FIP Brisbane 2023

81st FIP World Congress of Pharmacy and Pharmaceutical Sciences in Brisbane, Australia,
24 to 28 September 2023

Clinical biology

Prevalence of hepatitis B infection and screening for protective antibodies amongst pregnant women in Obio-Akpor LGA

Dr Amaka Marian Awanye[1], Dr Chidozie Ibezim[1], Pharm Lekara Teete[1], Pharm Emilia Ogagah[1], Dr Iheanyi Okonko[2], Prof Catherine Stanley[1]
[1]Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, University Of Port Harcourt, Rivers State, Nigeria,
[2]Department of Microbiology, Faculty of Science, University Of Port Harcourt, Rivers State, Nigeria
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Hepatitis B virus (HBV) infection is a major cause of liver-associated death and disability affecting over 350 million people globally. Individuals with chronic hepatitis B will progress to a more severe form of disease mainly cirrhosis, end-stage liver disease and hepatocellular carcinoma. Unvaccinated children exposed to HBV have >95% risk of developing a chronic infection as compared with infection in unvaccinated adults that have <2% risk of developing a chronic disease. This shows that there is a need to create awareness among pregnant women on the preventive and control measures which is affected by their knowledge, attitude and practices.

Objectives: The study is aimed to determine the knowledge and attitude of pregnant women towards HBV, the sero-prevalence of HBV infection as well as the level of protective antibodies among pregnant women attending ante-natal clinics in three selected health centers in Obio-Akpor LGA, Rivers State.

Methods: A cross-sectional study design using a random sampling technique was conducted between June to July 2022. Data from a total 103 consenting participants were collected using a structured questionnaire. Also, blood samples were collected and tested for the presence of HBV immunological markers such as Hepatitis B surface antigen (HBsAg) and Hepatitis B core protein IgM (anti-HBc IgM) and hepatitis B surface protein (anti-HBs) antibodies using immuno-chromogenic and enzyme-linked immunosorbent assay methods. Descriptive statistics was generated for variables including frequencies and percentages for categorical variables. Bivariate analyses were carried out and the Chi-square was used to test associations between variables using JASP® software version 0.17.1. A p-value of <0.05 was considered statistically significant.

Results: The overall knowledge of hepatitis was good, poor or no knowledge at all in 37%, 50% and 13% of study participants respectively. The overall attitude towards HBV vaccine was 94% positive. Age ($p < 0.001$) was significantly associated with good knowledge while level of education ($p = 0.009$) was significantly associated with positive attitude. A chronic HBV infection was detected in 1% of participants due to absence of Anti-HBc IgM. Three percent of participants have an acute infection due to presence of Anti-HBc IgM while protective antibodies were observed only in 48.54% of study participants (due to presence of anti-HBs). Only 17% could recall if they have been previously vaccinated against HBV. Frequency of alcohol consumption ($p = 0.04$), frequency of prescription drug consumption ($p = 0.008$), frequency of taking analgesics ($p = 0.001$), family history of liver disease ($p = 0.013$) and number of doses of HBV vaccine received ($p = 0.005$) were considered significantly associated with presence of protective HBs antibodies.

Conclusion: This study has demonstrated a poor knowledge of HBV. Although the incidence of HBV infection is low, the prevalence of protective antibodies was detected in less than half of the study participants. And this puts their babies at risk of chronic HBV infection. Furthermore, the high positive attitude towards receiving HBV vaccine suggests that increasing awareness and public health education should be recommended.

Proposal for setting up a forensic toxicology laboratory

Ms Kimberly Grima[1], Doctor Janis Vella Szijj[1], **Prof Lilian M. Azzopardi**[1]
[1]Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta , , Malta
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Absence of local forensic toxicology laboratories that focus on the analysis of drugs of abuse has resulted in the need to rely on external institutions leading to challenges in obtaining timely results.

Purpose: To propose the development plan for a practical, feasible and sustainable facility where analysis of drugs of abuse can be carried out for forensic purposes.

Method: The main process equipment used for the analysis of drugs of abuse was identified. Laboratory workflow was established and a list of instrumentation utilized for drug analysis, along with the respective specifications was drawn. Cost comparisons of equipment and instrumentation needed were made. The fixed capital investment (FCI) for setting up the laboratory was estimated using the percentage of delivered equipment method. Meetings were held with personnel involved in the regulation and analysis of drugs of abuse to review the proposed facility.

Results: Equipment identified included: analytical balance, automated immunoassay analyser, centrifuge, gas chromatography-mass spectrometry unit, liquid chromatography-mass spectrometry unit, laboratory freezer, laboratory refrigerator, laboratory water distiller, organic solvent evaporator, pH meter, ultrasonic bath, vortex mixer, hydrogen cylinder and nitrogen cylinder. The total cost of the main process equipment is €453,323.23. The Fixed Capital Investment cost is estimated to total €2,465,851.71 (±30%).
The laboratory proposal is based on the workflow which involves digitalised quality documents. Extraction of drugs or metabolites from analysis from the biological specimen is undertaken using protein precipitation or liquid-liquid extraction. Analytes are then screened for the presence of a specific drug using immunoassay techniques such as the enzyme multiplied immunoassay. Following screening tests, confirmatory tests are performed to confirm the identity of the drug using high-performance liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. After sample analysis is complete, data is analysed and evaluated by a forensic expert and recorded accordingly. Systems to ensure security of samples, data and reports are put in place.
The cost of developing the laboratory takes into account both direct and indirect costs, including purchased equipment, installation, and engineering and construction expenses. From feedback obtained from experienced stakeholders, the investment is deemed necessary given the need for establishing a sophisticated facility.

