plant material; macro- and microscopic evaluation; histochemistry and total flavonoid assessment. Standardization of CPL was done using High Performance Thin Layer Chromatography (HPTLC). Ethanolic extract of CPL leaves was administered in varying amounts of 200, 400, 800 mg/kg bw orally for 7 days to either Balb/C mice or Wistar rats (based on acute oral toxicity studies for dose definition). While pharmacological studies included evaluation of hind paw edema, delayed type (IV) hypersensitivity (DTH) response, assessment of phagocytic function by reticuloendothelial system (RES) and reduced glutathione content in the blood.

Results: Macroscopic study revealed that the leaf shape is cordate, colour-green, apex is obtuse at the base, and leaf margin was entire with pubescent surface. HPTLC finger print profile of CPLshowed identification of lupeol and β-Sitosterol as active compounds. Administration of CPL extract exhibited a significant anti-inflammatory effect in carrageenan-induced hind paw edema compared to carrageenan alone-induced group. Additionally,a dose dependent decrease inprimary and secondary antibody formation was noticed following CPL treatment, thereby suggesting its immunosuppressive potential. Compared to ascorbic acid-induced standard control group, CPL treatment in mice revealedlow antioxidant activity.

Conclusion: Overall, the present findings provide both in-vivo and in-vitro evidence of ethnopharmacological (immunosuppressive, anti-inflammatory and antioxidative) value of the ethanolic extract of Cissampelos pareira Linn. leaves.

Ivermectin effects on interleukin-6 induced nuclear translocation of STAT3 in a macrophage based cell line

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Introduction:
Inflammation is the bodies' natural defence to infection and tissue injury. More than 20% of the Australian population is living with disease with an underlying chronic inflammatory pathology. Core to an inflammatory response is the involvement of pro-inflammatory cytokines such as Interleukin-6 (IL-6). Cytokines such as IL-6 mediate down signal processes that result in the translocation of signalling proteins from the cytosol of immune cells to the nucleus where they interact with nuclear DNA. The STAT family are a group of proteins involved in this pathway, and of particular note, STAT3 is one of the predominant signal proteins

implicated in many inflammatory diseases as well as cancer. This study uses a high content imaging system to assess the translocation of STAT3 into the nucleus of macrophages treated with IL-6 and lipopolysaccharide (LPS), and examine the concentration dependent effect of Ivermectin on IL-6 induced translocation.

Purpose:
This study aimed to assess the effects of Ivermectin on the translocation of STAT3 from cell cytosol to the nucleus in a cell model for macrophages.

Methods:
THP-1 monocyte cells were cultured on 96 well high content imaging plates and differentiated by phorbol 12-myristale 13-acetate to take on a macrophage like state. THP-1 cells were then treated with LPS (1µg/ml) or IL-6 (30 ng/ml) for 20 minutes, 1 hr and 18hrs. The presence of STAT3 was assessed using antibodies against anti-STAT3 with Alexa Fluor 555 a goat anti-rabbit IgG as a secondary, and counter labelled with DAPI in order to assess colocalisation of STAT3 in the cell nucleus. Images were assessed using the ImageXpress high content imaging system.

Results:
Both LPS and IL-6 induced the translocation of STAT3 from the cytosol to the nucleus in PMA differentiated THP-1 cells. However, IL-6 had a strong effect on translocation even at 20 minutes while LPS was delayed with no translocation evident until 1 hr, indicative of the potential secondary nature of the effect as LPS elicits through other cytokine release, such as TNF-alpha and IL-6. In addition, there was a statistically significant decrease in the level of nuclear translocation of p-STAT3 in IL-6 stimulated macrophages treated with different Ivermectin concentrations (10µmol/L to 0.001µmol/L).

Conclusion:
Our study demonstrated early translocation of STAT3 with Il-6 treatment which could be blocked by pre-treatment with ivermectin treatment, describing a potential mechanism of action in immune cell modulation during inflammation and infection.

The therapeutic role of colchicine in vascular smooth muscle cell pathogenesis of atherosclerosis

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Background: Atherosclerosis begins with the retention of low-density lipoprotein (LDL) in the blood vessels. Under the stimulation of pro-atherosclerotic agonists such as transforming growth factor beta (TGFB), vascular smooth muscle cells produce proteoglycans with longer glycosaminoglycan (GAG) chains. Elongated GAG chains allow more positively charged LDL to bind, leading to the retention of LDL. Accumulation of LDL drives atherosclerosis to the inflammation phase, resulting in vascular smooth muscle cell proliferation and propagating plaque formation.
Colchicine is used for the treatment of inflammatory diseases such as gout and familial Mediterranean fever. Colchicine has been clinically investigated as a potential treatment for cardiovascular diseases. In COLCOT and LoDoCo2 clinical trials, patients with the history of coronary artery diseases experience fewer cardiovascular events after receiving colchicine treatment. The outcomes of these clinical trials reveal colchicine as a promising agent to manage atherosclerosis, in addition to conventional lipid-lowering therapy.

Purpose: Colchicine improved clinical outcomes in patients with atherosclerosis. However, the mechanism of action of colchicine in treating atherosclerosis, especially on vascular smooth muscle cells, is unclear. This project investigated the role of colchicine in vascular smooth muscle cells.

Method: Mouse aortic vascular smooth muscle cells (MOVAS) were treated with colchicine in the presence or absence of TGFB (2ng/mL). The expression of GAG synthesising enzymes and inflammatory cytokines was evaluated using qRT-PCR. Cell proliferation of MOVAS was assessed 72 hours post-treatment and was quantified as the number of cells at the end of the treatment.

Results: TGFB stimulated the mRNA expression of enzymes controlling GAG chain elongation, CHSY-1 and CHST-11, which were unaffected when treated with colchicine (1-10mM). In contrast, TGFB-stimulated mRNA expression of inflammatory markers, IL-6 and CCL2, was drastically inhibited by colchicine (10 mM). To assess the effect of colchicine on smooth muscle cell proliferation, MOVAS were treated with 5% FBS in the presence or absence of colchicine (3-100nM) for 72 hours. Colchicine (3-30nM) inhibited MOVAS cell proliferation by 30-50%, and complete inhibition was observed at 100nM.

Conclusion: Our results demonstrate that in vascular smooth muscle cells, colchicine inhibits inflammatory marker synthesis and vascular cell proliferation but not GAG chain elongation. These findings suggest that colchicine specifically targets atherosclerotic plaque in the inflammation phase, halting unstable plaque formation. Colchicine has the therapeutic potential to be used as a co-therapy in patients with atherosclerosis plaque rather than as a prophylactic treatment for atherosclerosis.

Cytotoxicity on MDA-MB-231 cells and antioxidant activity of tibig (Ficus nota Blanco Merr.)

