4. KONGRES SLOVENSKEGA TOKSIKOLOŠKEGA DRUŠTVA

KANABINOIDI POD DROBNOGLEDOM: NJIHOVA TOKSIČNOST IN UPORABNOST

CANNABINOIDS UNDER SCRUTINY: THEIR TOXICITY AND MEDICAL UTILITY

Ljubljana, 17.1.2019

Univerza *v Ljubljani*
Medicinska fakulteta

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Fakulteta *za farmacijo*

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Kanabinoidi predstavljajo raznoliko skupino snovi vključujoč endogene, rastlinske in sintetične kanabinoide. Zaradi kompleksnosti endokanabinoidnega sistema in raznolikosti kanabinoidov je prvi del kongresa namenjen osnovam za razumevanje njihovih učinkov, tako neželenih, kot želenih.
Indentifikacija kanabinoidnih receptorjev (CB1, CB2) in njihovih endogenih lipidnih ligandov je sprožila številne raziskave, ki so osvetlile delovanje endokanabinoidnega sistema in njegovo regulatorno funkcijo pri fizioloških ter patoloških procesih, predvsem njegovo nevromodulatorno in imunomodulatorno vlogo. Za receptorje CB1, ki so sklopljeni s proteinom G, je značilno, da je aktivacija odvisna od lokacije receptorja ali od interakcije med receptorjem CB1 in ostalimi ne-kanabinoidnimi receptorji v njegovi bližini, ki so ravno tako sklopljeni s proteinom G. Gre za nov koncept farmakološke učinkovitosti, ki ni ena sama, ampak je moč agonista odvisna od tipa celice in njenega stanja. To je verjetno tudi razlaga, zakaj povečano sproščanje endokanabinoidov v določenem primeru deluje »zaščitno«, v drugem pa vodi v nastanek neželenih učinkov. Trenutno je zato veliko pozornosti namenjeno, ne samo direktno delujočim agonistom kanabinoidnih receptorjev, ampak predvsem razvoju alosteričnih modulatorjev kanabinoidnih receptorjev, ki s svojo vezavo povečujejo aktivnost endokanabinoidov. V razvoju so tudi snovi, ki z zaviranjem njihovega privzema v celico ali razgradnje, povečujejo koncentracijo endokanabinoidov na njihovih receptorjih. Vendar pa je na žalost razvoj slednjih povezan s smrtnim izidom v klinični študiji. Eden od možnih vzrokov za nepričakovan akutni učinek je razlika med človekom in poskusnimi živalmi v izločanju preskusne snovi, kar kaže na velik pomen poznavanja farmakokinetike na tem področju. Predstavljeni so tudi akutni in kronični toksični učinki kanabinoidov, ki jih uporabljamo v medicinske namene ter ozadje dejstva, da ima kombinacija delta-9-tetrahidrokanabinola (THC) s kanabidiolom (CBD) manj neželenih učinkov kot sam THC. Rekreativna uporaba sintetičnih kanabinoidov predstavlja večji toksikološki problem, zato so le-ti predstavljeni v posebni sekciji. Imajo namreč resnejše akutne klinične učinke kot THC, ker imajo večjo afiniteto za vezavo na receptor CB1, obenem pa njihova toksičnost ni ustrezno preskušena.
V nadaljevanju kongresa je pozornost namenjena EU in nacionalni zakonodaji s področja kanabinoidov. Kljub pestrosti kanabinoidov se v medicinske namene zaenkrat uporablja le majhno število kanabinoidov, in sicer so to THC in njegovi stereokemični izomeri, vključno z dronabinolom, nabilonom kot sintetičnim analogom THC, in zdravilo v obliki oralnega pršila, ki vsebuje dva ekstrakta konoplje, od katerih je eden standardiziran na THC in drugi na CBD. Na podlagi Uredbe o razvrstitvi prepovedanih drog je v Sloveniji mogoče izdelovati magistralna in galenska zdravila iz kanabinoidov.

