International conference

Cannabis and science XI

Book of Abstracts

April 22, 2026
Brno Observatory and Planetarium

Main partner of the conference:

Zenplanto

We would like to thank our partners and supporters:

BMT MMM Group

LSZETIS VISION

AstraSana HOLDING AG

SensiQure

BIOSTER

Konopný táta

Semena konopí

sanlight better light, better plants

TSB

Magazín KONOPÍ

naarden

ecp ESTABLISHED TO CURE PEOPLE

Unique Therapeutics

FABRY Tech

Cannabilab E&H services, a.s.

semo

PRO NURSE Health

KARLSBAD WHISKY

phenomenex

CANNA

Konopeka

CzecHemp

Conference program

Time	Speaker	Title
9:00–9:20	Vlastimil Vajdák, Jiří Dušek, Václav Trojan	Conference start
9:20–10:05	Hanuš Lumír	Užívání THC u pediatrických pacientů se závažným onemocněním
10:05–10:50	Punja Zamir K.	Fungi and mycotoxins in cannabis flowers
10:50–11:25	Maksimova Viktorija	Enhancing therapeutic efficacy of cannabidiol through the development of advanced delivery systems
11:25–12:00	Klejbor Ilona	Golden Age or Autumn of Life? The Role of the Endocannabinoid System in Brain Aging
12:00–14:00	Lunch, informal discussion	
14:00–14:25	Lunerová Kamila	Miniaturizovaný kapalinový chromatograf a jeho aplikace na analýzu kanabinoidů
14:25–14:50	Bisceglia Francesco	German Medical Cannabis Market Development – Lessons learned
14:50–15:20	Babula Petr	Současné trendy ve farmakologii fytokanabinoidů
15:20–15:45	Vacek Jan	Farmakologie endokanabinoidního systému. Kanabidiol a jeho místo ve světě medicíny založené na důkazu
15:45–16:10	Landa Leoš	Využití léčebného konopí při terapii chronické bolesti
16:10–16:35	Řehulka Pavel	Vaporizované konopí v akutní léčbě migrény
16:35–17:00	Dostálová Alžběta Dorota, Hřib Radovan	Od indikace k léčivému přípravku

Speakers

Hanuš Lumír
Punja Zamir K.
Roszkowska Anna
Lunerová Kamila Trojan Václav, Kubů Pavel, Hrabák Aleš
Landa Leoš
Řehulka Pavel
Dostálová Alžběta Dorota, Hřib Radovan

Participants of the poster session

Agnieszka Mosińska
Katarzyna Sztormowska
Katarzyna Owczarek
Katarzyna Smarzewska
Katarzyna Woźniczka
Magdalena Kaszewska
Vaclav Trojan
Tomasz Bączek
Anna Roszkowska
Miloš Beran
Daniel Král
Josef Baltazar Šenkyřík
Vladan Ondřej
Hana Holcová Polanská
Alžběta Pávková
Michal Masařík
Jaleigh Morales
Chloe Earls
Jan Halámek

Abstracts

UŽÍVÁNÍ THC U PEDIATRICKÝCH PACIENTŮ SE ZÁVAŽNÝM ONEMOCNĚNÍM.

Lumír Ondřej Hanuš

School of Pharmacy, Hebrew University, Jerusalem, Israel

ÚVOD

Tetrahydrokanabinol (THC) představuje hlavní psychoaktivní složku konopí. U dětí je jeho použití výjimečné, přísně regulované a zvažuje se pouze tehdy, když selhávají standardní terapeutické možnosti. V pediatrii jsou kanabinoidy obecně používány s velkou opatrností, přičemž preferovanou látkou je CBD, nikoli THC.

MOŽNÉ TERAPEUTICKÉ INDIKACE THC U DĚTÍ

– Těžká nevolnost a zvracení při chemoterapii.
– Těžká, léčbě odolná spasticita.
– Paliativní péče.
– Výjimečné případy refrakterních epilepsií.

ZAKÁZANÉ NEBO NEVHODNÉ POUŽITÍ

THC není schváleno pro ADHD, úzkostné poruchy, autismus, poruchy spánku, depresi ani chronickou bolest (mimo paliativní kontext). Rekreační užívání je zakázané.

RIZIKA

– Narušení vývoje mozku.
– Zvýšené riziko úzkosti a psychózy.
– Riziko závislosti.
– Ovlivnění motoriky a koordinace.
– Kardiovaskulární účinky.
– Riziko náhodné intoxikace.

VÝVOJ MOZKU

Vývoj mozku pokračuje do cca 25 let. THC může negativně ovlivnit synaptické prořezávání, emoční regulaci a kognitivní funkce.

ZÁVĚR

THC má u dětských pacientů jen velmi omezené a přísně indikované použití, zejména v paliativní péči nebo při chemoterapií navozené nevolnosti. Preferovanou látkou zůstává CBD.

FUNGI AND MYCOTOXINS IN CANNABIS FLOWERS

Zamir K. Punja

[1] *Department of Biological Sciences, Simon Fraser University, Canada*

e-mail: punja@sfu.ca

INTRODUCTION

Cannabis (Cannabis sativa L.) grown for medicinal use is frequently contaminated by fungal pathogens that can be present within the harvested product. Regulatory limits are imposed on the colony-forming units (cfu)/g of total yeast and mold (TYM) to ensure consumer safety, and limits are imposed on certain mycotoxins. The objective of this research was to identify the variables influencing levels of fungal and yeast contaminants in cannabis samples and to establish if there is potential for mycotoxin production by some fungi.

MATERIALS AND METHODS

Samples of cannabis inflorescences were obtained fresh and after drying from four genotypes grown under greenhouse conditions over a 3-year period. They were homogenized and plated onto potato dextrose agar containing antibiotics to assess TYM [1]. Fungal species recovered were identified using molecular methods [2]. Variables affecting TYM examined in the study pre-harvest were presence of leaf litter, effect of air circulation and time of sampling, and phenotypic characteristics of the inflorescences. Post-harvest, the method of drying and extent of drying on TYM levels was examined. Naturally-infected and artificially--infected samples were analysed for mycotoxins by HPLC-MS/MS [3].

RESULTS

TYM was significantly influence by the cannabis genotype being grown. Phenotypic features enhancing TYM were large numbers of stigmas and inflorescence leaves surrounding the flowers. Leaf litter increased TYM while air circulation decreased cfu/g in the samples. Samples collected during summer months had higher TYM compared to other months. Drying of samples significantly reduced TYM, with hang-drying resulting in lower cfu/g compared to rack-drying. Over 23 species of TYM were identified in the samples, with Penicillium and Fusarium spp. being most prevalent. Mycotoxins produced by Fusarium, Penicillium and Alternaria spp. were detected in some naturally-contaminated samples. Artificially-inoculated tissues had significant accumulation of mycotoxins of Fusarium spp.

CONCLUSION

Cannabis samples may contain TYM and mycotoxins that exceed regulatory limits. Variables were identified that can reduce these levels during commercial production.