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Breast cancer is the most commonly diagnosed cancer among women and remains to be a national health priority in the Philippines. Current treatment options offer benefits alongside with their side and adverse effects, including highly expensive costs. Thus, finding natural compounds from plants may provide an alternative anticancer treatment in the future.
The aim of this study is to determine the cytotoxicity on MDA-MB-231 breast cancer cells and antioxidant activity of Ficus nota. The leaves of Tibig were macerated using methanol and have undergone subsequent solvent partitioning producing n-hexane, dichloromethane, n-butanol, and water solvent fractions.
Based on the results, it was demonstrated that the most active F. nota solvent fraction (MAFnF) that has a potential anticancer activity and a valuable cytotoxic agent against MDA-MB-231 cells is the dichloromethane solvent fraction. The determination of antioxidant activity was tested using FRAP assay (16.38±2.14 mcg/mL $FeSO_4$ concentration), DPPH scavenging assay (10.92 mcg/mL IC50), and NO scavenging assay (40.71 mcg/mL IC50).
The results showed that the dichloromethane solvent fraction exhibited the most significant antioxidant activity compared to the other F. nota solvent fractions. TPC and TFC were reported to be highest in dichloromethane solvent fraction having 22.67±3.36 mg GAE/g and 165.58±9.59 mg QE/g, respectively. The cytotoxicity of the MAFnF was performed using MTT assay with Doxorubicin as positive control. Potent cytotoxic activity against MDA-MB-231 breast cancer cells was observed at very low doses (7.028±0.02 mcg/mL IC50). The instrumental analyses were performed characterizing the active compounds in the MAFnF. Phenolics and flavonoid-like compounds were deduced to be present based on the IR spectra obtained in FTIR analysis and similar chromatograms with quercetin were observed in the HPLC analysis.
In conclusion, the study proved that F. nota is a rich source of potent antioxidants and can be a promising anticancer agent.

Multinational medical cannabis use patterns for mental and physical health conditions and pharmacy practice considerations

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Background: Medical use of cannabis is allowed in many countries and this has pharmacy practice implications. In the U.S., states set up their own systems due to federal prohibition. Canada, which permits medical and recreational cannabis use, manages medical cannabis federally, but provinces regulate retail sales. Australia, which permits medical use, has a highly regulated medical model.

Methods: In this paper, we describe the conditions for which medical cannabis is used across these three countries. We use self-reported data specifying reasons for medical use, product choice, and product composition from the 2021 survey wave of the International Cannabis Policy Study. Simple differences in weighted proportions reported across countries are assessed using t-tests. Regression models are then used to assess differences controlling for demographics.

Results: We find that the same reasons are generally given for medical use despite jurisdictional policy variance. Medical patients in all three countries report use primarily for pain (AUS: 39.8%; CAN: 51.0%; US: 51.5%), anxiety (53.8%; 51.5%; 56.0%), depression (44.0%; 35.8%; 41.4%), sleep problems (33.6%; 46.7%; 42.7%), headache (30.1%; 31.8%; 37.1) and PTSD (26.5%; 15.5%; 23.5%). Herb products followed by edibles were the top two products used across all countries, although half of all medical patients report using both, despite limited evidence for these modes of administration for medical use.

Conclusion: Despite jurisdictional differences in medical policies, evidence from self-reports suggest similar reasons for using medical cannabis and product choice. Implications for pharmacists will be discussed, including co-morbidity, drug-drug interaction, and patient counseling considerations.

Isolation and characterisation of andrographolide from andrographis paniculata and investigation of its interleukin-6 inhibitory activity

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Background: Interleukin-6 (IL-6) is a cytokine commonly produced during respiratory inflammation and is a potential target for the development of anti-COVID-19 drugs. Andrographolide, a diterpenoid lactone, has been shown to inhibit IL-6 binding to receptors in human cells.

Purpose: The aim of this study was to isolate andrographolide from Andrographis paniculata, characterize it, and investigate its effects on IL-6.

Method: The isolation of constituents was carried out by extraction with ethanol and purification by column chromatography. Characterization of the isolated compound was accomplished using ultraviolet and infrared spectrophotometry, as well as mass spectroscopy (MS), proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C-NMR).

Results: The isolation process resulted purified white crystals of andrographolide with a yield of 1.21%. An inhibition assay was performed on the purified andrographolide using a human IL-6 ELISA kit. The tested compound was shown to interact as an IL-6 inhibitor through a series of different testing stages, compared with the standard Tocilizumab. Tocilizumab and andrographolide showed IC_{50} values of 2.76 and 9.54 µM, respectively.

Conclusion: It was concluded that andrographolide has the potential to act as an IL-6 inhibitor, preventing the release of cytokines which may, consequently, be very useful in the treatment of COVID-19.

Can the peptide Nisin be used to kill Gram-negative bacteria?

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Background
Deaths caused by antimicrobial resistance (AMR) are rising fast and have reached over 1 million people yearly. This will likely increase to well over 10 million by 2050, that is more than cancer and diabetes combined. In the past 30 years very few, new antibiotics have reached the market, mainly due to a low financial incentive, but with the cost of AMR straining healthcare systems there is a renewed interest in the development of new antibiotics. One potential new antibiotic is the peptide nisin, used extensively in food industry to kill Gram-positive bacteria by binding to lipid II, but showing no activity on Gram-negative bacteria as it is unable to cross the outer membrane of these microbes. Lipid II is an established target for antibiotics, for example vancomycin towards which resistance is widespread. Notably, despite the extensive use of nisin over the past 70 years, the resistance towards this peptide is very rare and relates to its beneficial binding mode to lipid II.

Purpose
In this project we have developed a bioconjugate between the peptide nisin and a molecule targeting Gram-negative bacteria (a siderophore). The aim is to evaluate if nisin can kill Gram-negative bacteria as efficiently as Gram-positive bacteria if we can fashion a delivery system to overcome the outer membrane that protect Gram-negative bacteria.

Method
We designed our bioconjugate based on similar molecules reported in literature. Using known synthetic chemistry, we built an esterase sensitive linker molecule to which we attached the siderophore, and using copper-free click chemistry we added the nisin peptide molecule.

Results
The synthesis of the bioconjugate was optimized to yield the linker through a seven-step process in 57% yield, and the final product in 19% yield over 12 steps. The linker synthesis was developed based on known literature protocols, with reaction options explored to increase yield and evaluate the best route to generate the bioconjugate. Nisin is sensitive to copper and thus we needed to establish a synthetic protocol that allows use of strain-promoted copper-free chemistry. This work involved exploring options to remove traces of palladium while not involving silica gel as our siderophore ca bind this; palladium traces will prevent strain-promoted copper-free chemistry. We were successful in finding a route to use this type of chemistry and saw a marked increase in yield for the click-chemistry compared to copper-click reactions performed with nisin in the past.
Having the bioconjugate at hand the molecule is currently being evaluated for activity on the Gram-negative bacteria Pseudomonas aeruginosa, following established testing

protocols and using Gram-positive bacteria Staphylococcus aureus as control.

Conclusion
In this project we successfully built the first nisin-siderophore bioconjugate to evaluate if nisin can be used to treat troubling Gram-negative bacteria infections and become a new tool to use against antibiotic resistant bacteria.

Comparative antidiabetic effect of the aqueous methanol extract of Annona Muricata L. (annonaceae) leaves and fruits on alloxan-induced diabetic albino rats

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Background: Annona muricata (Annonaceae) grows in many tropical and subtropics of the world. The fruit has been researched to contain various constituents such as vitamins, magnesium, acetogenins. The fruits tree and its parts have been used extensively as an antidiabetic, antiparasitic, anticonvulsant, anticancer and antimalarial agents. Diabetes is a metabolic disease resulting in increased blood glucose (sugar) levels.

Purpose: This study investigated the phytochemicals screenings of the aqueous methanolic extracts of Annona muricata fruits and leaves and to compare their antidiabetic effects on alloxan induced diabetic rats
Methods: The filtrates of the macerated plant extract were collected using a filter paper concentrated in a rotary evaporator, evaporated to dryness and phytochemical screening was done. Doses of 200, 500 and 1000mg/kg each of the extracts were given orally for 6 days to the alloxan induced diabetic rats.