V zadnjem delu kongresa je pozornost namenjenja varnosti kanabinoidov pri medicinski uporabi. Zdravila, ki so pridobila dovoljenje za promet, imajo opredeljene posamezne indikacije, pri katerih sta bili dokazani učinkovitost in varnost uporabe teh zdravil. Posebej bodo predstavljene izkušnje z uporabo kanabinoidov pri zdravljenju trdovratnih epilepsij otrok in drugih nevroloških bolezni ter izkušnje pri uporabi v onkologiji. Predstavljene bodo tudi interakcije kanabinoidov z nekaterimi zdravili. Kanabinoidi predstavljajo pomembno dopolnilo v obstoječem naboru zdravil. Za njih pa veljajo enaka pravila kot za druga zdravila - morajo biti varna, kvalitetna in učinkovita. Jasna pa mora biti ločnica med njihovo rekreativno in medicinsko uporabo.

Upamo, da se bo za vsakega izmed udeleženca v programu tega kongresa našla tematika, ki ga bo še posebej pritegnila, morda se z njo tudi sam raziskovalno ukvarja. Želimo, da se znanja s tega področja povezujejo in dograjujejo tudi v slovenskem strokovnem ter znanstvenem prostoru, v prid bolnikom, ki uživajo kanabinoide, kakor tudi, da bi še povečali ozaveščanje uporabnikov, ki zlorabljajo predvsem nove sintetične kanabinoide.

Želimo vam prijetno druženje in čim več zanimivih izmenjav mnenj o tematikah, predstavljenih na kongresu!

Doc. dr. Katarina Černe
Prof. dr. Marija Sollner Dolenc
v imenu Organizacijskega odbora

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POVZETKI VABLJENIH PREDAVANJ

Endokanabinoidni sistem in raznolikost kanabinoidov: temelji za razumevanje učinkov

The endocannabinoid system as a target for therapeutic intervention

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The modern pharmacology of cannabinoids started in 1964 with the elucidation of the chemical structure of delta-9-tetrahydrocannabinol by Gaoni and Mechoulam. Since then, this area of research has witnessed a revolution in terms of the volume of scientific production, as well as in the detailed description of a novel physiological system (the endocannabinoid system, ECS). In addition, recent changes regarding the legal status of cannabinoid-based preparations in western countries, together with the expansion of the use of synthetic cannabinoids as drugs of abuse have brought renewed attention to the field.

The ECS is presently seen as a physiological system that plays relevant functions in the CNS and in the periphery, affecting for instance, the cardiovascular tone, the hepatic function, the regulation of appetite and the activity of neurons and glial cells. This wide variety of functions and processes regulated by the ECS reflects its high complexity and limits our current understanding of this system.

Among the different areas in which the ECS deserves attention, neurodegeneration is probably one of the most relevant. Intense research is currently focused on its potential therapeutic interest in, for instance, multiple sclerosis, Parkinson's, Huntington's or Alzheimer's diseases. Several elements of the ECS are involved in the pathogenesis of these diseases and are thus considered to be promising targets for the development of novel therapies.

Keywords: endocannabinoids, neurodegeneration, therapeutics

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Raznolikost kanabinoidov (endogeni, naravni, sintezni) in njihovih lastnosti

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Kanabinoidi, izolirani iz rastline *Cannabis sativa* L., so znani in uporabljani že več tisoč let, čeprav so njihov mehanizem delovanja dognali šele pred dobrimi tridesetimi leti, kristalno strukturo tarče pa šele pred dvema letoma. Med kanabinoide štejemo zelo raznolike substance, ki se v prvi vrsti razlikujejo po izvoru, saj so nekateri prisotni v naravi, predvsem v določenih rastlinah iz rodu Cannabis (fitokanabinoidi), drugi so endogenega izvora (endokanabinoidi), tretji pa pridobljeni kot rezultat racionalnih postopkov načrtovanja in sinteze farmacevtskih kemikov (sintezni kanabinoidi, kanabimimetiki). Strukturno gledano gre za zelo različne spojine, ki jim je skupno predvsem to, da modulirajo delovanje endogenega kanabinoidnega sistema, pri čemer pa lahko izražajo zelo različne učinke. V večini primerov gre za neposredno delujoče spojine, in sicer za ligande kanabinoidnih receptorjev CB1, ki se nahajajo predvsem v možganih, in CB2, ki so prisotni na celicah imunskega sistema. Ti ligandi lahko delujejo bodisi kot agonisti, antagonisti, inverzni agonisti ali pozitivni alosterični modulatorji enega (selektivni) ali obeh omenjenih receptorjev (neselektivni). Nekatere spojine pa se vpletajo v delovanje endogenega kanabinoidnega sistema tako, da vplivajo na biosintezo, razgradnjo ali privzem endogenih kanabinoidov. Kot zanimivost velja omeniti dejstvo, da paracetamol protibolečinsko delovanje doseže tudi preko modulacije endogenega kanabinoidnega sistema. V sklopu predavanja bom predstavil pregled različnih skupin kanabinoidov, njihov odnos med strukturo in delovanjem ter raznolik spekter učinkov. Na kratko pa se bomo dotaknili tudi njihovega terapevtskega potenciala, potenciala za zlorabo in problemov, ki so povezani s slednjima.