REFERENCES

[1] *Z.K. Punja, L. Ni, S. Lung, and L. Buirs. Frontiers in Microbiology 14, 1192035 (2023).*
[2] *Z.K. Punja. Canadian Journal of Plant Pathology 43, 88–100 (2021).*
[3] *Z.K. Punja, S.A. Tittlemier, and S. Walkowiak. Journal of Fungi 11, 528 (2025).*

Keywords: Fusarium; Mold; Mycotoxin; Post-harvest.

ENHANCING THERAPEUTIC EFFICACY OF CANNABIDIOL THROUGH THE DEVELOPMENT OF ADVANCED DELIVERY SYSTEMS

Viktorija Maksimova1, Mihail Aleksandrov1,2, Elena Drakalska Sersemova2

[1] *Department of Applied Pharmacy, Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia*
[2] *AuraPharm LLC, Kavadarci, North Macedonia*
[3] *Department of Pharmaceutical Technology, Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia*

e-mail: viktorija.maksimova@ugd.edu.mk

INTRODUCTION
Cannabidiol (CBD) has emerged as a promising non-psychoactive pharmacological effect, but it often demonstrates inconsistent efficacy in preclinical and clinical studies, largely due to its poor aqueous solubility, low bioavailability, and physicochemical instability [1].

MATERIALS AND METHODS
By reviewing different formulations as appropriate delivery systems for CBD, such as nanoparticles, liposomes, microcapsules, we have found microencapsulation as simple and economical procedure convenient for our experimental conditions. Microparticles were obtained with protein-based or polysaccharide coating (matrix) loaded with hemp seed oil and CBD using a complex coacervation method and pH-induced protein precipitation.

RESULTS
Our preliminary results indicate that the obtained microcapsules are in the size range ≈ 20–60 μm, characterized by span values ranging from 1.9 to 2.8, indicating moderate to broad distributions depending on the formulation parameters, and initially low zeta potential reflecting their low electrostatic repulsion.

CONCLUSION
Development of microcapsules as delivery systems is a promising strategy, but this procedure still requires a long optimization process to ensure the appropriate physicochemical characteristics of CBD.

REFERENCES
[1] *Aleksandrov M, Maksimova V, Smilkov K, Archives of pharmacy, Volume 76, 1, 1–24 (2026).*

Keywords: cannabidiol, delivery system, formulation, microencapsulation.

MINIATURIZED LIQUID CHROMATOGRAPH AND ITS APPLICATION FOR CANNABINOIDS ANALYSIS

Kamila Lunerová[1], Jozef Šesták[2], Dana Moravcová[2], Jakub Vaněk[1], Ivan Hamerník[3]

[1] *National Institute for NBC Protection, Brno, Czech Republic*
[2] *Institute of Analytical Chemistry of the Czech Academy of Sciences, Brno, Czech Republic*
[3] *INGOS, Ltd. Prague, Czech Republic*

e-mail: lunerova@sujchbo.cz

INTRODUCTION

The high-performance liquid chromatography (HPLC) is an important technique used in medical, pharmaceutical, food, environmental, forensic or industrial laboratories for analysis of drugs, toxins, natural compounds, metabolites and many others. The HPLC instruments are bulky benchtop devices and therefore their usage is limited for the laboratory use, in particular in tandem with mass spectrometry detection (LC-MS) [1, 2].

MATERIALS AND METHODS

We introduce miniaturized portable HPLC instrument (mini-LC) based on simplified capillary LC scheme with pressure resistance up to 100 MPa and enabling gradient elution using commercially available capillary microcolumns (0.3 × 50 mm). The system is equipped with unique compact optical detector capable of simultaneous multi-wavelength UV-Vis absorbance (200–400 nm) and fluorescence monitoring.

RESULTS

We applied this system for analysis of cannabinoids in ethanolic extracts of various Cannabis sp.cultivars. We used Waters nanoEaseTM M/Z HSS T3, 1.8 µm, 0.3 × 50 mm column, water as solvent A and methanol:acetonitrile (1:1) mixture as solvent B. Mobile phase flow rate was 10 µL/min. Mobile phase composition was: 80% (v/v) B, 50 µL → 80–95% (v/v) B in 20 µL → 95% (v/v) B, 10 µL. Absorbance detection was performed at 210 nm.

CONCLUSION

The miniaturized HPLC-UV/Vis/FLD system provides comparable results to the benchtop analytical HPLC system, if comparable separation conditions and detection system are used. Presented results demonstrate the capability of mini-LC to perform fast (up to 5 min) and repeatable separation and detection of cannabinoids in standard mixture and/or in complex matrices.

REFERENCES

[1] *Rahimi F. et al., Chromatographia **83**, 1165–1195 (2020), doi: 10.1007/s10337-020-03944-6.*
[2] *Šesták J., Planeta J., Kahle V., Anal. Chim. Acta 1112, 80–91 (2020), doi: 10.1016/j.aca.2020.03.020.*

Keywords: portable HPLC, cannabinoids

CURRENT TRENDS IN THE PHARMACOLOGY OF PHYTOCANNABINOIDS

Petr Babula[1]

[1] *Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Kamenice 5, Brno, CZ-625 00, Czech Republic*

e-mail: Babula@med.muni.cz

INTRODUCTION

Phytocannabinoid pharmacology is still often interpreted mainly through the classical cannabinoid receptors CB1 and CB2. This view is increasingly inadequate. Several plant-derived cannabinoids, especially cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabivarin (THCV), cannabinol (CBN), cannabichromene (CBC), and acidic cannabinoids such as CBDA, THCA and CBGA, show relevant activity at non-canonical molecular targets. CBD is the best-characterized example of this broader pharmacological profile. Its effects have been linked to modulation of 5-HT1A receptors, antagonism of GPR55, inverse agonism at orphan receptors GPR3, GPR6 and GPR12, and activation of PPARγ-dependent pathways. These mechanisms may contribute to anxiolytic, anti-seizure, neuroprotective, anti-inflammatory and metabolic effects. CBG differs substantially from CBD and appears to interact with α2-adrenoceptors and 5-HT1A receptors, supporting the view that minor phytocannabinoids are not simply weaker analogues of THC or CBD. Acidic cannabinoids further expand this concept by influencing calcium-dependent inflammatory signaling. The main message is that phytocannabinoids should be understood as multi-target ligands, not merely as modulators of the endocannabinoid system.