Results: Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, carbohydrates, tannins and reducing sugars. The aqueous methanol extracts of Annona muricata leaves and fruit; and glibenclamide (standard drug) produced significant ($p<0.05$) decrease in blood glucose in alloxan induced diabetic rats. At 1000mg/kg dose, the fruit extract yielded the highest (83.88%) antidiabetic effect compared to 47.86% effect produced by the leaves and glibenclamide.

Conclusion: This study suggests that aqueous methanol Annona muricata fruit extract could be more useful in the management of diabetes than its leaves.

Cytotoxicity and apoptosis mechanism of ethyl acetate extract of helminthostachys zeylanica on MCF-7 breast cancer cell line

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The root of Helminthostachys zeylanica, known as Tunjuk Langit, has been traditionally used by people of Inderalaya, South Sumatera for cancer treatment. Previous studies showed that H. zeylanica possesed potential cytotoxic activity against several cancer cell lines, however there is limited report of its cytotoxicity on MCF-7 breast cancer cell line. In addition, the mechanism of action remains unknown. Therefore, the objectives of this study were to evaluate the cytotoxic activity of ethyl acetate extract of H.zeylanica against MCF-7 cell line and to identify the apoptosis mechanism using Bax and Bcl-2 gene expression on MCF-7 cell line.
The ethyl acetate extract of H. zeylanica was obtained by ultrasound assisted maceration methods. The cytotoxic evaluation of ethyl acetate extract of H. zeylanica against MCF-7 cell line was determined by MTT-assay. The apoptosis mechanism was carried out by identifying the Bax and Bcl-2 gene expression on the RNA of MCF-7 cells treated with three series of concentrations of ethyl acetate extract (½ IC50, IC50 and 2IC50) using Polymerase Chain Reaction method. The result showed that the ethyl acetate extract exhibited cytotoxicity against MCF-7 cells with the IC50 value of 104.97 ppm. After treatment with the ethyl acetate extract, the MCF-7 cells displayed a high Bax gene expression and a decreasing Bcl-2 gene expression compared to control cell group.
These finding revealed that the ethyl acetate extract of H. zeylanica has moderate cytotoxicity against MCF-7 cell line and has the ability to induce the apoptosis in MCF-7 cells by increasing the expression of Bax gene as a pro-apoptotic protein and suppressing the expression of the Bcl-2 gene as an anti-apoptotic protein. Based on this study, the root of H. zeylanica has the potential to be developed as therapeutic agent for the treatment of breast cancer.

Vasorelaxant effect of ethyl acetate fraction of Hydrocotyle Javanica Thunb and its mechanism in spontaneously hypertensive rats aortic ring

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Hydrocotyle Javanica Thunb is derived from the same family as Centella asiatica, a local plant potentially claimed to possess antihypertensive effects but scientifically remains elusive. Thus, this study aims to investigate the antihypertensive and vasorelaxant activities of Hydrocotyle

Javanica Thunb extracts in spontaneously hypertensive rats (SHR). The most potent extract screened using non-invasive blood pressure measurement in which the animals were divided into seven treatment groups (control, verapamil (positive control), petroleum ether, chloroform, ethyl ecetate, methanol & water). The animals were treated orally at 500 mg/kg for 28 days prior with different Hydrocotyle Javanica Thunb extracts and controls, suggesting that methanol extract showed the best pharmacological action. Methanol extract was subjected to liquid-liquid fractionation by separation technique to obtain three fractions; hexane, ethyl acetate and water. The evaluation used various fractions of Hydrocotyle Javanica Thunb suggested that ethyl acetate fraction is the most potent fraction that exhibits anti-hypertensive effects prior proceed to mechanisms. Vasorelaxation effect of ethyl acetate fraction of Hydrocotyle Javanica Thunb was evaluated on the male SHR aortic ring. Isolated thoracic aortic rings were harvested and were subjected to endothelium-dependent relaxation studies, pre-contracted with phenylephrine (PE, 1 µM) and incubated with different antagonists such as NG-nitro-L-arginine methyl ester (L-NAME, 100 µM), indomethacin (10 µM), methylene blue (10 µM), atropine (1 µM), glibenclamide (10 Mm) and prazosin (0.01 µM). Incubation with prazosin, L-NAME and glibenclamide showed significantly potentiated vasorelaxation effect of Hydrocotyle Javanica Thunb.

These findings suggest that ethyl acetate fraction of Hydrocotyle Javanica Thunb possibly exerts vascular relaxation mechanisms in SHR mediated through endothelial-derived nitric oxide-cGMP relaxant pathway and prostacyclin pathways, activation of KATP channels and transmembrane calcium channel. Possible active compounds that contribute to the vasorelaxant effects are catechin, rutin and quercetin.

Modified synthesis of beta-amylid PET tracer [18F]FC-119S for our automated synthesizer

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Introduction:
[^{18}F]FC-119S ([2-(2-methylamino)pyridine-6-yl]-6-[(S)-3-fluoro-2-hydroxypropoxy]benzothazole), 1) exhibits a high binding affinity for Aβ1-42 protein aggregates[1] and enables visualization of amyloid plaques in the AD model 5xFAD mouse at early stage.[2] These reports prompted us to utilize 1 as an imaging marker for monitoring the production of our AD model animals. As 1 was a new PET tracer, we had to set up the radiosynthesis protocol to use 1 commonly for our PET facility. Here we describe the development of the preparation method of 1 using our automated synthesizer.

Methods:
In the literature 1 was synthesized by tertiary alcohol-promoted fluorination using the [^{18}F]fluoride ion eluted from anion exchange cartridge with nBu_4NHCO_3 and the reaction mixture was purified by HPLC for more than 20 min. We used K_2CO_3-eluted [^{18}F]fluoride ion for the fluorination in the presence of Kryptofix2.2.2 because it was the original protocol of our automation system. We tried to shorten the retention time of 1 during the HPLC purification since shorter total synthesis time is preferable for the PET tracer production.

Results:
As the literature described, the methanesulfonyl (Ms) precursor 2 with tetrahydropyranyl (THP)-protected hydroxy group was firstly used for the ^{18}F-labeling synthesis of 1. Fluorination of 2 using K[^{18}F] in DMSO at 100 °C and successive deprotection at the same temperature under acidic condition were carried out. In this case, a nonradioactive byproduct was appeared in front of 1 in the UV chromatogram during HPLC purification. The peak level of byproduct was high and separation of byproduct and 1 was low. Although radioactive 1 was obtained, it should be better to fix low resolution of the product separation up. Decreasing flow rate of eluent could improve the separation however the identification of byproduct might give us another solution. The byproduct was assumed as unfluorinated THP-deprotected Ms analog of 2 therefore we prepared the assumed compound 3 by the deprotection of THP group in 2 under the acidic condition. The HPLC analysis revealed retention time of 3 was corresponded to byproduct. Then we modified the leaving group of labeling precursor from Ms to p-toluenesulfonyl (Ts) because the byproduct derived from Ts precursor 4 must be more hydrophobic. Retention time of reverse-phase HPLC is governed by hydrophobicity and longer retention time is plausible for the corresponding deprotected Ts analog. Tosylation reaction for the terminal hydroxy group of glycol intermediate under conventional reaction condition was slow and low yield,however, it was improved when Bu_2SnO was added.[3] Finally Ts precursor 4 was obtained after THP protection. The ^{18}F-labeling synthesis of 1 from the precursor 4 was carried out and the desired 1 was obtained without the proximate UV peak.