Ključne besede: kanabinoidi, fitokanabinoidi, endokanabinoidi, sintezni kanabinoidi, odnos med strukturo in delovanjem, potencial za uporabo/zlorabo

Diversity of cannabinoids (endogenous, phyto-, synthetic) and their properties

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Cannabinoids, isolated from the plant *Cannabis sativa* L., have been known and used for thousands of years, although their mechanism of action was only established thirty years ago, while the crystal structure of their target was only solved two years ago. Cannabinoids are a very diverse group of substances, which primarily differ in origin, since some are present in nature, particularly in plants of the genus Cannabis (phytocannabinoids), others are of endogenous origin (endocannabinoids), while many have been obtained as a result of rational design and synthetic efforts by medicinal chemists (synthetic cannabinoids, cannabimimetics). Structurally speaking, these compounds are very different, the only thing they have in common being the fact that they modulate the endogenous cannabinoid system, which can result in distinct effects. In most cases, these compounds act directly via binding to the CB1 cannabinoid receptor, found primarily in the brain, and CB2 receptor, which is present in the cells of the immune system. These ligands may act as agonists, antagonists, inverse agonists or positive allosteric modulators of one (selective) or both of the said receptors (non-selective). Certain compounds interfere with the functioning of the endogenous cannabinoid system by affecting the biosynthesis, degradation or inhibition of endogenous cannabinoids. Interestingly, the analgesic properties of paracetamol, a very common drug used for pain relief, have also been attributed to its modulatory actions on the endogenous cannabinoid system. In the scope of the lecture, an overview of different groups of cannabinoids, their structure-activity relationship and the diverse spectrum of their effects will be presented. We will also briefly touch upon their therapeutic potential, the potential for abuse and the problems arising from these.

Keywords: cannabinoids, phytocannabinoids, endocannabinoids, synthetic cannabinoids, structure-activity relationship, use/abuse potential

Farmakokinetika kanabinoidov

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Navadna konoplja vsebuje več kot 60 kanabionoidnih spojin. Med posameznimi rastlinami konoplje obstajajo zelo velike razlike v njihovi vsebnosti. V telesu se te spojine presnavljajo po različnih, hkrati vzporednih in zaporednih poteh. Farmakokinetika kanabinoidov je poleg tega zelo odvisna tudi od načina aplikacije ter od odmerka. V zadnjih letih se opaža veliko povečanje zanimanja za terapevtsko uporabo derivatov konoplje na različnih področjih medicine, ki je tudi regulativno po svetu vse bolj podprta. Kljub naraščajoči uporabi kanabinoidov pa ostaja njihova farmakokinetika zaradi svoje kompleksnosti še ne povsem raziskana. Za nekatere predstavnike, npr. za kanabidiol obstaja dobro utemeljena domneva o korelaciji med odmerkom, plazemskimi in možganskimi koncentracijami in s tem farmakodinamskim učinkom, za nekatere druge, npr. THC, pa ne. Potrjen je tudi pozitivni vpliv sočasno zaužitih maščob na biološko uporabnost kanabinoidov, veliko pa ostaja še neraziskanega, npr. enterohepatična cirkulacija, različni vplivi na predsistemski in sistemski metabolizem, količina maščobnega tkiva pri posamezniku, vplivi različnih virov kanabinoidov (rastišče, čistost ekstrakta, vsebnosti), vpliv formulacij (vpliv ekscipiensov, velikosti, morfologije delcev), vpliv poti aplikacije ter interakcije z drugimi zdravili. Za varno in učinkovito uporabo kanabinoidov je torej ključna analiza farmakokinetičnih procesov in njihovo razumevanje in poznavanje pri različnih populacijah (otroci, starostniki, kahektični bolniki, nosečnice, idr.) ter v različnih kliničnih situacijah. Cilj prispevka je zato sistematičen pregled in identifikacija ter razlaga najpomembnejših procesov, ki lahko pomembno vplivajo na vrsto, intenziteto in trajanje učinkov zdravil s kanabinoidi. To znanje je nujno tudi za načrtovanje novih dostavnih sistemov s kanabinoidi in njihovimi kombinacijami za doseganje optimalnega terapevtskega učinka ter minimalnih neželenih učinkov teh zdravil.