Conclusion: Selection of appropriate instrumentation and equipment and robust laboratory workflow is essential to ensure efficient, robust and reliable analysis of drugs of abuse. Reliability of analytical test results is important as findings may serve as legal scientific evidence in judicial proceedings.

Analysis of minor cannabinoids

Mr Thoma Vella[1], Doctor Janis Vella Szijj[2], **Prof Lilian M. Azzopardi**[3]
[1]Department of Pharmacy, Faculty of Medicine, University of Malta, Msida, Malta, [2]Department of Pharmacy, Faculty of Medicine, University of Malta, Msida, Malta, [3]Department of Pharmacy, Faculty of Medicine, University of Malta, Msida, Malta
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Cannabis is increasingly researched due to its different therapeutic properties. The two major cannabinoids are Cannabidiol (CBD) and Tetrahydrocannabinol (THC). Minor cannabinoids include acidic minor cannabinoids such as Tetrahydrocannabinolic acid (THCA) and Cannabidiolic Acid (CBDA), neutral minor cannabinoids such as Cannabinol (CBN) and Cannabigerol (CBG), and varinic minor cannabinoids such as Cannabidivarin (CBDV) and Tetrahydrocannabivarin (THCV).

Purpose: To identify and compare analytical methods for determination of minor cannabinoids in different matrices.

Method: Systematic literature search was carried out to identify analytical methods used for determination of minor cannabinoids. Sources used were peer-reviewed journal articles published in English between 2015 and 2022 on the University of Malta's HyDI database using key words such as "determination of cannabinoids". Analytical procedures used were compared to identify the common parameters used for determination of minor cannabinoids.

Results: One hundred and one articles which described analytical techniques for the identification of minor cannabinoids were identified. Minor cannabinoids were determined using Ultra-high performance liquid chromatography (UHPLC) (n=69), Gas chromatography (GC) (n=133) and High-Performance Liquid Chromatography (HPLC) (n=228). The most commonly analysed minor cannabinoid was CBN which was determined using UHPLC (n=56), GC (n=100) and HPLC (n=189). Other common minor cannabinoids analysed were THCA and CBDA, using HPLC (THCA n=117) (CBDA n=116), UHPLC (THCA n=46) (CBDA n=40) and GC (THCA n=46) (CBDA n=24). The most commonly used detector for all techniques was Mass Spectrometer coupled to UHPLC (n=47) , GC (n=112) and HPLC (n=140).

Conclusion: HPLC is commonly used for analysis of minor cannabinoids. Unlike GC, derivatisation for determination of acidic cannabinoids is not required. Since acidic cannabinoids can decompose to their neutral counterparts at higher temperatures, HPLC is a more suitable technique than GC to determine their presence and quantity. CBN is the degradation product of THC and is commonly analysed

when determination of the total initial quantity of THC is required. Mass Spectroscopy offers superior selectivity and sensitivity when compared to other types of detectors and is commonly used for determination of minor cannabinoids which are usually present in smaller concentrations in cannabis when compared to other major cannabinoids.

Influence of different winemaking techniques on total phenolic content in wine of newly created grape variety Vožd

Mr Uroš Čakar[1], Mrs Ivana Plavšić-Janjatović[2], Mrs Nikolina Živković[2], Mr Aleksandar Petrović[2], Mrs Ljiljana Gojković-Bukarica[3]

[1]Faculty Of Pharmacy, University Of Belgrade, Belgrade, Serbia, [2]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia, [3]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

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

Background: The variety Vožd obtained from the crossing combination Začinak x Prokupac. Phenolic compounds are extracted from grape clusters during red grape maceration and fermentation and contribute to the quality of a red wine. The optimization of traditional winemaking methods, such as carbonic, thermo- and cold maceration, could be an interesting tool in order to obtain wine with a great phenolic characteristics.

Purpose: This paper presents influence of different maceration techniques (thermo maceration, carbonic and cold maceration) on the total phenolic content of Vožd wine.

Methods: The grape variety Vožd (vintage 2020) was harvested in optimal enological maturity which originated from vineyards belonging to the "Draskovic" winery in Vrsac (Serbia). After crashing and destemming, thermo maceration, cold and carbonic maceration were applied. Cold maceration was conducted at temperature of 4°C (4 days) and thermo maceration at temperature of 60°C (heated one hour). For carbonic maceration it was necessary usage of dry ice and that maceration lasted four days. After fermentation, pomace was separated and obtained wine samples were bottled and stored until analyses. Total phenolic content in wine samples was determined by the Folin–Ciocalteu's (FC) method using gallic acid as a standard.

Results: The highest total phenolic content was measured in wine which was subjected to heating on 60°C (2205.0 mg GAE/L). Treatment of cold maceration also showed a good extraction of these compounds in wine in amount of 2135.0 mg GAE/L. The technique, which led to lowest total phenolic content in wine, was carbonic maceration (1270.0 mg GAE /L).

Conclusion: Usage of high and low temperature as prefermentative treatments caused better phenolic extraction during vinification, compared to carbonic maceration.