Conclusions:
We synthesized ^{18}F-labeled FC-119S using Ts precursor under our automation system. The addition of Bu_2SnO was effective for the preparation of Ts precursor.

Discovery of novel quinoline derivatives as potent POLRMT inhibitors for the treatment of cancer

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Background: High oxidative phosphorylation (OXPHOS) happens in some tumors, which depends on OXPHOS for energy supply, particularly in slow-cycling tumor cells. Therefore, targeting human mitochondrial RNA polymerase (POLRMT) to inhibit mitochondrial gene expression emerges as a potential therapeutic strategy to eradicate tumor cells.

Purpose: This study aims to identify the quinoline derivatives as novel POLRMT inhibitors and explore its SAR led to the identification of an optimal compound L34, which exerted a strong antiproliferative effect on several cancer cells and decreased mitochondrial-related genes expression.

Method: The mitochondrial-related gene expression level using quantitative reverse transcription PCR (RT-qPCR) assay and antiproliferative activities using Sulforhodamine B (SRB) assay.

Results: A series of novel quinoline derivatives as POLRMT inhibitors were designed and synthesized. Among these compounds, an optimal compound L34 was recognized as the most promising POLRMT inhibitor. The RT-qPCR assay confirmed that compound L34 could effectively decrease the levels of relative mitochondrial mRNA. Moreover, it showed strong antiproliferation potency on several cancer cell types.

Conclusion: This study revealed that the novel quinoline derivatives show potential for the future development of POLRMT inhibitors.

Dotinurad treatment for hyperuricemia in patients with non-dialysis chronic kidney disease

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Background/aim: Chronic kidney disease is associated with increased risks of mortality and morbidity, and hyperuricemia is a risk factors for CKD. However, previous meta-analyses investigating the effects of UA-lowering treatments on the progression of CKD have had limited evidence. Dotinurad, a novel selective urate reabsorption inhibitor that inhibits URAT1, selectively reduces serum UA levels and may be a potential treatment option for hyperuricemia. However, real-world data on the use of dotinurad to treat hyperuricemia are limited. Therefore, this study aimed to evaluate the efficacy of dotinurad in patients with non-dialysis CKD.

Patients and methods: Twenty-one patients (15 male, 6 female; mean age, 71±11 years) with non-dialysis CKD were enrolled in this study. The average estimated glomerular filtration rate (eGFR) was 30.1±18.2 ml/min/1.73m2. Patients received dotinurad as first-line therapy, and the dose was gradually increased (1-3 mg) until the uric acid level reached the range of 6mg/dL. The time taken to achieve this was considered, and the observation period was 3.8±4.5 months.

Results: The administration of dotinurad effectively decreased uric acid levels, with an achievement rate of 76% for a uric acid level in the range of 6mg/dL.

Conclusion: Dotinurad is an effective and relatively well-tolerated agent for the treatment of hyperuricemia in patients with non-dialysis CKD.

Evaluation of the therapeutic effect and safety of REGKIRONA(Regdanvimab)

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Background: REGKIRONA®, the first domestic COVID-19 treatment drug, was released with conditional approval from the Ministry of Food and Drug Safety on February 5, 2021, and has been used for the treatment of all patients with mild to moderate severity in the high-risk group over 18 years of age. After the 3 phase clinical trial was completed, it was converted to an official license (September 17, 2021). On December 18, 2020, the hospital started operating a ward dedicated to infectious diseases to treat COVID-19 patients. However, since it has been less than a year since its release, there are few clinical cases reported in many medical institutions, so in this study, we evaluated the appropriateness of use, the efficacy and safety of treatment through clinical cases of patients who were hospitalized to the VHS medical center by October, 2021. This study also aimed to analyze the correlation with the patient's underlying disease through treatment failure cases.

Methods: From February to October 2021, we retrospectively analyzed the electronic medical records of patients who were hospitalized in the COVID-19 wards at the hospital and received REGKIRONA® treatment. In addition, regarding the criteria for the complete cure of COVID-19, refer to the “Criteria for Release of Quarantine” of the “Coronavirus Infectious Disease-19 Patient Treatment and Management of the Korea Centers for Disease Control and Prevention.”

Results: As a result of the study, most of the patients who were treated with REGKIRONA® while hospitalized at the hospital(n = 74) met the conditions for use, of which 65 were released from isolation (cure) and nine patients progressed to severe disease due to treatment failure. it was In addition, it took an average of 8.5 days from treatment to release of quarantine. Among the cured patients, adverse drug reactions occurred in 45 patients. 13(20.0%) of patients with headache, 9(13.8%) of patients with nausea and vomiting, 8(12.3%) of patients with muscle pain, and others(fever, chills, sleep disturbance) were observed. In the treatment failure group, 4(44.2%) of patients with nausea and vomiting, 4(44.2%) of patients with headache, 2(22.2%) of patients with chills, and 2(22.2%) of patients with dyspnea were observed.

Conclusions: Adverse drug reactions were reported in some of the patients who were admitted to the hospital and treated with REGKIRONA®, and although various side effects were reported compared to phase 2/3 study paper of

REGKIRONA®, most of them were mild and were immediately improved through supportive care, so they don't have clinical significance. And there was no injection-related adverse reaction, which was a serious adverse event that occurred in one case in paper of REGKIRONA®. After treatment most of the patients met the release criteria and were discharged, but 12.2% of patients progressed to severe disease due to treatment failure. In the treatment failure group, we tried to examine the underlying diseases, pre-treatment clinical symptoms, and clinical test values that could be classified as common, but the study subjects were limited. Therefore, it seems that continuous drug use evaluation is necessary in the future.

Development of Swietenia mahagoni seed as safe anti-diabetic adjuvant: A preclinical evaluation

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Swietenia mahagoni seed has been investigated around the globe for various health applications. The present study aims to evaluate the anti-diabetic potential of the seed using different in-vitro and ex vivo assays. The study also seeks to assess the cytotoxicity of the extract in normal cell lines (L6 cells) and brine shrimp lethality assay. The whole seed powder and the ethanol extract of the seed were used in the study. The phytochemicals viz., polyphenols, flavonoids, and tannins in the seed powder were evaluated. The anti-diabetic potential of the seed powder and the extract were evaluated by assessing the glucose adsorption potential, glucose uptake in Yeast cells, antiglycation activity, and glucose uptake in L6 myocytes. The antioxidant potential of the extract was evaluated using DPPH radical scavenging assay and 2',7'-Dichlorodihydrofluorescein Diacetate (DCFDA) Staining in L6 cells. The phytochemical evaluation showed the presence of polyphenols, flavonoids, and tannins in a significant quantity. In the DPPH assay, 100 µg of the extract shows radical scavenging activity similar to 50 µg of quercetin. In the DCFDA assay, It was observed that when compared to positive control cells (H2O2 treated cells), the extract treatment effectively reduced the fluorescence in the cells, indicating visual proof for free radical scavenging and cell-protecting ability. The seed powder adsorbed 40 % of the glucose in 3 hours of incubation. The Yeast cell glucose uptake and the L6 cell glucose uptake indicated the ability of the extract to enhance the glucose uptake by various tissues. The cytotoxicity assay showed the relative safety of the extract on the cultured cells with an IC50 value of 400µg, and no toxicity was observed against brine shrimps. the extract effectively reduced the formation of glycated end products or protein conjugates, as shown by the Antiglycation assay. Overall, the components of the mahagoni seed powder are safe and bear potential antioxidant and anti-diabetic ability. Further, research is underway to develop the swietenia mahagoni seed extract as a potential adjuvant in managing diabetes.