Keywords: phytocannabinoids; GPR55; 5-HT1A; PPARγ;

CANNABIDIOL AND ITS PLACE IN THE WORLD OF EVIDENCE-BASED MEDICINE

Jan Vacek, Jan Dehner

Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, Olomouc, 775 15, Czech Republic

e-mail: jan.vacek@upol.cz

The study of phytocannabinoids and cannabis products is a unique field in which purely professional argumentation influences, interacts with, and is influenced by many societal factors. These factors include the opinions of recreational users, self-medication patients; political, cultural, historical and executive attitudes, and last but not least, toxicological/addictological impacts in the context of chronic users. In the world of pharmacology, drug development or pharmacy practice, we would be hard-pressed to find similar parallels with other pharmaceutical products. The aim of this contribution is to 1/ define these parallels, 2/ further provide current insight into the pharmacology of cannabinoids (especially cannabidiol) and the evaluation of their safety [1], and 3/ provide an overview of available therapeutic strategies based on pure phytocannabinoids (not medicinal cannabis) [2, 3]. The pharmacology of phytocannabinoids will be discussed primarily in the context of neuromodulatory and anti-inflammatory (pleiotropic) effects. Therapeutic strategies will be mentioned within the framework of dermatological, dental, and general topical applications of phytocannabinoids. The lecture will emphasize the differences between knowledge based on the concept of evidence-based medicine vs. attitudes spread by the media and cultural trends. Such attitudes, according to the author of the contribution, limit the application of phytocannabinoids and cannabis products in practice.

REFERENCES

[1] *H. Polanska et al., Toxicology, 488, 153460 (2023).*
[2] *P. Jirasek et al., J. Periodont. Res., 59, 468–479 (2024).*
[3] *V. Student Jr. et al., Biomed. Pap., 170, 47–52 (2026).*

Keywords: cannabidiol; safety; topical application; pharmacology.

THE USE OF MEDICAL CANNABIS IN THE TREATMENT OF CHRONIC PAIN

Leoš Landa[1, 4], Radovan Hřib[2], Václav Trojan[3, 4], Kamila Ondráčková[2]

[1, 4] *Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2] *Centre for Pain Management, Department of Anesthesiology and Intensive Care, St. Anne's University Hospital, Brno, Czech Republic*
[3] *Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Brno, Czech Republic*
[4] *Cannabis Facility, International Clinical Research Centre, St. Anne's University Hospital, Brno, Czech Republic*

e-mail: landa@med.muni.cz

INTRODUCTION
One of the main areas of medical cannabis use is the treatment of pain, regardless of aetiology. In recent years, there have been significant advances in the production of individually prepared medical preparations, leading to the widespread use of medical cannabis on a routine basis. In light of this progress, we have decided to conduct this pilot research project with the aim of assessing (preliminarily determining) the effect of medical cannabis on patients suffering from chronic pain of various origins, mainly in terms of pain intensity and quality of life in these patients.

MATERIALS AND METHODS
The standardized EQ-5D-3L questionnaire (version for the Czech Republic) was used and administered to patients undergoing cannabis treatment at the Centre for Pain Management (St. Anne's University Hospital, Brno). A total of 31 patient questionnaires were collected. The questions focused mainly on pain intensity and a subjective assessment of overall health. The questionnaires were administered before the start of cannabis therapy and after six weeks of therapy. Patients received cannabis orally in the form of capsules or by inhalation using a vaporizer.

RESULTS
A clear, albeit non-significant, positive trend was observed in the subjective assessment of overall health status, and a statistically very significant improvement was noted in the assessment of pain intensity.

CONCLUSION
Results of this study – although in a pilot form with a limited number of subjects – conclusively demonstrated the positive effects of medical cannabis on pain perception and quality of life in patients suffering from chronic pain.

Keywords: Medical cannabis; Pain treatment; Quality of life

VAPORIZED CANNABIS FOR ACUTE MIGRAINE TREATMENT

Pavel Řehulka[1]

[1] *First Department of Neurology, Faculty of Medicine and St. Anne's University Hospital, Masaryk University, Brno, Czech Republic*

e-mail: pavel.rehulka@fnusa.cz

INTRODUCTION

In the Czech Republic medical cannabis is indicated as an adjunctive or supportive therapy for alleviating symptoms associated with serious diseases. Neurologists prescribe medical cannabis not only for strictly defined indications (e.g., chronic refractory pain, spasticity, Parkinsonian tremor), but also for other neurological conditions when its use is considered justified. Evidence on the efficacy of cannabis in migraine is derived mainly from retrospective analyses. Preclinical studies suggest that cannabinoids may exert their effects in migraine via ankyrin (TRPA1) and melastatin-8 (TRPM8) channels [1]. Only recently, the first evaluation of cannabis in the acute treatment of migraine has been conducted in a well-designed prospective study (randomized, double-blind, placebo-controlled, crossover design) [2]. In this clinical study involving 92 participants, vaporized cannabis demonstrated superior efficacy compared to placebo in the acute relief of migraine symptoms, including when different ratios of Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) were tested. Participants were randomized to treat four migraine attacks. Inhaled products consisted of vaporized dried female cannabis flowers heated to 180 °C: (1) 6% THC, (2) 11% CBD, (3) 6% THC + 11% CBD, (4) placebo. A standardized inhalation protocol was used (four inhalations: 5 seconds inhalation, 10 seconds breath-hold, 45 seconds normal breathing). The greatest therapeutic benefit compared to placebo was observed in the group receiving balanced 6% THC + 11% CBD. Two hours after vaporization, pain relief was achieved in 67% of patients, complete pain freedom in 35%, and freedom from the most bothersome symptoms (photophobia, phonophobia) in 60% of patients. The benefit persisted at 24 and 48 hours. No serious adverse events were reported; a minority of patients experienced mild intoxication, euphoria, or transient cognitive impairment [2]. One limitation of medical cannabis use is restriction on driving motor vehicles for approximately 6–8 hours, or up to 9–10 hours after administration [3].

MATERIALS AND METHODS

At the Headache Center of the First Department of Neurology, Faculty of Medicine, Masaryk University and University Hospital Brno, more than 1300 patients are regularly followed. In a pilot program including ten selected patients with resistant migraine (failure of > 3 preventive treatments), four patients accepted the option of treatment with vaporized cannabis (five patients declined due to preference for standard therapy or the need for daily driving; one patient agreed only to oral administration). Three women (aged 47–57 years) and one man (aged 36 years) inhaled vaporized cannabis at the onset of migraine attacks using a standardized method. Cannabis products were heated to 180 °C (two patients used a desktop vaporizer and two used a portable device). Each patient was prescribed 5 g of medical cannabis (maximum 0.5 g per day) containing 5.5% THC and 9.2% CBD, closely matching the protocol used in the referenced clinical study [2].

RESULTS

Two of the four patients (50%) experienced pain relief within 2 hours after inhalation of vaporized cannabis. Adverse effects (somnolence) were reported in one patient (25%). None of the patients continued treatment for more than several weeks (no further prescriptions were issued). All patients cited the inability to use cannabis acutely at the onset of migraine due to subsequent driving restrictions as the main reason for discontinuation (two patients lived in municipalities with fewer than 3,000 inhabitants, and two in municipalities with fewer than 10,000 inhabitants). Additionally, all patients had access to alternative

acute migraine treatments (triptans, nonsteroidal anti-inflammatory drugs), which they considered equally effective or superior to cannabis. The same reasons led six patients to decline participation in the pilot program.