Fruit wine active compounds and its activity on the enzymatic systems

Mr Uroš Čakar[1], Mrs Nikolina Živković[2], Mrs Ivana Plavšić-Janjatović[2], Mrs Mirjana Čolović[3], Mrs Danijela Karstić[4], Mr Ivan Stanković[1], Mr Aleksandar Petrović[2], Mrs Brižita Đorđeević[1]

[1]Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, [2]Faculty of Agriculture, University of Belgrade, Belgrade, Serbia, [3]Vinča Institute of Nuclear sciences - National Institute of the R, Belgrade, Serbia, [4]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

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

Background: Plum is fruit which is rich source of many biological active compounds with beneficial health effects. As a processed plum is possible to consume in different forms as a jam, compote and juice. During the processing thermolabile compounds (such as phenolic compounds) destroyed and its content decrease. It problem could be solved by the processing of plum into the wine which does not involve application of high temperature. Produced wine are rich source of phenolic compounds.

Purpose: The aim of this study was to determine phenolic profile, and activity of fruit wine on enzymatic systems in vitro and lipid peroxidation.

Method: Production of fruit wine was conducted by different controlled conditions of microvinifications. Wines were produced with addition of sugar and enzymatic preparation and without addition. Two different yeasts were used in separate fermentations. Phenolic profile was evaluated by UPLC TQ-MS/MS. Level of lipid peroxidation (malondialdehyde (MDA) level) and activity of enzymes of antioxidant protection (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were evaluated on synaptosomes. Synaptosomes were isolated from the brain of Wistar albino rats.

Results: Plum wine analysis conducted by UPLC TQ-MS/MS showed presence of phenolic acids and flavonoids. The most dominant compound was chlorogenic acid with the content 195.23 to 221.57 μg/ml. Among other phenolic acids were detected p-coumaric (1,75-3,51 μg/ml), protocatehuic (11.51-21.43 μg/ml), gallic (14.87-23.77 μg/ml) and caffeic (4.51-10.71 μg/ml). Flavonoids detected in plum wines were catehin (8.31-15.21 μg/ml), epicatehin (32.21-51.73 μg/ml) and quercetin (18.57-32.87 μg/ml). Antioxidant activity of above mentioned phenolic compounds affected on level of lipid peroxidation and activity of enzymes of antioxidant protection on isolated synaptosomes in which were experimentally induced oxidative stress by hydrogen peroxide. The MDA level was in range (1.25-2.41 nmol/mg), while SOD activity was in range (4.52-6.77 U/mg). Activity of GPx was in range (0.0197-0.0215 U/mg) as well as CAT (0.042-0.065 U/mg). Higher content of quantified phenolic compounds, as well as decrease of MDA content and increase of SOD, GPx and CAT activity were observed in wines produced with addition of sugar and enzymatic preparation.

Conclusion: Plum wine is rich source of phenolic acids and flavonoids which exhibit beneficial health effect on the human organism. Beside quantified compounds in this study other biologically active principles are responsible for decrease of MDA and activation of SOD, GPx and CAT. The ability of plum wine to decrease MDA level and activate enzymes of antioxidant protection could be used against free radicals and prevention of oxidative stress.

Tetracycline resistance modulating effect of bixin on Staphylococcus aureus expressing Tet(K)

Ms Hyun-A Cho[1]
[1]University College London (ucl), London, United Kingdom
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Multidrug resistance (MDR) of bacterial pathogens is a critical public health concern and calls for collaborative global action. In light of this challenge, academia is actively exploring strategies to overcome MDR, including the development of drug efflux pumps inhibitor. Bixin is a carotenoid pigment extracted from the seed of Bixa Ornella and is known to possess biological properties, including antimicrobial activities. Studies reported the effect of bixin in modulating resistance against the Tet(K) efflux protein-expressing strain of Staphylococcus aureus.

Objectives: Thereby, the study aimed to corroborate the resistance modulatory effect of bixin on Tet(K) expressing S. aureus and examine the effect of bixin on the Tet(K) efflux protein. Ultimately the study aimed to prospect the potency of bixin as the Tet(K) efflux pump inhibitor (EPI) and a potential antibiotic adjuvant.

Methods: The broth dilution method determined the minimum inhibitory concentration (MIC) of tetracycline against four strains, including Tet(K) phenotype S. aureus. To evaluate the synergy between bixin and tetracycline, the broth checkerboard microdilution method was performed, which also determined the MIC of bixin. The fold decrease of tetracycline MIC and fractional inhibitory concentration (FIC) were calculated to identify the most synergistic concentration of tetracycline and bixin. Fluorescence spectroscopy was utilised to examine the effect of bixin and carbonyl cyanide m-chlorophenyl hydrazone (CCCP), respectively, on tetracycline efflux of S. aureus with Tet(K) transporter.

Results: Respective MIC of tetracycline for Tet(K) and NorA S. aureus, MSSA, and E. coli were 32, 0.25, 0.25, and 2 µg/mL. In the presence of bixin, there was a 256-fold decrease in tetracycline MIC to 0. 125 µg/mL for Tet(K) expressing S. aureus. Bixin MIC was identical to tetracycline, i.e. 32 µg/mL. The most synergistic concentration combination of tetracycline and bixin against the Tet (K) strain of S. aureus were 0.125 and 16 µg/mL. Tetracycline efflux decreased by 52.18% in the presence of bixin. However, the efflux assay with CCCP signified the need for further optimisation of efflux assay conditions.