Effect of oleanolic acid on ischemic heart failure

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Objectives: Oleanolic acid (OA), a natural product, is a hepatoprotective drug used clinically, which has various biological activities such as anti-cancer, anti-osteoporosis, anti-obesity, anti-diabetes, anti-inflammation, antioxidant, etc. Recent studies have found that OA alleviates pressure overload-induced myocardial remodeling. However, its role in ischemic heart failure (IHF) remains unclear. This study aims to explore the therapeutic effect and mechanism of OA in IHF.

Methods: Myocardial infarction model was established through coronary artery occlusion by left anterior descending ligation in mice. OA (20 mg/kg/d) or Metoprolol (40 mg/kg/d) was given by continuous gavage for 28 days. The protective effects of OA on IHF were examined by echocardiography measurement, histological and TUNEL analysis. At the cellular level, the hypoxia model of cardiomyocytes was constructed. The apoptosis and inflammation of cardiomyocytes were detected. The protein and mRNA expression of AMPK and ESRRA in mice heart tissues and cardiomyocytes were detected by Western blot and RT-PCR.

Results: OA treatment significantly improved cardiac function of IHF mice and the symptoms of heart failure. Compared with the model group, the apoptosis of cardiomyocytes, inflammatory infiltration and fibrosis area of the heart tissue in OA group were decreased.OA increased mRNA and protein expression levels of AMPK and ESRRA in IHF mice. Meanwhile, OA reduced the apoptosis of cardiomyocytes and the expression of inflammatory cytokines caused by hypoxia.

Conclusions: OA exhibits cardioprotective effect on IHF by anti-inflammatory, anti-apoptotic and anti-fibrotic effects through AMPK-ESRRA signaling pathway, OA holds great promise as a preventive and therapeutic agent for IHF and related diseases.

Bivalent kappa acting opioid peptide constructs improves the stability while maintaining efficacy in kappa opioid receptor activity

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The opioid pandemic is a problem within the healthcare system as patients suffering from acute and chronic pain prescribed with opioid analgesics suffer from harmful side effects. Opioids act on opioid receptors (OPrs) in central nervous system (CNS) and peripheral nervous system (PNS) to reduce the nociceptive signal transduction by inhibiting the production of cyclic adenosine monophosphate (cAMP). Subsequently, activation of OPrs in the CNS leads to side effects such as addiction, euphoria and sedation, driving opioid abuse. OPrs are classified into three subtypes, Mu-OPr (MOPr), Delta-OPr (DOPr) and Kappa-OPr (KOPr) and distributed throughout the CNS and PNS. Peripheral acting opioids have capabilities to reduce the nociception signal while avoiding the CNS. Dynorphin is an endogenous opioid peptide produced by the nervous system and immune cells, acting on KOPr which are primarily distributed in the PNS. The drawback of dynorphin is susceptibility to enzymatic metabolism, hampering in vivo potency. A strategy employed to improve the stability and potency of dynorphin involved synthesising bivalent constructs of dynorphin 1-7 (Dyn 1-7), as it is the shortest and most potent fragment of dynorphin with KOPr selectivity. Linkers of varying lengths and composition were trialled, with Dyn1-7 pharmacophores were further stabilised for increased stability.

Method: Peptides were synthesised in-house using a Biotage® Initiator+ Alstra automated peptide synthesiser via solid phase peptide synthesis. Transfected HEK293 cells overexpressing MOPr, DOPr and KOPr were used to assess cAMP modulation of these bivalent constructs. cAMP is measured using a Perkin Elmer Alphascreen cAMP detection kit. Potencies were determined by the IC50 produced in a concentration curve for KOPr. A high-low micromolar screen was performed for MOPr and DOPr. The stability of peptides synthesised were assessed by exposing 1µM of peptide in biological media such as rat blood plasma, assay medium Hank's Balance Salts Solution (HBSS) and bovine trypsin in ammonium carbonate solution at 37°C and sampled at differing timepoints up to 120min. The samples were analysed via liquid chromatography mass spectrometry (LC-MS) for peptides presence and cleaved fragment. All analysis were performed using Graphpad Prism software.

Results: Synthesised peptides were deemed acceptable for in vitro assessments with purities >90% attained by HPLC. Peptides show potencies (IC50) in KOPr ranging from 0.9 nM to 3.2nM, and low selectivity to MOPr and DOPr. In HBSS, insignificant degradation was observed with 80-99% remaining in solution after 2 hrs, supporting the stability of the peptide in the cAMP assay. Plasma stability of lead bivalents indicated half-lives up to 100 min, while trypsin half-lives extend up to 1 hour.

Conclusion: The stabilised bivalent constructs display exceptional potency (sub-nanomolar IC50) and extended stability in rat plasma and trypsin. In vivo evaluation in pain models is the subject of future studies.

Liraglutide combined three-dimensional cell therapy: The potential on improving cell transplantation therapy for nerve repair

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Background: Cell transplantation has emerged as a potential strategy for nerve repair. Olfactory ensheathing cells (OECs) are one of the promising cell options due to the easy accessibility and treatment outcomes. However, limitations on OEC-based therapies still exist. One major issue is the poor quality of transplanted cells, including low cell viability and unfavourable cell properties. Therefore, improving cell survival and properties of OECs aiming to improve the nerve repair purpose have received considerable attention.

Purpose: The present study explores the possibility of combining drug treatment (liraglutide) and three-dimensional (3D) cell culturing techniques to improve OEC properties.

Method: OECs were pre-treated with vehicle or liraglutide and the cell viability and properties (migration and morphology) were evaluated. The pre-conditioned cells were then formed into 3D spheroids. The spheroids were analysed for cell migration in a spheroid spreading assay or were co-cultured with dorsal root ganglion (DRG) spheroids for neuronal interaction investigation.

Results: The results indicated that liraglutide improved OEC survival, migration, and morphology. The liraglutide-treated OEC spheroids also possessed better spreading capabilities. Furthermore, in the co-cultured DRG model, liraglutide-treated spheroids enhanced neuronal attraction and interaction.

Conclusion: Liraglutide can improve OEC viability and therapeutically relevant properties. Moreover, combination of liraglutide treatment and 3D culture technique can be a promising strategy for optimising OEC-based cell therapy for nerve injury treatment.

LL1, a novel small molecule inhibitor, overcomes oxaliplatin resistance in colorectal cancer by targeting STAT3 signaling and crosstalk with tumor-associated macrophages

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Background: Colorectal cancer (CRC) is the second most common cause of cancer-related deaths worldwide. And oxaliplatin is a first-line chemotherapy drug used to treat CRC. However, acquired resistance progression is a major cause of oxaliplatin treatment failure. The STAT3 family appears to regulate CRC resistance; furthermore, it plays a central role in malignant cycles within immunosuppressive tumor microenvironments. However, it remains unclear whether STAT3 regulates oxaliplatin-induced CRC resistance or immune crosstalk within tumor microenvironments. Therefore, we identified LL1 as a relatively specific STAT3-targeted inhibitor through small molecule screening; this inhibitor was used in combination with oxaliplatin to treat oxaliplatin-resistant CRC progression in this study.