CONCLUSION

Predictors of both non-initiation and early discontinuation of cannabis treatment for migraine include: (1) Anticipated driving restrictions requiring patients to decide in advance whether cannabis use is feasible (limiting its use in acute situations), (2) Availability of effective alternative treatments with more convenient routes of administration or satisfactory efficacy (e.g., commonly available oral analgesics). The results may be limited by the small sample size and by negative expectations toward cannabis compared to standard treatments (potentially higher nocebo response).

REFERENCES

[1] *De Petrocellis L, Vellani V, Schiano-Moriello A, et al. Plant- -derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. The Journal of Pharmacology and Experimental Therapeutics, 325, 1007–1015 (2008).*

[2] *Schuster NM, Wallace MS, Marcotte TD, et al. Vaporized cannabis versus placebo for acute migraine: A randomized, double-blind, placebo-controlled crossover trial. Headache, 66, 365–376 (2026).*

[3] *Trojan V, Landa L, Hrib R, et al. Assessment of delta-9-tetrahydrocannabinol (THC) in saliva and blood after oral administration of medical cannabis with respect to its effect on driving abilities. Physiological Research, 71, 703–712 (2022).*

Keywords: Migraine; Cannabis; Pain Management; Nocebo Effect.

FROM INDICATION TO MEDICINAL PREPARATION: WHAT, WHEN, WHERE AND WITH WHOM?
(Konopí a věda XI, Brno, 22. 4. 2026)

Alžběta Dorota Dostálová[1], Radovan Hřib

[1] *Oddělení příprav léčiv, Nemocniční lékárna; Centrum pro léčbu bolesti, ARK, Fakultní nemocnice u sv. Anny v Brně, Czech Republic*

INTRODUCTION

Medical cannabis has been legally available in the Czech Republic as a magistral preparation since 2013. Despite growing clinical experience, the pathway from initial indication to delivery of the medicinal product remains complex. This presentation explores the practical clinical and pharmaceutical workflow at the pain management centre and hospital pharmacy of FN u sv. Anny v Brně, using a dialogue between a pain specialist (MUDr. R. Hřib) and a pharmacist (PharmDr. Ing. A. D. Dostálová). As noted by Prof. Hanuš, cannabis is a remarkable medicine – but not a panacea: it does not work for every patient, disease, or disease stage.

MATERIALS AND METHODS

Based on retrospective clinical experience from ARK and the Hospital Pharmacy at FN u sv. Anny v Brně. Patient selection, indications, product selection, dosing strategies, and dispensing processes were reviewed and structured into a dialogue-based educational format. Available cannabis forms include dried female flowers and liquid extracts in three groups: high-THC/minimal-CBD, balanced THC/CBD, and high-CBD/minimal-THC.

RESULTS

Patients referred by specialists or GPs present with chronic pain of various origins. Cannabis is used primarily within multimodal analgesic therapy (modified WHO analgesic ladder) or as monotherapy. High--THC preparations are preferred for pain, initiated nocturnally via oral route (capsules or solution). The pharmacy prepares capsules (from dried flowers), oral solutions/drops, topical ointments (Unguentum simplex), and suppositories. Dispensing takes approximately 2 working days. Patient co-payment is 10% of total cost; monthly costs range from under 200 CZK (capsules) to ~1,400 CZK (vaporisation). Key challenges include THC/CBD concentration limits in extracts, reporting requirements for low-cost care settings, three-month dispensing logistics, and prescription complexity for GPs – for which a simplified three-code classification (dominant THC, balanced, dominant CBD) has been proposed.

CONCLUSION

The journey from indication to delivery of medicinal cannabis requires close physician–pharmacist collaboration. Standardising prescription processes, adjusting regulatory limits, and simplifying reporting would improve patient access and outcomes. The proposed three-code KLP classification may facilitate prescribing, particularly for general practitioners.

REFERENCES

[1] *Juřica J. Farmakokinetika a lékové interakce kanabinoidů. In: Landa L. (ed.) Léčebné konopí v současné medicínské praxi. Praha: Grada Publishing; 2020, 68–82. ISBN 978-80-247-3967-0.*
[2] *Landa L, Jurica J, Sliva J, Pechackova M, Demlova R. Medical cannabis in the treatment of cancer pain and spastic conditions and options of drug delivery in clinical practice. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 2018;162(1):18–25.*
[3] *Hřib R, Pecháčková M, Šaloun J. Kanabinoidy v léčbě chronické neonkologické bolesti. In: Landa L. (ed.) Léčebné konopí v současné medicínské praxi. Praha: Grada Publishing; 2020, 83–96. ISBN 978-80-247-3967-0.*

Keywords: medical cannabis; pain management; magistral preparation; THC; CBD

Posters

APPLICATION OF *IN VIVO/EX VIVO* SPME-LC-MS/MS APPROACH FOR PROFILING OF CBD, ITS METABOLITES AND ENDOCANNABINOIDS IN RAT BRAIN AND BLOOD SAMPLES

Agnieszka Mosińska[1], Katarzyna Sztormowska[2], Katarzyna Owczarek[3], Katarzyna Smarzewska[4], Katarzyna Woźniczka[1], Magdalena Kaszewska[1], Vaclav Trojan[5, 6], Tomasz Bączek[1], Anna Roszkowska[1]

[1] *Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland*
[2] *Department of Pharmacology, Medical University of Gdańsk, Gdańsk, Poland*
[3] *Department of Biochemistry, Medical University of Gdańsk, Gdańsk, Poland*
[4] *Centre of Neurology and Neurochemistry, University Clinical Hospital in Wrocław, Wrocław, Poland*
[5] *Cannabis Facility, St. Anne's University Hospital Brno, Brno, Czech Republic*
[6] *Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Brno, Czech Republic*

INTORDUCTION

In recent years, there has been a growing interest in research focusing on the endocannabinoid system and the influence of phytocannabinoids on its functioning [1–3]. The analysis of both phyto- and endocannabinoids is difficult because these compounds are often present in animal/human tissues in trace amounts, and their determination requires highly sensitive analytical instruments. Also, low stability of those compounds requires fast and precise sample handling and analysis.

MATERIALS AND METHODS

This research focused on the application of biocompatible SPME probes with C18 extraction phase for isolation of selected phyto- and endocannabinoids from blood and brain samples under in vivo and ex vivo conditions, respectively. SPME combined with LC-MS/MS analysis was applied for measuring the levels of CBD and its metabolites (7-OH-CBD and 7-COOH-CBD), and endocannabinoids (AEA and 2-AG) in real biological samples.

RESULTS

SPME enabled the isolation and analysis of CBD and its metabolites. These compounds were monitored during pharmacokinetic studies in blood and brain structures (cerebellum, cerebral medulla, amygdala, hippocampus). All studied brain structures exhibited the highest levels of CBD 2 hours after CBD oral administration, except for the hippocampus (the highest level was detected at 4-th hour). The concentration of 2-AG in the brainstem and the amygdala remained relatively stable during the 6-hour experiment. In the hippocampus and cerebellum, the levels of 2-AG were slightly decreasing over time.