Conclusions: These results suggest the tetracycline resistance modulating effect of bixin on S. aureus with the Tet(K) gene. Albeit the direct impact of bixin on Tet(K) protein was not determined, this study suggests that bixin decreases tetracycline diffusion across the S. aureus membrane based on established carotenoid-membrane interaction. Further optimisation of assay conditions and confirmation of the Tet(K) expression via PCR is recommended before efflux assay and cell membrane fluidity assay to substantiate the bixin-membrane interaction.

The first patients with AIDS in Portugal and the HIV-2 discover: Odette Ferreira's record notebooks of patient data and LAV (HIV) detection tests (1984-1985)

Dr Paula Basso[1], **Mrs. Rosário Lourenço**[1]
[1]Pharmacy Museum /ANF, Lisboa, Portugal, [2]the Faculty of Science and Technology of the Universidade Nova de Lisboa , Lisboa, Portugal
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Odette Ferreira (1925-2018), professor and researcher at the Faculty of Pharmacy, University of Lisbon (FFUL), joined in September 1984 the retrovirus research team led by Luc Montagnier of the Pasteur Institute of Paris (IPP)´s Viral Oncology Unit. She returned to Lisbon with the mission of carrying out a seroepidemiological study on LAV (Lymphadenopathy Associated Virus or HIV) infection in the population residing in Portugal and simultaneously identifying the virus in Portugal. This study integrated patients hospitalized with a mysterious illness at Hospital Egas Moniz and allowed the discovery of HIV-2 at the IPP, in September 1985.

Purpose: To analyze the personal and seroepidemiological data of the first people infected with the AIDS virus or who belonged to risk groups (homosexuals, hemophiliacs, prostitutes, injecting drug users, hemodialysis, and poly-transfusion patients) in Portugal, between October 1984 and December 1985. The conclusions of this seroepidemiological study on LAV (HIV) infection in the population residing in Portugal were presented at the II European Congress of Clinical Microbiology in Brighton on 5th September 1985 by Odette Ferreira, Luc Montagnier, Sophie Chamaret and Denise Guetard, without knowing that three of the patients were in fact, infected with an unknow virus at that time.

Method: Analyze the data collected by Odette Ferreira and compare these data with the official data provided by annual reports published by the National Institute of Health (INSA) between 1984 and 1985.

Results: Comparing the official data from 1984 and those collected by Odette Ferreira and having started the HIV detection tests only in October 1984, the researcher immediately detected 7 cases, compared to the 2 annual official cases. Regarding deaths, official data and those of Odette Ferreira coincide in only 1 death.

In 1985, the data are even more divergent, with the FFUL team detecting 151 cases of AIDS in Portugal and officially there were only 18. As for deaths, official data refer to 6 cases and the FFUL team, 20 deaths.

The data results from the first patients and tests for the detection of (HIV) by Odette Ferreira were from Out-Dec.1984: HIV1 (7); Homosexuals (5); Heterosexuals (3); Hemophiliac (2); Polytransfused (1); Male (10); Caucasian race (10).

In 1985, were HIV1(143); HIV2 (8) registered a posteriori in April 1986; homosexuals (52); heterosexuals (99); hemophiliac (18); polytransfused (4); blood donor (3); drug addict (12); hemodialysis (29); Male (121); Women (30) Caucasian race (141); African race (10).

Conclusion: Odette Ferreira registered data referring to 685 HIV diagnostic tests, among which she detected 158 positive cases, 527 negative cases and 21 deaths. Comparing these data, the dimension of the reality of the AIDS disease in Portugal is visible, which the official report ignored and did not mention.

There was an epidemiological situation that was not officially transmitted, either in terms of positive cases or deaths.

This seroepidemiological study allowed the isolation and discovery of a second AIDS virus, thanks to the collaboration between the FFUL teams and the IPP.

Induction of extravillous trophoblast cells (HTR-8/SVneo) invasion by co-culture with human umbilical vein endothelial cells (HUEhT-1)

Dr Tomohiro Nishimura[1], Ms Yukika Tajima[1], Ms Yukako Harada[1], Dr Saki Noguchi[1], Dr Masatoshi Tomi[1]
[1]Keio University, Tokyo, Japan
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Purpose: Preeclampsia (PE) is one of the most important complications worldwide during pregnancy. The etiology of PE has been elucidated for a long time and the remodeling process of spiral artery in the early pregnancy is thought to be the primary cause. Fetal extravillous trophoblasts invade the uterine endometrium and replace uterine vascular endothelial cells to reconstruct the uterine spiral artery in the first trimester of pregnancy, ensuring blood supply from the mother to the placenta. Placental ischemia caused by impaired remodeling has been observed. However, the mechanism of endothelial cell-directed invasion of extravillous trophoblasts is unknown. In this study, the authors aimed to evaluate the effects of co-culturing the human extravillous trophoblast cell line HTR-8/SVneo cells with the human umbilical vein endothelial cell line HUEhT-1 cells on their invasive ability, chemotaxis performance, and proliferative capacity. In the invasion experiments, the authors examined the effects on the invasive ability of HTR-8/SVneo cells using cobblestone-like and capillary-like conditions that reflect the pre- and post-tubule formation of HUEhT-1 cells, respectively.