Ključne besede: tetrahidrokanabinol, kanabidiol, farmakokinetika, absorpcija, distribucija, metabolizem, eliminacija

Cannabinoid pharmacokinetics

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Common cannabis contains more than 60 cannabinoid compounds. There is large variability in their content between individual cannabis plants. In the body, these compounds are metabolized by various parallel and sequential pathways. The pharmacokinetics of cannabinoids is also highly dependent on both the route of administration and the dosage. In recent years, there has been a noticeable increase in interest in the therapeutic use of cannabis derivatives in various fields of medicine, which is also increasingly supported globally by regulative authorities. Despite the rising use of cannabinoids, their pharmacokinetics has not yet been fully studied, due to its complexity. For some compounds, for example for cannabidiol, there is a well-founded hypothesis about correlation between dose, plasma and brain concentrations and, ultimately, the pharmacodynamic effect; while for some others, e.g., THC, this is not the case. The positive effect of concomitantly consumed fats on the bioavailability of cannabinoids has already been established, but much still remains to be elucidated, e.g., enterohepatic recirculation, various effects on the pre-systemic and systemic metabolism, the amount of fatty tissue in each individual, the effects of different origins of cannabinoids (plants, purity, content), the effect of formulations (influence of excipients, size, particle morphology), route of application and interactions with other drugs. Analysis of pharmacokinetic processes and understanding them in various populations (children, elderly, cachectic patients, pregnant women, etc.) and in specific clinical situations is therefore crucial for the safe and effective use of cannabinoids. The goal of this contribution is a systematic review and identification of the most important processes that can have key effects on the type, intensity and duration of the effects of cannabinoid medicines. This knowledge is also crucial for the design of new delivery systems with cannabinoids and their combinations with optimal therapeutic and minimal adverse effects.

Keywords: tetrahydrocannabinol, cannabidiol, pharmacokinetics, absorption, distribution, metabolism, elimination

Toksikološke lastnosti ligandov kanabinoidnega sistema

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Iz rastline *Cannabis sativa* L. so do sedaj izolirali že več kot 100 fitokanabinoidov, poleg njih pa obstaja več kot 550 sintetičnih spojin, ki delujejo na kanabinoidne receptorje CB1 in CB2. Prav tako je treba omeniti, da nobeden od ligandov kanabinoidnih receptorjev ni popolnoma CB1- ali CB2-specifičen. Zato se učinki vsakega od njih razlikujejo, ne le zaradi različne moči na kanabinoidnih receptorjih, ampak tudi zato, ker lahko delujejo na druga ne-CB1 in ne-CB2 prijemališča (npr. receptor TRPV1, receptor GPR55, napetostno-odvisne ionske kanale, transporterje za kateholamine). Obenem je to lahko razlog za razlike v interakcijah z zdravili, ki jih jemljemo sočasno, poleg aktualnejših farmakokinetičnih razlogov, za kar pa so potrebne nadaljne raziskave.

Najpogosteje proučevani kanabinoid je Δ^9-tetrahidrokanabinol (THC). THC je delni agonist na obeh kanabinoidnih receptorjih, vendar je njegov psihoaktivni učinek povezan predvsem z aktivacijo receptorjev CB1. Receptor CB1 je eden izmed metabotropnih receptorjev z največjo ekspresijo v osrednjem živčevju, z izjemo možganskega debla. Slednje je lahko razlog zakaj THC ni povezan z nenadno smrtjo zaradi depresije dihanja. Čeprav so akutni učinki na osrednji živčni sistem THC jasno opredeljeni, je tveganje za ireverzibilne nevropsihološke učinke THC kot neodvisnega dejavnika, zlasti pri pediatrični populaciji in mladostnikih, potrebno nadalje raziskati za pojasnitev povezave. Za razliko od THC, fitokanabinoid kanabidiol (CBD) nima psihoaktivnih učinkov, vendar lahko pri sočasni uporabi vpliva na nekatere učinke THC. CBD, ki nima pomembne afinitete za CB1 in CB2, aktivira ali zavira številne uveljavljene ter domnevne farmakološke tarče. CBD je kot aktivna snov v zdravilu Epidiolex, ki se daje oralno, pred kratkim opravil nadzorovana klinična preskušanja, da so ocenili njegovo varnost pri zdravljenju redkih epileptičnih sindromov pri otrocih. Največjo zaskrbljenost glede varnosti so predstavljale povišane vrednosti transaminaz. Zato je treba izvesti postmarketinški nadzor toksičnosti za jetra.

