Pharmacy Education (2024) 24(7) 73 - 74
https://doi.org/10.46542/pe.2024.247.7374

CONFERENCE ABSTRACTS

FIP CAPE TOWN 2024

82nd FIP World Congress of Pharmacy and Pharmaceutical Sciences in Cape Town, South Africa, 1 to 4 September 2024

Clinical biology

Determination of cannabinoids in plasma

Neve Borg, Janis Vella Szijj, Anthony Serracino-Inglott

Department of Pharmacy, Faculty of Medicine and Surgery, University of Malta

Introduction: The use of cannabis in the medical industry is increasing. Tetrahydrocannabinol (THC)'s psychoactive properties and Cannabidiol (CBD)'s analgesic, neuroprotective, anticonvulsant, anti-inflammatory and anti-emetic properties contribute to the different conditions for which medical cannabis can be used.

Objectives: To develop and validate a quick and efficient method to quantify THC and cannabidiol CBD in plasma using High-Performance Liquid Chromatography (HPLC) coupled with Ultraviolet (UV) detection.

Methods: An analytical method to determine the concentration of THC in plasma was developed, and analytical parameters, including stationary phase, mobile phase, detector, sample preparation technique, and biological matrix, were identified.

The method was validated for accuracy, intra-day and inter-day precision, linearity, selectivity and stability, and compliance with the International Council of Harmonisation 1 (ICH) guidelines.

Results: Protein precipitation was performed on plasma samples containing different concentrations of THC using ice-cold acetonitrile in a 1:1.5 plasma-to-acetonitrile ratio, followed by vortex mixing, centrifugation and filtration using syringe filters. The sample was then analysed using the ACE C18 chromatographic column as the stationary phase (250 x 4.6mm; 5µm i.d) and a 30:70 water with 0.01% acetic acid: acetonitrile with 0.01% acetic acid as the mobile phase. THC was detected using a UV spectrophotometer at a wavelength of 225nm and eluted at a retention time of 15.14 minutes.

During method validation, the method was found to have valid inter-day precision, selectivity and linearity.

Conclusions: The developed method demonstrated robustness whilst utilising equipment that is accessible in analytical settings. Further research that is now proposed is to apply the developed and validated method to determine CBD in the plasma of patients. Determination of cannabinoids in patients' biological fluids can help provide more pharmacokinetic information, leading to better dosing of cannabinoids and increased patient safety.

Impact of fetal ezrin deficiency on decidual immune cells in mice model

Tomohiro Nishimura, Mayuko Yamanaka, Ryo Mizokami, Saki Noguchi, Masatoshi Tomi

Keio University Tokyo, Japan

Introduction: Fetal mice lacking the ezrin gene display fetal growth restriction (FGR) after gestational day (GD) 15.5. Ezrin serves as a crucial cross-linker protein between a membrane protein and cytosolic actin, exhibiting abundant expression in the placenta among the ERM protein family. Intriguingly, the fetal ezrin gene knockout also unveils an absence of ezrin in the maternal decidua, a significant tissue for immunotolerance toward the semi-allogenic fetus. It has been reported that fetus-derived invasive trophoblasts interact with maternal immune cells in the decidua. The objective of this study is to elucidate the impact of fetus-derived ezrin on decidual immune cells, influencing both fetal growth and immunotolerance.

Methods: Ezrin+/- heterozygous knockout mice were interbred to produce wild-type and ezrin knockout offspring, enabling analysis of gene expressions and immune cell distribution in the placenta. The pregnant mice were subcutaneously administered tacrolimus at 0.1 mg/kg (treatment group) or vehicle alone (non-treatment group) every other day from GD 8.5. mRNA and protein levels of IL-6 were analysed by RT-qPCR and ELISA, respectively. Classical M1 and M2 macrophages were analysed in the decidua by flow cytometry using CD86 and CD206 as markers. Regulatory T (Treg) cells were also examined using CD4 and Foxp3 as markers.