Purpose: This study aims to demonstrate that LL1 can reverse oxaliplatin resistance in colorectal cancer by inhibiting STAT3 phosphorylation and blocking crosstalk between tumor-associated macrophages and resistant cells.

Methods: Oxaliplatin-resistant HCT 116 and HCT 8 colorectal cancer cell lines were constructed. These cell lines were then used to evaluate the reversal of LL1 on oxaliplatin resistance in colorectal cancer using CCK8 determination, Colony formation, Flow cytometry, Western blot analysis, and in vivo oxaliplatin-resistant xenotransplantation models. Additionally, co-culturing colorectal cancer-resistant cell lines with THP-1 macrophages was used to determine the malignant phenotype of CRC-resistant cells as well as macrophage polarization and signal secretion.

Results: This study shows that oxaliplatin resistance in CRC may be promoted by the STAT3/TWIST signaling pathway; furthermore, tumor-associated macrophages (TAMs) enhance CRC cell proliferation, invasion, and resistance through IL-6 activation of the IL-6/STAT3 pathway in oxaliplatin-resistant cells. LL1 can block their crosstalk by inhibiting STAT3 phosphorylation.

Conclusion: When multiple signaling pathways converge on STAT3, STAT3 plays a key role in tumor growth and resistance. Therefore, targeting STAT3 for inhibition may be a promising strategy to provide more effective clinical treatment for oxaliplatin-resistant CRC. LL1 is a novel STAT3 inhibitor that makes colorectal cancer cells sensitive to oxaliplatin both in vitro and in vivo while also blocking crosstalk between macrophages and resistant cells. These findings provide preclinical evidence of oxaliplatin-resistant CRC and further verify that LL1 is an important immunotherapy adjuvant for oxaliplatin resistance in CRC.

Discovery of novel ALK degraders based on the hydrophobic tagging technique

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Background: As a promising therapeutic strategy for designing TPD molecules that have long been underestimated, the hydrophobic tagging (HyT) technology shows several advantages over PROTACs, but also suffers many disadvantages, particularly, the limited number of usable HyT tags restrains the rapid development and widespread applications of HyT technology.

Purpose: This study aims to investigate the lengths of linkers, types of hydrophobic tags, and structures of solvent-exposed moieties that affect the efficiency and potency of the resulting HyT-based degraders。
Method: Binding affinities using time-resolved fluorescence resonance energy transfer (TR-FRET) and antiproliferative activities [3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide] (MTT) methods, ALK relative protein levels were detected by western blotting.

Results: Bornane was discovered as an unprecedented hydrophobic tag in this study and was used to degrade the ALK fusion protein by linking it to ALK inhibitors. The most promising degrader, B36, potently reduced ALK levels through Hsp70 and the ubiquitin-proteasome system (UPS) in vitro without compensatory upregulation. Furthermore, B36 exhibited a significant tumor-inhibiting effect in vivo with moderate oral bioavailability.

Conclusion: This study revealed that the discovery of novel hydrophobic bornane tags shows promise for the future development of TPD technology.

Silybin inhibits hepatocytes to produce and secrete succinate to reverse liver fibrosis

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There are no effective drugs currently for the progression of steatohepatitis to liver fibrosis. Silybin is widely used in the treatment of non-alcoholic steatohepatitis (NASH). This study aimed to reveal the mechanism of silybin in NASH-liver fibrosis transformation. Mice were fed with CDAHFD for six weeks to induce liver steatosis as well as mild liver fibrosis. Succinate accumulated in hepatocytes during NASH because the tricarboxylic acid cycle blocked. We detected the progress of synthesis and efflux of succinate from mitochondria to extracellular and the activation of hepatic stellate cells. We found that SDHA, the subunit of succinate dehydrogenase, decreased in CDAHFD-fed mice livers and the overall activity of SDH decreased as well. The lipomics

study of liver mitochondrial membrane showed that the proportion of PE, PS, PC and PI decreased significantly and the proportion of PG and phosphatidic acid increased significantly in CDAHFD-fed mice livers. The CL level decreased significantly which was an important anchor in SDH. Silybin restored the expression of CRLS1 and partially restored the mitochondrial membrane phospholipids level to normalize the tricarboxylic acid cycle. After siRNA interfered with CRLS1 expression, the SDH activity of HepG2 cells decreased. In HepG2 cells and primary hepatocytes, silybin alleviated the increase of succinic acid efflux caused by PA. After PA stimulated HepG2 cells, the succinic acid transporter SLC25A10 on the mitochondria did not change significantly, but the expression of succinic acid transporter MCT1 on the cell membrane increased significantly. AZD3965, the inhibitor of MCT1, decreased succinic acid efflux into the medium. Silybin reduced succinic acid efflux by reducing the expression of MCT1.The expression of fibrosis-related markers in LX-2 cells were increased by the conditioned medium of HepG2 cells treated with PA or by direct administration of succinic acid, but decreased by the conditioned medium of HepG2 cells treated with silybin-PA or by direct administration of GPR91 inhibitor. In sum, in the mitochondria of CDAHFD mice, the decrease of CRLS1 expression leads to the disorder of phospholipid in mitochondrial membrane, which further leads to the decrease of SDH activity and succinate accumulation. Succinate accumulated in mitochondria was transferred outside through MCT1. Silybin inhibits NASH-induced liver fibrosis by inhibiting both the synthesis and efflux of succinate from mitochondria to extracellular to activate hepatic stellate cells.

Development of highly potent AKR1C3 inhibitors to restore the chemosensitivity of drug-resistant breast cancer

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Background: The incidence of breast cancer ranks first among all female malignant tumors. Drug resistance, which leads to rapid recurrence or progression of cancer and ultimately death of the patient, is one of the prime basis of breast cancer treatment failure. It has been reported that aldo-keto reductase 1C3 (AKR1C3) is overexpressed in breast malignancy and is correlated with tumor invasiveness, aggressiveness, and chemotherapy resistance.

Purpose: This study was designated to discover novel AKR1C3 inhibitors and evaluate their ability to reverse drug resistance of breast cancer cell line.

Method: HipHop model and pharmacophore model-base virtual screening was performed to discover lead compound. Then based on the analysis of the binding mode of S19-1001 with AKR1C3, four ground of rational drug design was performed to improve the potency of the scaffold. After in vitro enzyme inhibiting evaluation on AKR1C3 of all derivatives, the most potent compound S19-1035 was chosen to determine the crystal structures in complex with AKR1C3. In vitro cellular toxicity assay on breast cancer cell line was also performed to determine the anti-proliferation effect of S19-1035 when administrated alone or in combination with doxorubicin.

Results: Lead compound S19-1001 was screened out and showed moderate potency (IC50 = 233 ± 1.5 nM). 37 derivatives were then synthesized and the most potent compound S19-1035 exhibited an IC50 value of 3.04 ± 0.3 nM. Co-crystal structures of AKR1C3 with S19-1035 was further resolved to analyze the detailed binding information. S19-1035 could occupy the binding site of AKR1C3 in a “V” shape and display strong π-alkyl and π-σ interactions with multiple hot-residues (Trp-86, His-117, Phe-306, Tyr-319) in different sub-pockets. In vitro cellular assay was performed and high dosage S19-1035 (100 μM) displayed no anti-proliferation effect on breast cell lines (MCF-7, MDA-MB-231 and doxorubicin-resistant cell line MCF-7/doxorubicin) under long-term exposure (96 h), which proved the cellular safety of S19-1035. Moreover, in vitro synergistic assay showed that 10 μM S19-1035 could efficiently reverse the drug resistance of MCF-7/doxorubicin in combination with 50 μM doxorubicin and improve the cellular toxicity of doxorubicin. Chou-Talalay analysis showed that the combination index values between S19-1035 and doxorubicin were 0.27 (strong synergism), which indicated S19-1035 could significantly restore the sensitivity of drug-resistant cells to doxorubicin.