Methods: Extracellular matrix-coated Transwells seeded with HTR-8/SVneo cells were placed on wells of

cobblestone-like HUEhT-1 cells cultured on collagen I or capillary-like HUEhT-1 cells cultured on extracellular matrix gel. The invasive ability was evaluated by the number of cells that passed through the Transwell after 20 hours after the co-culture. Chemotaxis performance was also evaluated by the same analysis using uncoated Transwells. Cell proliferation capacity was evaluated using Cell Counting Kit-8.

Results and Discussion: When co-cultured with cobblestone HUEhT-1 cells, the invasive ability of HTR-8/SVneo cells increased in a density-dependent manner with HUEhT-1 cells, while chemotaxis performance and proliferation ability remained unchanged. These results suggest that the invasive ability of HTR-8/SVneo cells is increased through the enhancement of extracellular matrix degradation by the involvement of HUEhT-1 cells. In addition, the invasive ability of HTR-8/SVneo cells was decreased in a density-dependent manner with HUEhT-1 cells when co-cultured with capillary -like HUEhT-1 cells.

Conclusions: Co-culture with cobblestone-like HUEhT-1 cells promoted the invasion of HTR-8/SVneo cells. Thus, these results indicate that the promotion of extracellular matrix degradation by the involvement of cobblestone-like endothelial cells may induce the invasion of trophoblasts into the uterine endometrium.

Changes of steroid hormones and the effects of therapeutic drugs on it in postmenopausal patients with rheumatoid arthritis: A case-control study

Ms Qing Shu[1], Ms Yingying Zhang[1], Prof Weihong Ge[1]
[1]Nanjing Drum Tower Hospital, Nanjing, China
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Objectives: Steroid hormones play an important role in the pathogenesis of rheumatoid arthritis (RA). However, there is rare research on the levels of steroid hormones and their metabolites in Chinese RA patients with or without methotrexate (MTX) or glucocorticoid (GC) treatment.

Methods: The enrolled patients were divided into RA-Untreated group (n=23) and RA-Treated group (n=65)which was further divided into RA-MTX (n=35) group and RA-MTX+GC (n=30) group. Age and sex matched healthy people were served as controls (n=50). The concentrations of 36 steroid hormones from RA and controls were measured by liquid chromatography/tandem mass spectrometry.

Results: The cortisol (F), progesterone (P), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone (T), dihydrotestosterone (DHT), unconjugated estradiol (Free E2), conjugated + unconjugated estradiol (Total E2), conjugated + unconjugated 16-hydroxyestrone (Total 16-OH-E1), conjugated + unconjugated 16-epiestriol (Total 16-Epi E3), conjugated + unconjugated 17-epiestriol (Total 17-Epi E3) level in control group were significantly higher than RA and

RA-Untreated group (FDR<0.05), while conjugated + unconjugated 2-hydroxyestrone (Total 2-OH-E1) was the opposite (FDR<0.05). In addition, there were significant difference in the concentrations of aldosterone (ALD), unconjugated estrone (Free E1), conjugated + unconjugated estrone (Total E1), Total E2, unconjugated 2-methoxyestrone (Free 2MeO-E1), conjugated + unconjugated 4-hydroxyestrone (Total 4-OH-E1), and Total 16-Epi E3 between the RA-Untreated and the RA-Treated groups (FDR<0.05). Compared with RA-Untreated , RA-MTX had significantly higher levels of ALD, T, DHT, Total E2, Total 16-Epi E3, but significantly lower Total E1 level (FDR<0.05). Compared with RA-MTX , RA-MTX+GC had significantly lower levels of F, DHEA, DHEAS, androstenedione (AD), T, DHT, Free E1, Total E1, Free E2, conjugated + unconjugated 2-methoxyestrone (Total 2MeO-E1), Free 2MeO-E1, conjugated + unconjugated estriol (Total E3) and significantly higher Total 4-OH E1 (FDR<0.05).

Conclusions: Impaired steroid synthesis was found in RA patients, and MTX could help RA patients restored some abnormally reduced steroid hormones to a level similar to that of the healthy controls. Although corticosteroids treatment has improved patients' symptoms or laboratory indicators clinically, it has exacerbated the degree of abnormal hormone levels.

Glutaredoxin 1 regulates cholesterol metabolism and gallstone formation by influencing protein S-glutathionylation

Ms Yan Xia[1], Professor Professor Li[1], Prof. Jinhan He[1]
[1]Sichuan University, Chengdu, China
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Cholelithiasis is a worldwide common disease, with a prevalence ranging from 10% to 15% in adults, and increasing with age. More than 90% of gallstones are cholesterol gallstone disease(CGD).
Although the cholesterol supersaturation in gallbladder bile is the prerequisite for its pathogenesis, its mechanism is still not clear. It has now been firmly established that a large part of biliary cholesterol secretion is driven by the action of the transporter ABCG5/8, and enhanced by secretion of biliary phospholipid and bile salts. Along with this, the sterol transporters ABCG5/ABCG8 or bile acids synthesis genes are closely related to the incidence of cholesterol gallstones. However, ABCG5/ABCG8 also affect the risk of sitosterolemia in the opposite direction. Thus, ABCG5/ABCG8 are not direct targets for treating CGD.
Post-translational modifications (PTMs) such as phosphorylation, glycosylation, ubiquitination and acetylation play significant roles in the regulation of cholesterol homeostasis. Similar to other PTMs, studies have suggest Glutaredoxin-1 (Glrx1) and Glrx1-related protein S-glutathionylation are increasingly being observed to drive various physiological and pathological processes, especially in metabolic diseases such as diabetes, obesity and fatty liver.