CONCLUSIONS

The presented SPME-based approach for simultaneous analysis of phyto- and endocannabinoids in biological samples in a novel solution in monitoring of those compounds close to real-time during pharmacokinetic studies. SPME probes facilitated extraction of even trace levels of analyzed compounds, and open the possibility to further investigations of the endocannabinoid system under in vivo conditions in blood and also in brain structures.

REFERENCES

[1] *Roszkowska A., Klejbor I., Bogusiewicz J., Plenis A., Bojko B. et al., Monitoring of age- and gender-related alterations of endocannabinoid levels in selected brain regions with the use of SPME probes, Metabolomics, 2023, 19(4):40.*

[2] *Woźniczka K., Konieczynski P., Plenis A., Bączek T., Roszkowska A., SPME as a green sample-preparation technique for the monitoring of phytocannabinoids and endocannabinoids in complex matrices, J Pharm Anal, 2023, 1117–1134.*

[3] *Magdalena Kaszewska, Katarzyna Woźniczka, Katarzyna Sztormowska-Achranowicz, Agnieszka Mosińska, Václav Trojan, Patrik Schreiber, Nikolas Balog, Tomasz Bączek, Anna Roszkowska, Perspectives of cannabis-based medicines in a view of pharmacokinetic studies of Δ9-THC and CBD in humans, Biomedicine & Pharmacotherapy, Volume 192, 2025, 118673*

OPTIMIZATION OF SPME-LC-MS/MS METHODOLOGY FOR ANALYSIS OF PHYTO- AND ENDOCANNABINOIDS IN SERUM SAMPLES

Magdalena Kaszewska[1], Agnieszka Mosińska[1], Katarzyna Woźniczka[1], Anna Roszkowska[1]

[1] Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Gdańsk, Poland

INTRODUCTION

The endogenous cannabinoid system is a crucial component of the organism, maintaining proper body homeostasis through its involvement in numerous physiological processes, including energy metabolism, memory regulation, and motor activity. The system comprises multiple receptor types – most notably CB1 and CB2 along with their endogenous ligands and the enzymes responsible for biosynthesis and degradation of small molecules named endocannabinoids (ECBs) [1]. In addition to ECBs, these receptors are also activated by exogenous compounds, such as plant-derived cannabinoids (phytocannabinoids, PCBs) [2].

MATERIALS AND METHODS

The aim of this study was to develop and optimize a methodology for the simultaneous analysis of selected ECBs (AEA, NADA, 2-AG) and PCBs (CBD, and 7-COOH-CBD) along with two deuterated internal standards, AEA-d11 and CBD-d3, in serum samples using solid-phase microextraction (SPME) coupled with liquid chromatography tandem mass spectrometry (LC-MS/MS). Optimization of the sample preparation step encompassed the selection of extraction time, desorption time, and appropriate solvents for analyte desorption from the C18 coating.

RESULTS

Under optimized conditions, analytes were extracted from serum samples over a period of 30 minutes. Following the extraction step, the analytes were desorbed from C18 coating (extraction phase) into a MeOH/IPA mixture (50:50, v/v) for 20 minutes. The resulting extracts were subsequently subjected to LC-MS/MS analysis. Chromatographic separation was achieved on an ACE Excel C18-AR column (100 × 2.1 mm, 1.7 µm), with a single run time of 4.5 minutes. All analyses were conducted in positive ionization mode.

CONCLUSION

The optimized SPME-LC-MS/MS method enabled the successful separation and quantitative analysis of all target compounds, including three deuterated internal standards, in serum samples within a short analysis time. The developed method provides a robust and reliable foundation for further investigation of ECB and PCB levels in real biological samples.

REFERENCES

[1] *Roszkowska A, Klejbor I, Bogusiewicz J, Plenis A, Bojko B, et al. Monitoring of age- and gender-related alterations of endocannabinoid levels in selected brain regions with the use of SPME probes. Metabolomics. 2023;19(4):40.*

[2] *Woźniczka K, Konieczynski P, Plenis A, Bączek T, Roszkowska A. SPME as a green sample-preparation technique for the monitoring of phytocannabinoids and endocannabinoids in complex matrices. J Pharm Anal. 2023:1117–1134.*

SAFETY ASSESSMENT ON CBD-RICH HEMP EXTRACT IN SUB-CHRONIC CROSS-SEX STUDY WITH RATS

Jan Dehner[*,a,b], Hana HolcovaPolanska[*,c], Katerina Petrlakova[d], Sanja CavarZeljkovic[b,e], Tibor Beres[b], Michal Hendrych[f], Jan Storch[g], Petr Tarkowski[b,e], Michal Masarik[c,d], Petr Babula[c], Jan Vacek[a,]

[a] *Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic*

[b] *Czech Advanced Technology and Research Institute (CATRIN), Palacky University, Slechtitelu 27, 783 71 Olomouc, Czech Republic*

[c] *Department of Physiology, Faculty of Medicine, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic*

[d] *Department of Pathophysiology, Faculty of Medicine, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic*

[e] *Centre of the Region Hana for Biotechnological and Agricultural Research, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Crop Research Institute, Slechtitelu 29, 78371 Olomouc, Czech Republic*

[f] *First Department of Pathology, St. Anne's University Hospital and Faculty of Medicine, Masaryk University, Pekarska 664/53, 602 00 Brno, Czech Republic*

[g] *Department of Advanced Materials and Organic Synthesis, Institute of Chemical Process Fundamentals of the Czech Academy of Sciences, v. v. i., Rozvojova 135, 165 02 Prague, Czech Republic*

[*] Both authors contributed equally.

Cannabidiol (CBD) is a phytocannabinoid of Cannabis sativa L., and is currently increasingly widely used for medical purposes. Here we focus on the safety and pharmacokinetics of CBD-rich full-spectrum hemp extracts (77% w/w) in male and female rats. Subchronic toxicity testing for 90 days was conducted using doses of 0.5, 5, 10, and 35 mg CBD extracts/kg/day orogastrically administered. No adverse effects and disturbances in organs or body weight, behaviour, locomotion, food intake or morbidity/mortality were observed. The pathomorphological examination did not show any changes in the gastrointestinal tract and liver. Blood cell analyses showed a significant difference between control and the treated animals in leukocyte, mean corpuscular haemoglobin concentration, mean corpuscular volume of erythrocytes, and number of neutrophils and monocytes. However, blood cell analysis showed significant sex-dependent differences, such as haemoglobin and erythrocyte count. In the treated animals, the changes of levels of ions (Ca^{2+}, Na^{+}, K^{+}, Cl^{-}), alkaline phosphatase, and creatinine were also observed in both sexes. Males showed decreased alanine transaminase levelsand females showed hyperalbuminemia. CBD was quantified in treated animals in dose dependent manner. The accumulation of CBD in individual tissues increased in the following order: brain, serum, liver, heart, kidneys, muscles, and skin. The results showed sex-dependent latent disruption of kidney and liver homeostasis, most probably reversible in nature.