Conclusion: In summary, this work identified a novel class of AKR1C3 specific inhibitors with nanomolar inhibition potency and good adjuvant effect with doxorubicin on drug-resistant breast cancer, providing a good foundation for the development of new combination chemotherapeutic drugs.

Discovery of novel NLRP3 inflammasome inhibitors by an in-house database driven identification from natural product oridonin

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Background: Modern science considers inflammation to be a double-edged sword in the whole life of the body. Excessive or unreasonable activation of inflammasomes may lead to the release of pro-inflammatory cytokines, subsequently triggering pyroptotic cell death. Targeting NLRP3 inflammasome with inhibitors is anticipated to provide novel strategies for NLRP3-driven inflammatory diseases.

Purpose: This study aims to identify a promising drug candidate targeting NLRP3 inflammasome with new scaffold and explore its SAR led to the identification of an optimal compound 32, which exerted strong anti-inflammation effect.

Method: By screening our in-house database of oridonin and further optimizing the hit at C-7 hydroxyl group, a series of novel derivatives were obtained and tested using BMDMs upon challenge with LPS/ATP to measure their inhibitory potency on the release of IL-1β by enzyme-linked immunosorbent assay, and anti-inflammatory activities were assayed in monosodium urate (MSU)-induced gouty arthritis model.

Results: An attractive skeleton 5 was identified and new derivatives were further designed and synthesized from hit 5 and the optimal compound 32 displayed two digital nanomolar inhibition on NLRP3 inflammasome. Moreover, diverse agonists-induced activation of NLRP3 could be impeded by compound 32 without altering NLRC4 or AIM2 inflammasomes. Importantly, compound 32 possessed tolerable pharmaceutical properties and exhibited significant anti-inflammatory activity in MSU-induced gouty arthritis model.

Conclusion: This study provided a promising drug candidate targeting NLRP3 inflammasome with new scaffold, which might serve as a tool for further investigation.

Wogonin alleviates inflammation and oxidative stress in acute lung injury via targeting PPARα

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Acute lung injury (ALI) is a common and severe lung disease characterized by uncontrolled inflammatory responses and oxidative stress. Inhibiting inflammation and oxidative stress has been recognized as one of effective strategies to attenuate ALI. In our present study, Wogonin (WOG), a natural flavonoid compound derived from the root of Scutellaria baicalensis., exhibited significant anti-inflammatory and anti-oxidant activities in ALI animal models and alveolar macrophages model. And mechanism study found that WOG activated PPARα by upregulating its expression and phosphorylation. The study will provide evidence for the development of therapeutic drugs for ALI, and bring reference to the application of natural products from traditional Chinese medicine.

Impact of PCSK9 inhibitors on platelet reactivity among patients undergoing primary percutaneous coronary intervention

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Aim: The main objective of this study was to investigate the impact of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors on parameters detected by Thrombelastography (TEG) in patients with acute coronary syndrome (ACS) who underwent primary percutaneous coronary intervention (PCI).

Methods: During January 2022 to March 2022, this prospective study consecutively included 219 ACS patients receiving primary PCI in Zhongshan Hospital, Fudan University. According to whether postoperative PCSK9 inhibitors were used, patients were divided into the PCSK9 inhibitor group and the statin-only group. 1:2 propensity score matching method was used to balance baseline characteristics between two groups. MAADP (adenosine diphosphate-induced maximal amplitude) was measured by TEG 72-96 h after PCI. All patients were followed up clinically for 6 months.

Results: After matching, there were 55 and 110 patients in PCSK9 inhibitor group and the statin-only group. The MAADP levels in patients with PCSK9 inhibitors were significantly lower than the statin-only patients (12.10 [7.10-20.60] mm vs. 21.95 [14.08-30.00] mm, P=0.034). Multivariate linear regression analyses indicated that the use of PCSK9 inhibitors was independently associated with MAADP levels (β=0.242, P<0.001). No significant differences between groups were observed for the clinical outcomes. Conclusion: PCSK9 inhibitor administration could inhibit platelet activation. This study provided a certain basis for the use of PCSK9 inhibitor in ACS patients with normal serum lipid levels.

Genistein prevents cigarette smoke-induced airway inflammation and oxidative stress in cells and rats by modulating cAMP/PKA/NFκB signaling

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Chronic obstructive pulmonary diseases (COPD) is a lung disease caused by chronic exposure to cigarette smoke (CS). High dietary intake of isoflavones has been reported to reduce the risks of COPD and improve lung function, however, the mechanisms were unclear so far. The present study aims to investigate the effect and mechanism of a

principal isoflavone genistein in preventing CS-induced COPD. Human bronchial epithelial BEAS-2B cells were pretreated with genistein or different pharmacological agents 1 hour prior to exposure to cigarette smoke medium (CSM). The cell culture medium and cell lysates were collected for further experiments. In in vivo study, male Sprague-Dawley rats were administrated with genistein or vehicle by oral gavage, and exposed to either CS or sham air (SA) 1 hour daily. After 8 weeks' exposure, lung tissues and the bronchoalveolar fluid (BALF) were collected for further study. In cells stimulated with CSM, the levels of IL-8 and malondialdehyde (MDA) were increased, and the activities of superoxide dismutase (SOD) and catalase (CAT) were decreased; these changes were reversed by genistein. CSM increased phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2), p38, inhibitor of kappa B α (IκBα) and NFκB p65; these increases were down-regulated in cells pretreated with genistein. Genistein also reduced CSM-induced increase in 5'-AMP level, and decreased cyclic adenosine monophosphate (cAMP) level and protein kinase A (PKA) activity. In CS-exposed rats, alveolar enlargement was observed, which was ameliorated by genistein. CS-exposure also increased the levels of cytokine-induced neutrophil chemoattractant-1 (CINC-1), IL-6 and monocyte chemotactic protein 1 (MCP-1) in BALF, which were attenuated by genistein. Moreover, genistein reduced CS-induced elevation of MDA and normalized the activities of SOD and CAT in rat lung homogenates.
In conclusion, genistein can reduce CSM-induced airway inflammation partly through activation of cAMP/PKA/NFκB pathway, and partly through inhibition of ERK1/2 and p38 and reduction of oxidative stress. Genistein might be a potential therapeutic agent for preventing the development of COPD.

The oncogenic role of cyclin-dependent kinase inhibitor 2C in lower-grade glioma

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Background: Lower-grade gliomas (LGGs) are slow-growing, indolent tumors that usually affect younger patients and present a therapeutic challenge due to the heterogeneity of their clinical presentation. Dysregulation of cell cycle regulatory factors is implicated in the progression of many tumors, and drugs that target cell cycle machinery have shown efficacy as promising therapeutic approaches. However, no comprehensive study has examined how cell cycle-related genes affect LGG outcomes.

Methods: The cancer genome atlas (TCGA) data were used as the training set for differential analysis of gene expression and patient outcomes; the Chinese glioma genome atlas (CGGA) was used for validation. Levels of one candidate protein, cyclin-dependent kinase inhibitor 2C (CDKN2C), and its relationship to clinical prognosis were determined using a tissue microarray containing 34 LGG tumors. A nomogram was constructed to model the putative role of candidate factors in LGG. Cell type proportion analysis was performed to evaluate immune cell infiltration in LGG.