Predstavitev bo povzela kar je znano o akutnih in kroničnih toksikoloških učinkih, katere študije še manjkajo in kaj so negotovosti v zvezi z varnostjo eksogenih kanabinoidov.

Ključne besede: kanabinoidi, akutna toksičnot, kronična tokisčnost, študije na živalih, podatki od ljudi

Toxicological properties of cannabinoid receptor ligands

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More than 100 phytocannabinoids have been isolated from the plant *Cannabis sativa* L. and more than 550 chemical compounds that can interact with two types of cannabinoid receptors: CB1 and CB2. It is also worth noting that none of the cannabinoid receptor ligands are entirely CB1- or CB2-specific. The effects of each of these ligands therefore differ not just because of different potency at cannabinoid receptors but also because they can interact with other non-CB1 and non-CB2 targets (e.g., TRPV1 receptor, GPR55 receptor, voltage-gated ion channels, neuronal transporters of catecholamines). As a consequence, the potential for drug-drug interactions of these compounds can also vary.

The most studied phytocannabinoid is Δ^9-tetrahydrocannabinol (THC). THC is a partial agonist at both cannabinoid receptors, but its psychotomimetic effect is produced primarily via activation of the CB1 receptor. The CB1 receptor is one of the G protein-coupled receptors expressed at the highest level in the central nervous system, with the noteworthy exception of the brain stem, which may help to explain why THC is not associated with sudden death due to respiratory depression. Although acute cognitive and other effects of THC are clearly defined, the risk of irreversible neuropsychological effects of THC on its own, especially in the paediatric/adolescent population, needs further research to elucidate the association. Unlike THC, phytocannobinoid cannabidiol (CBD), does not appear to have psychotomimetic effects but may interact with some of the effects of THC when co-administered. CBD, which lacks significant affinity for CB1 and CB2, activates or inhibits a number of established and putative pharmacological targets. CBD administered orally has recently undergone well-controlled clinical trials to assess its safety when treating orphan pediatric epilepsy sindromes. Transaminaze elevation has been identified as a safety issue of concern. Postmarketing surveillance for liver toxicity must therefore be performed.

This presentation will summarize what is known about acute and chronic toxicological effects, what studies are still lacking and uncertainties surrounding the safety of exogenous cannabinoids.

Keywords: cannabinoids, acute toxicity, chronic toxicity, animal studies, human data

POVZETKI VABLJENIH PREDAVANJ

Sintetični kanabinoidi in rekreativna uporaba

Synthetic cannabinoids: A threat to health security

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One of the unintended effects of globalisation is that new and recurring threats capable of severely damaging public health can emerge and spread faster than ever before. Infectious diseases, such as Ebola, and the market in fake medicines, are just two examples of such serious cross-border threats. Drug markets have not been immune to these global changes either, with the market in new psychoactive substances (NPS) growing rapidly over the last decade. Currently, the EMCDDA monitors more than 700 NPS through the EU Early Warning System. This includes a growing number of highly potent substances, such as the synthetic cannabinoids (SCs). Since 2008, 190 SCs have been detected on the drug market in hundreds of products, making them the largest group of substances monitored by the EMCDDA. These substances pose a high risk of life-threatening poisoning to users, can cause explosive outbreaks that can overwhelm local healthcare systems, and can pose a risk of occupational exposure. They are also relatively cheap and easy to conceal and smuggle, with a few grams sufficient to make many thousands of doses for the drug market. Not only do these offer economic incentives to suppliers, but they also reduce the risks of being caught. Originally sold as "legal" replacements to cannabis, in some places synthetic cannabinoids have become a drug of choice among vulnerable groups, such as the homeless and prisoners, as powerful and cheap intoxicants that are used for their "mind-numbing" effects. Their high potency is also responsible for explosive outbreaks of poisonings, ranging in size from a handful of victims to over 800 people. Such outbreaks can rapidly overwhelm emergency responders and hospital emergency departments. While the number of SCs appearing for the first time each year is slowing and there has been a drop in the amount of bulk powders seized, they continue to be available across much of Europe. Since 2016, the EMCDDA has risk assessed five SCs that have caused serious concern at European level: MDMB-CHMICA, AB-CHMINACA, ADB-CHMINACA, 5F-MDMB-PINACA, and CUMYL-4CN-BINACA. Together, these substances have been involved in more than 100 deaths. This presentation examines the health security threat posed by synthetic cannabinoids and what agencies can do to strengthen preparedness and response activities to reduce the threat to public health.