However, the exact mechanism of Glrx1 on cholesterol metabolism remains unclear, and its role in gallstone formation has not been reported.

Purpose: As CGD is closely related to cholesterol metabolic disorder. The present study aims to investigate whether Glrx1 and Glrx1-related protein S-glutathionylation play a role in the regulation of cholesterol metabolism and gallstone formation in a diet induced gallstone model.

Methods: The authors first investigated whether Glrx1 plays a role in gallstone formation in lithogenic diet-fed mice using immunoblotting and quantitative real-time PCR. Then a whole-body Glrx1-deficient (Glrx1-/-) mice and hepatic-specific Glrx1-overexpressing (AAV8-TBG-Glrx1) mice were generated, in which the authors analyzed to analyze the effects of Glrx1 on lipid metabolism upon LGD feeding. Quantitative proteomic analysis and immunoprecipitation (IP) of glutathionylated proteins were performed.

Results: The authors found that protein S-glutathionylation was markedly decreased and the deglutathionylating enzyme Glrx1 was greatly increased in the liver of lithogenic diet-fed mice. Glrx1-/- mice were protected from gallstone disease induced by a lithogenic diet because their biliary cholesterol and cholesterol saturation index (CSI) reduced significantly. Conversely, AAV8-TBG-Glrx1 mice showed greater gallstone progression with increased cholesterol secretion and CSI. Further studies showed that Glrx1-overexpressing greatly induced bile acid levels and/or composition to increase intestinal cholesterol absorption by upregulating Cyp8b1. In addition, liquid chromatography-mass spectrometry and IP analysis revealed that Glrx1 also affected the function of asialoglycoprotein receptor 1 (ASGR1) by mediating its deglutathionylation, which further inhibited its degradation, thereby altering the expression of LXRα and controlling cholesterol secretion.

Conclusion: These findings present novel roles of Glrx1 and Glrx1-regulated protein S-glutathionylation in cholesterol metabolism and gallstone formation. These data advises Glrx1 significantly increased the risk of gallstone formation by simultaneously increasing bile-acid-dependent cholesterol absorption and ASGR1- LXRα-dependent cholesterol efflux. This study suggests the potential effects of inhibiting Glrx1 activity to treat cholelithiasis.

Glutaredoxin-1 alleviates acetaminophen-induced liver injury by decreasing its toxic metabolites

Dr Ying Xu[1], Prof. Yanping Li[1], Prof. Jinhan He[1]
[1]Department of Pharmacy, Institute of Metabolic Diseases and Pharmacotherapy, West China Hospital, Sichuan University, Chengdu, China
Posters Tuesday, September 26, 2023, 12:30 PM - 2:30 PM

Background: Acetaminophen (paracetamol, APAP) is a widely used antipyretic and analgesic in clinical practice. However, an excessive intake of APAP can result in severe liver damage and even acute liver failure (ALF). The principal

cause of APAP-induced hepatotoxicity is the excessive production of N-acetyl-p-benzoquinone imine (NAPQI), which is a toxic metabolite of APAP. S-glutathionylation is a reversible redox post-translational modification, and protein S-glutathionylation levels are changed during APAP-induced liver injury. This is a potential mechanism of APAP-induced hepatotoxicity. Glutaredoxin-1 (Glrx1) is a glutathione-specific thioltransferase and highly expressed in liver. Glrx1 is a primary enzyme to catalyse deglutathionylation and plays a key role in redox signaling and homeostasis.

Purpose: The aim of this study was to determine whether and how Glrx1 plays a role in APAP-induced liver injury.

Methods: The Glrx1 knockout mice (Glrx1-/-) and liver-specific Glrx1 overexpression mice (AAV8-Glrx1) were generated and subjected to APAP-induced liver injury. The levels of three metabolites APAP-GSH, APAP- CYS and APAP-NAC were assessed by HPLC-MS/MS technology to indicate the formation of the toxic metabolite NAPQI. Pirfenidone (PFD), an anti-fibrosis drug used in clinic, is a potential inducer of Glrx1. PFD was administrated preceding APAP to assess its protective effects.

Results: The hepatic expression of total protein S-glutathionylation (PSSG) increased and Glrx1 decreased in APAP-induced liver injury in mice. Glrx1-/- mice were more sensitive to APAP-induced hepatotoxicity, with higher serum ALT and AST levels, and more necrosis areas in liver. Glrx1-/- mice also had higher oxidative stress, more toxic metabolites of APAP, and more pronounced activation of p-JNK and p-ERK. Furthermore, Glrx1 deficiency led to an increase in the total hepatic PSSG levels and the S-glutathionylation levels of Cyp3a11, which could be a contributing factor to the enhanced activity of Cyp3a11. Conversely, AAV8-Glrx1 mice were protected from APAP-induced hepatotoxicity by reducing oxidative stress, decreasing toxic metabolites of APAP and inhibiting the activation of p-JNK and p-ERK. Preceding PFD administration upregulated Glrx1 expression and alleviated APAP-induced liver injury by decreasing oxidative stress and inhibiting the activation of p-JNK and p-ERK.