Results: Various genes encoding cell cycle regulatory factors showed increased expression in LGG and was significantly related to isocitrate dehydrogenase and chromosome arms 1p and 19q mutation status. CDKN2C expression independently predicted the outcome of LGG patients. High M2 macrophage values and elevated CDKN2C expression were associated with poorer prognosis in LGG patients.

Conclusion: CDKN2C plays an oncogenic role in LGG, associated with M2 macrophages.

Antimicrobial action and reversal of resistance in MRSA by difluorobenzamide derivatives targeted at FtsZ

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RFMO-04 - Rapid Fire Session Monday, P1-P2, September 25, 2023, 2:30 PM - 4:00 PM

Background
The WHO recognizes antibiotic resistance as a top five global burden of disease. This threat necessitates urgent new therapies with novel mechanisms of action. In bacteria, the filamentous temperature sensitive Z-ring (FtsZ) protein represents an attractive target for the development of new antimicrobial agents as it plays a crucial role in cell division.

Purpose
This study sought to characterize the antimicrobial activity of five difluorobenzamide derivatives with non-heterocyclic substituents attached through the 3-oxygen.

Method
In this study, the difluorobenzamide derivatives of 3-methoxybenzamide were synthesized. Microscopic analysis was performed to determine if the derivatives inhibited bacterial cell division. On-target activities were assessed through their effects on the polymerization and GTPase activity of purified FtsZ. Reversal of resistance was tested in combination with beta-lactam antibiotics that are used in clinical practice. In silico docking was used to assess the

compound binding site in vivo and in vitro toxicity using mammalian tubulin, liver cells and red blood cells were performed.

Results
The compounds displayed antimicrobial activity against the pathogenic bacterium methicillin resistant Staphylococcus aureus as well as clinically pathogenic MRSA strains. The compounds could act synergistically with beta-lactam antibiotics, suggesting there may be a potential for reversal of resistance. The compounds effectively inhibited cellular division as observed in the 'ballooning' phenotype and their ability to enhance GTPase activity and FtsZ polymerization in vivo. Additionally, off-target analysis was undertaken to ensure the compounds would not display haemolytic activity, cytotoxicity against mammalian cells and a nematode model in vitro.

Conclusion
This study identified promising new antimicrobial compounds that selectively inhibit bacterial cell division. It is also the first report of FtsZ-targeting compounds that could reverse the resistance of a beta-lactam antibiotic in clinically resistant MRSA. These derivatives are therefore promising compounds for further development as antimicrobial agents or as resistance breakers to re-sensitive MRSA to beta-lactam antibiotics.

Investigation for neuroprotective activity of Iris kashmiriana for treatment of dementia of Alzheimer's type

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RFMO-04 - Rapid Fire Session Monday, P1-P2, September 25, 2023, 2:30 PM - 4:00 PM

Background: Flavonoids has an ability to combat with oxidative stress caused in Alzheimer's disease. Iris kashmiriana rhizome is a rich source of isoflavonoids and isoflavones like irigenin, nigricin, irilone, and irisolidone.

Purpose: The present study was designed to evaluate neuroprotective activity of Iris kashmiriana bioactive fraction in ICV-STZ induced neurodegeneration in rats.

Materials and methods: Rhizome part is defatted using petroleum ether and 90% hydroethanolic extract is prepared. Plant fractions using chloroform, ethyl acetate, n-butanol as solvent were prepared from the extract. An in-vitro screening tests include Nitric oxide, H202 and DPPH scavenging assays. Rat (pheochromocytoma) PC12 cell lines of extract and fractions against amyloid-β and. In in-vivo study employing Wistar rats weighing 250-280g was performed. Streptozotocin at 3 mg/kg via ICV route was given on first day and the bioactive fraction (butanolic fraction) at (80 mg/kg p.o.) was administered daily for 21 days. A statistical analysis was carried out using Graph pad Prism 5.

Results: In in-vitro tests the bioactive fraction prevents cytotoxicity in DPPH, NO and H2O2 with IC50 values 30.36±0.12 µg/ml, 78.8±0.13µg/ml, 26.19±0.11µg/ml respectively. The concentrations of cells showed significant increase when treated with bioactive fraction (Effect shown was 90% at concentration 10 µg/ml, 110% at concentration 50 µg/ml, 120% at concentration 100 µg/ml and 92% at concentration 150 µg/ml). ICV-STZ treated animals exhibited memory deficits in the Morris water maze (mean dwell time 17±4 for Normal control, 30±1 for Sham, 16±1 for STZ, 28±5 for STZ+ n-butanolic fraction), Y maze (Alteration% was 45±1 for normal, 43±0.5 for Sham, 28±1.5 for STZ, 29±0.5 for STZ+ n-butanolic fraction), open field test (mean time spent 14±4 for normal, 13±4 for Sham, 7±0.5 for STZ, 12±4 for STZ+ n-butanolic fraction) and photo actometer test (mean ambulatory score was 450±50 for normal, 460.16±10 for Sham, 380.5±30 for STZ, 480±30 for STZ+ n-butanolic fraction). Administration of butanolic fraction produced significant restoration of memory dysfunction. Biochemical estimations of thiobarbituric acid reactive substances (0.6±0.4 for normal, 0.3±0.01 for Sham, 1.3±0.1 for STZ, 0.2±0.01 for STZ+ n-butanolic fraction), reduced glutathione and acetylcholinesterase levels (0.6±0.2 in normal, 0.6±0.4 in Sham, 1.1± 0.3 in STZ, 0.3± 0.5 in STZ+ n-butanolic fraction) in brain.

Conclusion: The findings of the present study revealed that the extract and fractions of Iris kashmiriana possess protective role against Alzheimer type dementia in ICV-STZ rats as can be seen from its anti-oxidant, anti-inflammatory and neuroprotective potential. The rhizomes of this plant can therefore be used to manage neurodegenerative disorders including AD.

CYP4Z1: A potential target for breast cancer treatment by killing cancer stem cells

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Objective: Breast cancer stem cells (BCSCs) are considered to be one of the roots contributing to the chemoresistance and metastasis in breast cancer. Our and other studies have shown that CYP4Z1 expression is increased in breast cancer tissues and positively correlated with breast cancer progression. This study aims to reveal the critical roles and underlying mechanisms of CYP4Z1 in the signal activation of BCSCs through CYP4Z1 transgenic mice and further screened out a better CYP4Z1 inhibitor.

Methods: We constructed CYP4Z1 transgenic mice and combined with breast cancer spontaneous model MMTV-PyMT mice to reveal the effects of CYP4Z1 in breast cancer occurrence and progression. Additionally, the underlying mechanisms were elucidated using drug genomics, metabolomics, and molecular interaction analysis. Furthermore, we chemically synthesized and screened out CYP4Z1 inhibitors through enzyme activity detection.

Results: CYP4Z1 was found to contribute to PyMT-induced breast cancer occurrence and progression. And compound 7c was screened out to be a better CYP4Z1 inhibitor, which can specifically kill BCSCs by targeting CYP4Z1.

Conclusion: CYP4Z1 can be a novel drug target for breast cancer and compound 7c might be used for breast cancer treatment by targeting CYP4Z1. And compound 7c can be a novel lead compound for CYP4Z1 inhibitor.