Keywords: health security, synthetic cannabinoids, early warning systems, globalization, legal highs, new psychoactive substances, outbreaks, risk assessment

Novi sintetični kanabinoidi - zloraba v slovenski študentski populaciji

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V času od oktobra 2017 do vključno aprila 2018 je bila izvedena anketa o uporabi novih psihoaktivnih snovi (NPS) s posebnim poudarkom na sintetičnih kanabinoidih (SK) in katinonih (SKA) med študenti Univerze v Ljubljani. Spletna anketa nudi lahko dostopno ter anonimno reševanje, preko pošiljanja spletne povezave do vprašalnika predstavnikom letnikov na različnih fakultetah, objavljanja na njihovih spletnih straneh in družabnih omrežjih ter po študentskih domovih v Ljubljani – tako smo pridobili naključnost vzorca. Namen ankete je bilo zbiranje podatkov o novih psihoaktivnih snoveh, za katere imamo v Republiki Sloveniji zelo malo relevantnih podatkov, tako o uporabi kot prepoznavnosti le-teh.

V vprašalnik so bila poleg demografskih vprašanj (spol, starost, kraj stalnega in začasnega bivališča, fakulteta, letnik študija) vključena tudi vprašanja o poznavanju termina NPS, o pogostosti kombiniranja NPS z alkoholom, klasičnimi drogami ter zdravili na recept, o poznavanju in uporabi novih drog iz skupine sintetičnih kanabinoidov, katinonov ter ostalih predstavnikov psihoaktivnih snovi, prav tako tudi starost ob prvem stiku z določeno drogo in časovni interval uporabe, način preskrbe z drogo, osebna mnenja uporabnikov o njihovih učinkih ter povezave neto zaslužka/prejemka in tedenske/mesečne porabe denarja za nakup drog ter alkohola.

Za analizo je bilo ustreznih 516 anket. Med vsemi anketiranci je (vsaj) enega izmed navedenih sintetičnih kanabinoidov prepoznalo 339 vprašanih (65,7 %), poskusilo pa 69 vprašanih (13,4 % anketiranih). Prepoznavnost posameznih sintetičnihnih kanabinoidov v anketi je v povprečju 5,4 %. Pri oceni lastnega znanja vseh vprašanih o nevarnosti uporabe sintetičnih kanabinoidov, ki jo je anketiranec izrazil na podlagi številčne lestvice od 1 do 5, kjer 1 predstavlja pomanjkanje seznanjenosti z informacijami o nevarnostih SK, je kar 34 % izbralo najnižjo vrednost, le 5 % jih je menilo, da so zelo dobro informirani (vrednost 5). Povprečje je bilo 2,3, iz česar lahko zaključimo, da je splošna informiranost o tej vrsti novih psihoaktivnih snovi slaba. Zato je pomembno, da se mlade preko različnih kampanj in delavnic ozavešča o nevarnostih uporabe te vrste drog.

Ključne besede: sintetični kanabinoidi, nove psihoaktivne snovi, zloraba

New synthetic cannabinoids - abuse in the Slovenian student population

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A survey on the use of new psychoactive substances (NPS) was conducted between October 2017 and April 2018, with a special focus on synthetic cannabinoids (SK) and cathinones (SKA), among students of the University of Ljubljana. The online survey was easily accessible and anonymous; by sending a web link to the questionnaire to students' representatives at various faculties, publishing it on their websites and social networks, and within student dormitories in Ljubljana, we obtained a randomness of the sample. The purpose of the survey was to collect data on new psychoactive substances, for which there are very few relevant data in the Republic of Slovenia, on both their use and visibility.