Conclusion: The authors have successfully identified the role of Glrx1-mediated protein S-glutathionylation in the context of APAP-induced liver injury. Glrx1 deficiency led to an increased S-glutathionylation of Cyp3a11, which may result in the elevation of Cyp3a11 activity and subsequent increase in the formation of N-acetyl-p-benzoquinone imine (NAPQI). Upregulating of Glrx1 expression may be a promising avenue for clinical management of APAP-induced liver injury.

A novel method for rapid bacterial sensitivity test in just two hours

Assistant professor Mohamed Abd El Gawad[1], **Mr Ahmed Abu Tahoun**[1], Ms Alaa Emad ElDin[2]
[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Misr University for Science and Technology (MUST), 6 October city, Egypt, [2]Faculty of science, Zagazig University, Zagazig, Egypt
RFTU-02 - Rapid Fire Session Tuesday, M1-M2, September 26, 2023, 11:00 AM - 12:30 PM

Background: Antibiotic resistance is a major global public health concern. Traditional bacterial sensitivity testing methods can take up to 48 hours, delaying the administration of effective treatment. Therefore, there is a need for a rapid and reliable bacterial sensitivity testing method.

Purpose: In this study, the authors aimed to develop a novel method for rapid bacterial sensitivity testing in just 2 hours.

Method: This novel bacterial sensitivity testing method was tested on three models of bacteria: E. coli, Staphylococcus aureus, and Klebsiella pneumoniae, as models for gram-negative and gram-positive bacteria. Antibiotics in their raw form (amoxicillin, Imipenem, ceftriaxone, Vancomycin, levofloxacin, and tetracycline). The bacteria were prepared in nutrient broth supplemented, followed by exposure to raw antibiotics for 40 minutes at 37°C. Next, the bacteria were centrifugated and incubated in 1 ml of 10% salt (NaCl and KCl) for 60 minutes with measured added free amino acids (proline and glycine betaine), then treated with ninhydrin reagent. The method involved the detection of quantitative free amino acids produced and uptaken by bacteria after treatment with salt. sensitive bacteria were unable to produce or uptake the measured free amino acids, leading to an abundance of free amino acids in the outer media of the bacteria (the solution), which gave a colorimetric result upon treatment with ninhydrin. On the other hand, Bacteria that were resistant to the antibiotic produced and uptook all measured free amino acids from the solution, leading to a negative result with ninhydrin treatment. The extent of the color change was then quantified using spectrophotometry. A control sample, which contained bacteria without any exposure to antibiotics, was used to establish a baseline for the color change. The absorbance of each sample was measured at a wavelength of 570 nm, and the difference between the absorbance of the control and test samples was calculated to determine the level of antibiotic sensitivity. The data collected from the experiments were analyzed using statistical software, and the results were presented as mean ± standard deviation.

Results: The results of these experiments showed that this method could accurately determine the antibiotic sensitivity of bacteria within just 2 hours. The developed method achieved an accuracy of 90% in determining bacterial sensitivity to antibiotics in 100 samples. Additionally, the authors found that the color change could also differentiate between Gram-positive and Gram-negative bacteria. Gram-

positive bacteria produced a yellow color due to the high concentration of produced proline as osmoprotectant which reacts with ninhydrin reagent giving a yellow product before dark purple appears, while Gram-negative bacteria produced a dark purple color due to the high concentration of produced glycine betaine.

Conclusion: The developed method provides a reliable and rapid way to determine bacterial sensitivity to antibiotics, with results available within 2 hours. This method has the potential to be an alternative to traditional bacterial sensitivity testing methods as a microfluidic chip that can be a point-of-care test, and it can also be used as an alternative to gram staining to differentiate between gram-positive and gram-negative bacteria.

Assessing the role of cardiomyocyte senescence and secreted factors in anthracycline-induced cardiotoxicity

<u>Ms Laura Katherine Booth</u>[1], Ms Ray Alsuhaibani[1], Dr Rachael Redgrave[2], Dr Simon Tual-Chalot[2], Dr Maria Camacho Encina[2], Ms Yasemin Ekinci[1], Dr Omowumi Folaranmi[2], Professor Ioakim Spyridopoulos[1], Dr Jason Gill[1], Dr Gavin Richardson[2]
[1]Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom, [2]Biosciences Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom
RFTU-02 - Rapid Fire Session Tuesday, M1-M2, September 26, 2023, 11:00 AM - 12:30 PM

Background: The prescription of anthracycline chemotherapies (such as doxorubicin) is a mainstay intervention for oncologists treating many malignancies, but these drugs have well-established adverse drug reactions, including delayed-onset cardiotoxicity, which can culminate in heart failure (anthracycline-induced cardiotoxicity, AIC). Anthracyclines can induce cardiomyocytes to senescence principally through DNA damage, and while it is known that this process contributes to disease, the exact mechanisms are not well understood. One possibility is that senescent cardiomyocytes and their senescence-associated secretory phenotype (SASP) drive the subclinical, structural changes that precede measurable, functional changes in the heart: late-stage AIC is often characterised by a phenotype associated with senescence & cardiac ageing, e.g. fibrosis and maladaptive remodelling.

Purpose: The authors hypothesised that senescent cardiomyocytes contribute to AIC via a pro-remodelling secretory phenotype. Accordingly, the authors aimed to establish in vitro models of doxorubicin-induced cardiomyocyte senescence, and assess the pro-remodelling cellular phenotype of these cardiomyocytes (e.g. expression of pro-remodelling cytokines).