IN VITRO CYTOTOXIC ACTIVITY OF SOLANINE ON SELECTED CANCER CELL LINES

Piotr Graczyk[1], Natalia Kownacka[1], Olga Kuduk[1],Justyna Stefanowicz-Hajduk[1]

[1] *Department of Biology and Pharmaceutical Botany, Medical University of Gdansk, Poland*

e-mail: petrilasendri21@gumed.edu.pl

INTRODUCTION

Solanine is a glycoalkaloid found in plants of the Solanaceaefamily.The highest amount of solanine can be found in green parts of tubers, sprouts, and leaves of potatoes *(Solanum tuberosum)*,which originate from South America and now are cultivated worldwide [1]. Solanine is known for its anti-pyretic,anti-inflammatory, and antibiotic activity [2]. Recently, attention has been drawn to its potential anticancer properties, and the initial results have been promising [3].In our study, we evaluated in vitro cytotoxicity properties of solanine. The cytotoxicity was estimated on human cancer cell lines such as gastric cancer – AGS, melanoma – A-375 and cervical cancer – HeLa, and non-cancer human fibroblasts.

MATERIALS AND METHODS

Solanineobtained from Phytolab (Germany)was dissolved in DMSO at the concentration of 20mg/mL.

The human gastric adenocarcinoma AGS, cervical cancer HeLa, and melanoma A-375 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Fibroblasts were obtained from LGC Standards (Germany).AGS cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F-12. HeLa, A-375 cells and fibroblasts were maintained in Dulbecco's Modified Eagle's Medium (DMEM) with low glucose and high glucose, respectively. All media were supplemented with 100 units/mL of penicillin, 100 µg/mL of streptomycin, and 10% (v/v) fetal bovine serum (FBS) (Merck Millipore, Burlington, MA, USA). The cells were incubated at 37 °C with 5% CO_2.

To estimate the cytotoxic effect of solanine, an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed. All cell lines were seeded in 96-well plates at a density of 5×10^3 cells/well and treated for 24 hours with the plant metabolite at concentrations of 1.5–22.5 µg/mL for AGS and HeLa, and 0.5–10 µg/mL for A-375. The DMSO concentration did not exceed 1% (v/v) for all the cell lines.The data are expressed as percentage of viability and also as IC_{50} values (µg/mL). Oxaliplatin was used as a positive control.

RESULTS

Solanine exhibited cytotoxic activity against all tested cell lines, with the strongest effect observed in melanoma A-375 cells, which showed an IC_{50} value of 3.41 ± 0.20 µg/mL.In the case of gastric cancer AGS cells and cervical cancer HeLa cells, the IC_{50} values were 6.30 ± 0.35 µg/mL and 6.73 ± 0.64 µg/mL, respectively.The viability of A-375 cells decreased from 86 ± 5% at a concentration of 1 µg/mL to 19 ± 3% at 10 µg/mL. In contrast, AGS and HeLa cell viability decreased from 78 ± 6% to 22 ± 2% and from 81 ± 1% to 23 ± 2%, respectively, over the concentration range of 3 to 22.5 µg/mL.

CONCLUSION

Results of our experiment demonstrate that solanine possesses significant cytotoxic activity against human cancer cell lines in vitro. The strongest effect was observed in melanoma A-375 cells, suggesting a higher sensitivity of this cell type to the compound. While AGS and HeLa cell lines also responded to solanine treatment, their IC_{50} values were moderately higher. These findings support the potential of solanine as a candidate for further investigation. Additional studies including mechanism of action and in vivo activity are necessary to fully evaluate its therapeutic potential and safety profile.

Keywords: solanine; cytotoxic effect; cancer cells; Solanaceae

REFERENCES

[1] *L. Bejarano, Journal of the Science of Food and Agriculture, 80, 2096–2100 (2000).*

[2] *S.H. Hassan, S. Gul, H.S. Zahra, A. Maryam, H.A. Shakir, M. Khan, and M. Irfan, Nutrition and Cancer, 73,1541–1552(2020).*

[3] *S. Luo, G.J. Tian, F.X. Yu, and Z.D. Wen, Translational Cancer Research, 10, 1578–1582 (2021).*

MONITORING OF THE LEVEL OF ENDOGENOUS CANNABINOIDS IN THE HEART WITH THE USE OF SPME-LC-MS/MS METHOD

Hanna Grodzka, Martyna Grochowalska, Julia Zadrożna, Tomasz Bączek, Anna Roszkowska

Department of Pharmaceutical Chemistry, Medical University of Gdansk, Poland

e-mail: anna.roszkowska@gumed.edu.pl

INTRODUCTION

The endocannabinoid system (ECS) is a network of lipid signalingmolecules, theirreceptors, and metabolizingenzymes, alsoactive in the heart [1]. Endocannabinoidssuch as anandamide (AEA), 2-arachidonoylglycerol (2-AG), and N-arachidonoyldopamine (NADA) arelocallyproduced in cardiomyocytes, the endothelium, and smoothmusclecells [2]. Under pathologicalconditionslikehypertensionorheartfailure, ECS activityintensifies, influencingvasculartone, oxidativestress, and inflammation [2]. The aim of thisstudy was to develop a sensitivemethod for determiningendocannabinoidlevels in hearttissueusing SPME-LC-MS/MS, to assesstheirdistribution in variousanatomical regions, and to comparelevels in humanexplantedhearts and rat hearts (WistarHanstrain).

MATERIALS AND METHODS

Solid-phase microextraction (SPME) was used for extraction, followed by liquid chromatography. Optimization included sorbent selection, extraction/desorption time, and sample pH. Different coatings were tested for efficiency and matrix compatibility. Extractions were performed on homogenized and non-homogenized rat heart tissue. Extraction conditions were adapted based on previous studies [3].

RESULTS

The method was applied to analyze endocannabinoids in 3-month-old male rat hearts using biocompatible C18 probes (4 and 10 mm). Ten-minute static extraction enabled effective compound collection without additional processing. The method showed good repeatability and stability. Different heart regions were sampled to assess potential variation in endocannabinoid distribution. Extractions were successful in both homogenized and intact tissue samples, and the probes retained performance even after autoclaving.

CONCLUSION

SPME showspotential for analyzing biological tissues in both research and clinical settings, with key advantages such as real-time applicability and suitability for monitoring endocannabinoids in heart regions.

REFERENCES

[1] *O'Sullivan S. E., in: Pertwee R. G. (ed.) Endocannabinoids. Springer, 393–412 (2015)*

[2] *Sierra S., Luquin N., Navarro-Otano J., Clin. Auton. Res., 28(1), 35–52 (2018).*

[3] *Roszkowska A., et al., Metabolomics, 19:40 (2023).*

Keywords: Endocannabinoids; Heart; SPME; LC-MS/MS.