In addition to demographic questions (gender, age, place of permanent and temporary residence, faculty and study year), questions were included on knowledge of the term NPS, the frequency of combining NPS with alcohol, classical drugs and prescription drugs, knowledge and use of new drugs from the group of synthetic cannabinoids, cathinones and other representatives of psychoactive substances, as well as the age at first contact with a certain drug, and the time interval of use, drug supply, personal opinions of users about their effects and net earnings/money to buy drugs and alcohol.

Five hundred and sixteen responses were relevant for the analysis, of which 339 students (out of the 516 survey participants, so 65.7%) recognized at least one of the mentioned synthetic cannabinoids and 69 students (13.4%) had used at least one of the substances before, at least once. The visibility of individual synthetic cannabinoids in the survey was on average 5.4 %. In assessing each of the respondents' own knowledge about the risk of using synthetic cannabinoids, which the respondent expressed on the basis of a numerical scale from 1 to 5, where 1 represented a lack of familiarity with information on the dangers of SK, 34 % selected the lowest value and only 5 % of them thought they were very well informed (value 5). The average was 2.3. It can be concluded from this data that general information about this type of new psychoactive substances is poor. It is therefore important that young people are made aware of the dangers of using this type of drug, through various campaigns and workshops.

Key words: synthetic cannabinoids, new psychoactive drugs, abuse

Primeri zastrupitev s sintetičnimi kanabinoidi v kliničnem laboratoriju

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Kmalu po prvi identifikaciji sintetičnih kanabinoidov v izdelkih za rekreativno uporabo pred približno desetletjem so se kliniki in laboratoriji soočili s problematiko prepoznavanja uživanja teh psihoaktivnih snovi oz. njihove identifikacije v kliničnem okolju. Za razliko od tradicionalnih drog je klinična prezentacija pri zlorabi oz. predoziranju s sintetičnimi kanabinoidi raznolika in velikokrat nepredvidljiva, predvsem kadar je pri posamezniku že prisotna npr. neka psihiatrična motnja.

Obstoječi presejalni testi (na kanabis) za dokazovanje prisotnosti sintetičnih kanabinoidov v bioloških vzorcih niso ustrezni, razvoj in uporabnost novih testov za rutinsko uporabo pa omejuje veliko število strukturno različnih predstavnikov znotraj te skupine novih psihoaktivnih snovi ter dejstvo, da je v biološkem materialu zaradi večje jakosti delovanja prisotna zelo nizka koncentracija osnovne substance, ki je velikokrat podvržena tudi intenzivnemu metabolizmu in ima zelo kratko razpolovno dobo. Vse našteto prav tako močno otežuje njihovo določanje v bioloških vzorcih s pomočjo kromatografskih tehnik, sklopljenih z masno spektrometrijo, ki sicer predstavljajo potrditvene metode za identifikacijo in/ali kvantifikacijo teh substanc. Zaradi pojavljanja vedno novih spojin predstavljajo sintetični kanabinoidi z analitičnega vidika velik izziv tudi zaradi časovnega zamika med njihovih vstopom na trg rekreativnih drog in razpoložljivostjo ustreznega referenčnega materiala/standardov oz. masnih spektrov, ki so osnova za njihovo določanje z omenjenimi metodami. Podobno velja tudi za podatke glede optimizacije teh analiznih metod.

Upoštevaje vse našteto je v kliničnem okolju nujna dobra komunikacija in sodelovanje z oddelki, saj je laboratoriju pri analizi lahko v veliko pomoč poznavanje okoliščin zastrupitve oz. informacija glede zaužitih substanc ali pa analiza samih produktov, ki jih je posameznik zaužil, v kolikor je našteto mogoče pridobiti.

Ključne besede: sintetični kanabinoidi, nove psihoaktivne snovi, klinični laboratorij, zastrupitev, masna spektrometrija

Synthetic cannabinoid intoxication: Case reports in clinical laboratory analysis

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Soon after the first positive identification of synthetic cannabinoids in products for recreational use about a decade ago, clinicians and laboratories were faced with a challenge of how to recognize abuse of these psychoactive substances and their identification in the clinical setting. Unlike with traditional drugs, the clinical presentation of synthetic cannabinoids intoxication can be very diverse and often unpredictable, especially when someone already suffers from a medical condition (e.g., a psychiatric condition).