Methods: Human AC16 cardiomyocytes were exposed to 500 nM doxorubicin, for 3 hours (representing a transient, sublethal and clinically relevant dose) or exposed to vehicle control. Cardiomyocytes were allowed to recover for 10 days

before analyses commenced. Markers of senescence were evaluated at the transcript level (via quantitative polymerase chain reaction, qPCR), and at the protein level (via immunocytochemistry, ICC), and heart failure-associated transcripts were also assessed. Parallel studies were conducted in human stem cell-derived cardiomyocytes (iPSC-CMs) to validate this model. Conditioned media was collected from senescent/non-senescent AC16 cardiomyocytes for 48 hours and applied to human cardiac fibroblasts (HCFs) for 10 days, whereupon phenotypic changes in HCFs were evaluated using qPCR and ICC. Secreted factors in the conditioned medium were explored using a cytokine array.

Results: Senescence and heart failure transcripts were significantly upregulated in these two independent cellular models (AC16 cardiomyocytes and iPSC-CMs) at 10 days post-doxorubicin *vs* control (p21 3.2-fold, p16 1.3-fold, PURPL 8.9-fold, GDF15 18.9-fold). This data was validated at a protein level. Interestingly, senescent AC16 cardiomyocytes secreted a functional SASP capable of inducing myofibroblast activation, identified by increased expression of IL-8, periostin and αSMA-positive stress fibres in otherwise unstimulated primary HCFs. Cytokine array analysis of conditioned media collected from doxorubicin-induced senescent cardiomyocytes identified a cardiomyocyte SASP comprising several proteins involved in immune cell recruitment (MCP-1: 470 *vs* 2 pg/mL), inflammation (IL-6: 22 *vs* 0 pg/mL) and myocardial remodelling (FGF-2: 41 *vs* 7 pg/mL).

Conclusion: The authors have established in vitro models for AIC, showing that sublethal doxorubicin exposure induces senescence in human cardiomyocytes. This is accompanied by expression of markers related to heart failure. Senescent cardiomyocytes release a pro-remodelling SASP which promotes phenotypic changes in HCFs. Overall, these findings indicate that senescent cardiomyocytes can induce remodelling in the myocardium, which is a driver of early-stage AIC. The functionality of the cardiomyocyte SASP, and especially its role in cell-cell crosstalk, remains underappreciated in AIC and will form the basis of further work. Our data suggests that pharmacologically targeting cardiomyocyte senescence may be a therapeutic strategy to prevent or slow AIC: a hypothesis the authors will pursue in future studies.

Foeniculum vulgare Mill. alleviates microglia-mediated neuroinflammatory responses and neurotoxin-induced behavioral responses in cellular and experimental models of Parkinson's disease

Prof Sushruta Koppula[1], Prof Ramesh Alluri[2], Professor and Principal Sumalatha Gindi[3], Prof Spandana Rajendra Kopalli[4]
[1]Department of Biomedical and Health Sciences, Konkuk University, Chungju, South Korea, [2]Department of Pharmacology, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, India, [3]Department of Pharmacology, VIKAS Institute of Pharmaceutical Sciences, Rajahmundry, India, [4]Department of Integrative Bioscience and Biotechnology, Sejong University, Seoul, South Korea
RFTU-02 - Rapid Fire Session Tuesday, M1-M2, September 26, 2023, 11:00 AM - 12:30 PM

Background: Foeniculum vulgare Mill., from the family Apiaceae is an aromatic tropical herb cultivated in several countries including Asia and Mediterranean regions with various pharmacological properties.

Purpose: The aqueous fruit extract of F. vulgare (FVE) was evaluated for its potential anti-neuroinflammatory properties in cellular and experimental models of Parkinson's disease (PD).

Methods: Lipopolysaccharide (LPS)-stimulated BV-2 microglia-mediated neuroinflammation in vitro and 1-methyl-4 phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced PD animal model in vivo was investigated.

Results: FVE (25, 50, and 100 μg/mL) attenuated the LPS-stimulated increase in NO production and suppressed the altered levels of iNOS and COX-2 expression. Further, the enhanced proinflammatory mediators including interleukin-6 and tumor necrosis factor-alpha were also significantly inhibited by FVE in LPS-stimulated BV-2 cells. Furthermore, the disrupted antioxidative enzyme status such as superoxide dismutase, catalase, and glutathione in LPS-stimulated BV-2 cells was significantly ameliorated by FVE. Mechanistic studies revealed that FVE exhibited its anti-neuroinflammatory effects by mediating the NF-κB/MAPK signaling. In vivo studies, FVE (100, 200, and 300 mg/kg) ameliorated the MPTP (25 mg/kg, i.p.)-induced changes in locomotor, cognitive, and behavior functions evaluated by rotarod, passive avoidance, and open field test significantly ($p < 0.05 \sim p < 0.001$). High-performance thin-layer chromatography fingerprinting analysis of FVE exhibited several distinctive peaks with rutin, kaempferol -3-O-glucoside, and anethole as identifiable compounds.

Conclusion: Our study indicated that FVE restored the altered oxidative enzyme status and attenuated the neuroinflammatory processes in LPS-stimulated BV-2 microglia via regulating the NF-κB/MAPK signaling. Further, FVE restored the MPTP-induced behavioral deficits in vivo indicating the potential role of FVE in ameliorating microglia-mediated oxidative stress and neuroinflammation seen in various neurodegenerative disorders including PD.