PROFILING OF PHYTOCANNABINOIDS IN CBD- AND CBG-DOMINANT CANNABIS PLANTS WITH TWO EXTRACTION APPROACHES ALONG WITH HPLC-UV ANALYSIS

Stefan Bagieński[1],Piotr Gołaś[1], Gracjan Maślanka[1], Adrian Górski[1],Katarzyna Woźniczka[1], Vaclav Trojan[2,3], Patrik Schreiber[2], Nikolas Balog[2], Anna Roszkowska[1]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, Gdańsk, Poland*

[2] *Cannabis Facility, St. Anne's University Hospital Brno, Brno, Czech Republic*

[3] *Department of Natural Drugs, Faculty of Pharmacy, Masaryk University, Brno, Czech Republic*

e-mail: katarzyna.wozniczka@gumed.edu.pl

INTRODUCTION

The increasing value of the medical cannabis market observed in recent years is associated with growing competitiveness among its producers. In raw cannabis plant material, acidic forms of major and minor cannabinoids (phytocannabinoids (PCs)) dominate, such as cannabigerolic acid (CBGA), and cannabinolic acid (CBDA). Currently, a gold standard method described in Cannabis flos monograph of the European Pharmacopoeia comprises the analysis of acidic and neutral forms of PCs in Cannabis spp. inflorescences with the use of ethanolic extraction [1]. As a complementary technique for extraction of PCs from collected plant inflorescences, solid-phase microextraction (SPME) can be implemented [2,3].

METHODS AND MATERIALS

Both techniques were used for extraction of PCs from small amounts (100 mg) of homogenized samples of CBG- and CBD-dominant cannabis flowers. Traditional extraction technique required the use of 10 mL of ethanol for each sample.

SPME-based extraction utilized C18 extraction phase. HPLC-UV analysis of obtained extracts was performed in gradient elution mode. The separation of analytes was performed with the use of Acsentis Express C18 chromatographic column (15 × 4.6, 2.7 µm) with total analysis time of 12.5 min.

RESULTS

The obtained results reveal differences in the composition of PCs between both variants of cannabis plants. In CBD-dominant plant, CBDA and its neural form – cannbidiol (CBD) dominated, whereas in CBG-dominant plant, CBGA and its neutral form – cannabigerol (CBG) was detected. In addition, in CBD-dominat plants small levels of other PCs were detected, including cannabinol (CBN).

CONCLUSION

The obtained results were compared in the respect to the amount of extracted PCs, and also sensitivity and selectivity of those two different extraction approaches. SPME coupled to HPLC-UV can be used as quality control tool to monitor the content of particular PCs during post-harvest processing and storage of medicinal cannabis products.

REFERENCES

[1] *https://dl.novachem.com.au/files/Ph%20Eur%203028%20Cannabis%20flos.pdf*

[2] *Woźniczka K, Trojan V, Urbanowicz K, Schreiber P, Zadrożna J, Bączek T, Smoleński RT, Roszkowska A. In vivo profiling of phytocannabinoids in Cannabis spp. varieties via SPME-LC-MS analysis. Anal Chim Acta, 2024, 1306:342621.*

[3] *Woźniczka K., Konieczyński P., Plenis A., Bączek T., Roszkowska A., SPME as a green sample-preparation technique for the monitoring of phytocannabinoids and endocannabinoids in complex matrices, J Pharm Anal, 2023, 10, 1117–1134.*

Keywords: Phytocannabinoids, SPME, Ethanolic extraction, HPLC-UV

Abstract Miloš Beran

In general, fogponics, a more advanced form of aeroponics, is considered a very promising growing method with exceptionally high yields combined with minimized water consumption and great future potential, given its fundamental independence from the external environment conditions. However, there is a great lack of experimental data to support the previous claims. We will present results of a fogponic growing experiment compared to a concurrent hydroponic control. Fogponics uses aerosol with droplet sizes typically in the range of 1 to 10 micrometers. This technology uses the surface of piezoelectric discs, vibrating at a high frequency, typically in the range of 1–2 MHz, or ultrasonic nebulizers. A general disadvantage of all ultrasonic aerosol generators is the relatively low power and therefore the low density of the aerosol. The submicron component of the aerosol is only minimally represented. In contrast, our new generation mist system has a much higher power and creates unique aerosols with a higher density and a predominant submicron component. Given that the size of aerosol nanodroplets is comparable to the size of capillary root pores, we assume the possibility of direct penetration of nutrient solution nanodroplets into these pores. Another new factor is the use of compressed air for cutting the aerosol droplets. Air entering the aeroponic chamber at a relatively high speed can hypothetically significantly increase the important transport of air oxygen by the roots and thus the growth rate of the cultivated plants. We have managed to complete the entire plant growing cycle with the fogponic system with promising results. The fogponics system, equipped with complete automatic control, a sensor system and a touch screen, was protected by a utility model in the Czech Republic. However, further growing experiments will be necessary for definitive conclusions in comparing fogponic and hydroponic growing technology.

Beyond the Wall: Molecular Evidence of Early Regenerative Potential in Cannabis sativa Protoplasts

Protoplast-based technologies provide a powerful platform for genome editing, somatic hybridization, and non-chimeric transformation in plants. However, in Cannabis sativa, complete plant regeneration from protoplasts remains unresolved, limiting advanced biotechnological applications. In this study, we tested protoplast isolation across multiple cannabis cultivars and evaluated the influence of donor material age and cultivation conditions. The highest yields were obtained from 1–2-week-old in vitro-germinated seedlings of cv. 'USO 31', reaching up to 9.9×10^6 cells·g^{-1} with viability as high as 83%. Using a modified regeneration medium originally developed for Arabidopsis thaliana, we initiated cell divisions leading to microcallus formation. RT-qPCR analysis of selected marker genes revealed significant upregulation of the proliferation marker PCNA and the auxin-responsive gene IAA-2 during early cultivation, indicating re-entry into the cell cycle. Oxidative stress-related genes showed dynamic regulation, with APX upregulation suggesting activation of antioxidant defences, while ABA-related markers (PP2C-1, LEA34) remained downregulated, indicating limited abiotic stress. Collectively, our findings provide some of the first molecular evidence for regenerative potential in cannabis protoplast cultures, linking early cell cycle re-entry with coordinated oxidative stress management. These insights lay the groundwork for developing a protoplast-to-plant regeneration system in C. sativa and open new avenues for precision breeding and genome engineering.