Results: IL-6 expression in the placenta of mice was significantly higher than that of mice at gestational day 15.5. mRNA expression of IL-6 in the decidua was higher in ezrin knockout mice than in mice, but those in the junctional zone and labyrinth did not significantly change. These results suggest that the decidua associated with the fetus induces an immunological response such as inflammatory status. M1 macrophages, which produce pro-inflammatory cytokines, were increased in the decidua of fetal mice compared to that of mice, while M2 macrophages, which produce anti-inflammatory factors, were not increased. Treg cells, which suppress immune responses, were significantly reduced in the decidua of fetal mice compared to that of mice. Further analysis involved an immunosuppressant, tacrolimus, in pregnant Ez+/- mice, indicating prevention of the decrease in fetal body weight and decidual Treg cells in mice at GD 15.5.

Conclusions: These results suggest that impaired expression of ezrin in the fetus induces inflammatory conditions in the decidua through M1 polarisation of macrophages, the increase of IL-6, and the decreased level of Treg cells. It is possible that fetus-derived ezrin is partly involved in the maintenance of immunotolerance in the maternal decidua.

Untargeted serum metabolomics and lipidomic revealed potential biomarkers for diagnosing Parkinson's disease and monitoring its progression

Lina A. Dahabiyeh[1], Refat M. Nimer[2], Jeremiah D. Wells[3], Oliver Fiehn[3]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, The University of Jordan, Amman, Jordan

[2]Department of Medical Laboratory Sciences, Jordan University of Science and Technology, Irbid, Jordan

[3]West Coast Metabolomics Center, University of California, Davis, Sacramento, California, United States

Introduction: Parkinson's disease (PD) stands as the second most prevalent neurodegenerative disorder, and it is the most common age-related ailment. Despite its prevalence, the underlying causes of PD remain largely elusive, with around 95% of cases being idiopathic. Furthermore, accurate diagnosis is challenging, and current medications only improve motor function without slowing down or reversing the disease progression. Therefore, there is an urgent need to provide a better understanding of the underlying pathophysiology of PD, identify new potential diagnostic and prognostic biomarkers and discover promising therapeutic targets for drug development.

Methods: Serum samples from 50 patients with different stages of idiopathic PD (early, mid and advanced) and 45 age-matched controls were subjected to biphasic liquid extraction. Moderate-to-highly polar metabolites were profiled using hydrophilic interaction liquid chromatography-mass spectrometry (HILIC-MS/MS) and gas chromatography-mass spectrometry (GC-TOF MS) based metabolomics approaches. Non-polar metabolites and lipids were profiled using reversed-phase LC-MS lipidomics. Annotated metabolites and lipids were analysed using MetaboAnalyst and Simca P+14. Metabolites and lipids with variable importance in projection (VIP) score > 1 and P-value <0.05 were considered significant.

Results: A total of 169 lipids and 212 metabolites were significantly altered (in both univariate analysis and multivariate partial least square-discriminant analysis (PLS-DA)) in patients with PD compared to controls. Among the upregulated metabolites and lipids in PD are cysteine-S-sulfate, N-acetyl tryptophan and saturated lysophosphatidylcholines (LPC 17:0, 16:0, 15:0). Lower levels of N-acetylaspartic acid, phosphatidyserines (e.g. PS 40:4, PS 16:0_22:4), sphingomyelins (SM 42:1) and ceramides (e.g. Cer 40:0, 42:0) were detected in PD patients compared to controls. Set enrichment analysis revealed a decrease in xanthines, including caffeine and its downstream metabolites, in patients with PD relative to controls and in advanced PD versus early-stage PD. This suggests that caffeine and its metabolites might have a potential role in protecting against neuronal damage and decelerating PD progression among early-stage PD patients. Furthermore, PD progressed from early to advanced stages with decreasing levels of lysophosphatidylinositols LPI 20:4. Conversely, cysteine-S-sulfate and LPC-O 20:0 showed an increase in their levels with disease progression. A panel of seven metabolites resulted in a strong receiver-operator curve (ROC) with high classification accuracy (AUC = 0.977), suggesting it could be useful for diagnosing idiopathic PD.

Conclusion: The study shows an intriguing number of robust changes in specific serum lipids and metabolites that may become useful for diagnosing PD and its progression once panels have been validated in larger clinical trials and prospective studies. Unusual metabolites like cysteine-S-sulfate and LPI 20:4 might point to therapeutic targets that could enhance the development of novel PD treatments, such as N-methyl-D-aspartate (NMDA) antagonists and GPR55 agonism, respectively.