Daniel Král
Josef Baltazar Šenkyřík
Vladan Ondřej

NOVEL MODULATORY PATHWAYS IN OSTEOSARCOMA: ROLE OF PHYTOCANNABINOIDS AND VITAMIN D3

Hana Holcová Polanská [1, 2, 3], Alžběta Pávková [2], Michal Masařík [1, 2, 3]

[1] *Michal Masařík Research Group, Faculty of Medicine, Masaryk University, Brno, CZ*
[2] *Department of Physiology, Faculty of Medicine, Masaryk University, Brno, CZ*
[3] *Department of Pathophysiology, Faculty of Medicine, Masaryk University, Brno, CZ*

e-mail: hana.holcova.polanska@med.muni.cz

INTRODUCTION

Osteosarcoma is an aggressive bone tumor affecting mainly adolescents and young adults. Because current therapies remain limited, new biological approaches are needed [1]. Phytocannabinoids such as cannabidiol (CBD) and cannabigerol (CBG) show anti inflammatory, antioxidant, and antitumor effects and act through multiple signaling pathways linked to cell growth, migration, and metabolism [2]. Vitamin D3, through its receptor VDR, further regulates proliferation, differentiation, and angiogenesis [3]. Since both phytocannabinoids and vitamin D3 influence key processes relevant to tumor progression, their combined effects may offer a promising strategy for modulating osteosarcoma cell behavior.

MATERIALS AND METHODS

SAOS 2 osteosarcoma cells were cultured in DMEM F12 medium supplemented with 10% FBS and antibiotics under standard conditions (37 °C, 5% CO_2). Cell proliferation was assessed by real time monitoring using the Incucyte® system after treatment with CBD, CBG, and vitamin D3 at defined concentrations for 138 hours. Cell migration was evaluated using a wound healing assay with the Incucyte® system; cell movement into the wound area was monitored for up to 100 hours following treatment application. Colony formation was analyzed by seeding low density cultures in 6 well plates, treating them for three weeks, and staining colonies with Trypan Blue for visual quantification. Cell metabolism was measured using the Seahorse XFp Analyzer to determine glycolytic activity and mitochondrial ATP production after exposure to CBG and CBG + vitamin D3, following manufacturer's instructions.

RESULTS

CBD and CBG showed concentration dependent effects on SAOS 2 cells. The strongest growth inhibition was observed with CBD 5 µM + vitamin D3 and similarly with CBG 5 µM + vitamin D3. In the migration assay, the lowest motility was recorded for CBD 5 µM + vitamin D3, while CBG also reduced migration, particularly when combined with vitamin D3. In the colony formation test, the fewest colonies appeared in the CBD 5 µM + vitamin D3 group, consistent with growth curve results. Metabolic analysis showed increased glycolytic inhibition in cells treated with CBG 5 µM and CBD 5 µM + vitamin D3, with no major differences in mitochondrial ATP production.

CONCLUSION

CBD and CBG, particularly in combination with vitamin D3, reduced proliferation, migration, and colony forming capacity of SAOS 2 osteosarcoma cells, with the strongest effects observed for CBD 5 µM + vitamin D3. These findings suggest that phytocannabinoids together with vitamin D3 may represent a promising complementary approach for modulating osteosarcoma cell behavior.

REFERENCES

[1] *A. Abarrategi, J. Tornin, L. Martinez-Cruzado, A. Hamilton, E. Martinez-Campos, J.P. Rodrigo, M.V. González, N. Baldini, J. Garcia-Castro, R. Rodriguez, Stem Cells International 2016 (2016) 3631764.*
[2] *M. Vigano, L. Wang, A. As'sadiq, S. Samarani, A. Ahmad, C.T. Costiniuk, Front. Immunol. 16 (2025).*
[3] *D. Feldman, A.V. Krishnan, S. Swami, E. Giovannucci, B.J. Feldman, Nat Rev Cancer 14 (2014) 342–357.*

Keywords: Phytocannabinoids; Vitamin D3; Osteosarcoma Cell Line.

ADVANCING PORTABLE DIAGNOSTIC TECHNOLOGIES FOR BIOMEDICAL, FORENSIC, AND SECURITY APPLICATIONS

Jaleigh Morales [1], Chloe Earls [1], Jan Halámek [1]

[1] *Department of Chemistry, Middle Tennessee State University, USA e-mail: jan.halamek@mtsu.edu*

INTRODUCTION

The increasing prevalence of illicit substances, particularly marijuana and fentanyl-related compounds, presents significant challenges for law enforcement and public health. Current toxicological detection methods, including gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), require centralized laboratory infrastructure and invasive sampling, limiting their applicability in rapid-response scenarios [1]. There is a critical need for portable, rapid, and costeffective detection strategies that can be deployed in the field. This study explores immunological and biochemical approaches for the detection of tetrahydrocannabinol (THC) metabolites and fentanyl analogs. Specifically, competitive immunoassays were used to detect THC-COOH in fingerprint sweat, while enzyme inhibition assays were employed to evaluate fentanyl detection through butyrylcholinesterase (BChE) activity. These approaches aim to provide a foundation for the development of rapid, on-site diagnostic tools [2].

MATERIALS AND METHODS

THC metabolite detection was conducted using a competitive immunoassay targeting THC-COOH in fingerprint sweat samples collected from both nonusers and active marijuana users. Absorbance changes were measured to evaluate antigen-antibody interactions via UV-Vis spectroscopy. For fentanyl detection, BChE enzyme activity was assessed using Ellman's assay. Various fentanyl analogues were introduced at concentrations ranging from 10^{-12} M to 10^{-7} M, and enzyme inhibition was monitored over time intervals between 90 and 600 seconds.

RESULTS

Fingerprint samples from active THC users showed decreased immunoassay absorbance compared to controls, indicating detectable THC metabolites in sweat. Fentanyl analogues inhibited BChE activity in a concentration-dependent manner, with detectable effects as low as 10^{-12} M and within 90 seconds. These results highlight the sensitivity and rapid response capability of enzyme-based detection systems.

CONCLUSION

This study demonstrates proof-of-concept for portable, rapid detection of illicit substances using immunological and enzymatic methods. THC use can be identified through fingerprint sweat analysis, while fentanyl analogues can be detected via BChE inhibition. These approaches offer significant advantages, including low cost, fast analysis time, and minimal sample preparation. The results support the development of field-deployable diagnostic tools for use in forensic investigations, law enforcement, and public health settings.

REFERENCES

[1] *E. Brunelle, B. Thibodeau, A. Shoemaker, J. Halámek, ACS Sensors, 4, 3318 (2019).*
[2] *V. Rania, A. Newland, L. Halámková, V. Trojan, R. Hřib, J. Halámek, ACS Omega, 9 (38) (2024).*

Keywords: THC detection; fentanyl; enzyme inhibition; portable diagnostics

Scientific committee

Prof. MUDr. Alexandra Šulcová, CSc.
Prof. Jan Halámek, Ph.D.
Dr. hab. Anna Roszkowska
MVDr. Ing. Václav Trojan, Ph.D.
Ing. Helena Pluháčková, Ph.D.

Organising committee

MVDr. Ing. Václav Trojan, Ph.D.
MUDr. Radovan Hřib
Ing. Helena Pluháčková, Ph.D.
Agnieszka Mosińska
Daniel Dostál