Synthetic cannabinoids are typically not identified by screening (marijuana) drug tests. The development and usability of new tests for their routine testing is limited by the vast number of structurally unrelated compounds represented within this group of new psychoactive substances. Furthermore, due to their high potency, the concentration of the parent compound in biological specimens is usually very low, and since they are intensively metabolized, their half-life is also very short. Hyphenated techniques (chromatography combined with mass spectrometry) are used for confirmation of their identity and/or quantification. However, the use of these is also hampered by the above-mentioned factors. The constant inflow of new compounds onto illicit drug markets and the delay to when reference materials and mass spectra for these compounds are available are additional challenges for their analysis. Similarly, data regarding optimization of the methods used are usually not readily available.

Taking all of this into account, good communication between clinicians and laboratories is essential, since the circumstances of the intoxication, information on the consumed compounds and analysis of the products involved, if available, can be of great assistance to the laboratory.

Keywords: synthetic cannabinoids, new psychoactive substances, clinical laboratory, intoxication, mass spectrometry

POVZETKI VABLJENIH PREDAVANJ

Kanabinoidi in zakonodaja

Kako je regulirana uporaba kanabinoidov?

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Kanabinoidi so raznolika skupina snovi, ki se vežejo na kanabinoidne receptorje v telesu in možganih ter povzročijo učinke podobne tistim, ki jih povzroči rastlina *Cannabis Sativa* L. Pogosto jih delimo v (i) endokanabinoide, ki so naravni endogeni ligandi in nastajajo v človeškem in živalskem organizmu; (ii) sintezne kanabinoide, ki se izdelujejo v laboratoriju, npr. dronabinol (sintezni tetrahidrokanabinol) ali nabilon, ki sta učinkovini različnih zdravil; (iii) fitokanabinoide, ki se v naravi nahajajo v rastlini *Cannabis Sativa* L. pa tudi v nekaterih drugih rastlinah. Medtem ko je regulacija zdravil s sinteznimi kanabinoidi v osnovi enaka kot za ostala zdravila, pa zakonodaja o fitokanabinoidih ni tako premočrtna.

Mednarodno pravo ne preprečuje uporabe fitokanabinoidov ali konoplje za zdravljenje določenih indikacij. V skladu s konvencijami Združenih narodov je potrebno uporabo drog pod mednarodnim nadzorom omejiti na »medicinske in znanstvene namene«. Če se država odloči, da bo dovolila gojenje kanabisa za medicinske namene, 28. člen Konvencije iz leta 1961 (Enotna konvencija o mamilih, dopolnjena leta 1972) določa sistem zahtevanih kontrol, po drugi strani pa Konvencija o psihotropnih snoveh iz leta 1971 in Konvencija proti nezakonitemu prometu z narkotičnimi in psihotropnimi snovmi iz leta 1988 nadzorujeta promet s tetrahidrokanabinolom.

V nekaterih državah EU imajo dovoljenje za promet zdravila s sintezno pridobljenim tetrahidrokanabinolom in nabilonom v kapsulah ter standardiziranim ekstraktom konoplje v oralnem pršilu. Nekaj držav članic EU dovoljuje uporabo cvetov konoplje in pripravkov iz njih v obliki magistralnih zdravil. Nobena država pa ne dovoljuje kajenja konoplje za medicinske namene.
V Sloveniji Uredba o ravrstitvi prepovedanih drog za uporabo v medicinske namene dovoljuje tetrahidrokanabinol, nabilon in konopljo.

Ključne besede: kanabinoidi, tetrahidrokanabinol, zakonodaja, medicinska uporaba

How is the use of cannabinoids regulated?

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Cannabinoids are a varied group of substances that bind the cannabinoid receptors of the body and brain and have similar effects to those produced by the plant *Cannabis Sativa* L. They are often classified as (i) endocannabinoids, which are natural endogenous ligands produced by human and animal organisms; (ii) synthetic cannabinoids that are created in the laboratory, e.g., dronabinol (synthetic tetrahydrocannabinol) or nabilone, which are the active compounds of various medicinal products; (iii) phytocannabinoids, which only appear in nature in the plant *Cannabis Sativa* L. and some other plants. While the regulation of synthetic cannabinoids in medicinal products is basically the same as for other medicinal products, the legislation for phytocannabinoids is not so straightforward.

International law does not prevent phytocannabinoids or cannabis being used as a medicine to treat defined indications. According to UN conventions, drugs under international control should be limited to »medical purposes«. Article 28 of the 1961 Convention (Single Convention on Narcotic Drugs, amended in 1972), describes the system of controls required if a country decides to permit the cultivation of cannabis for medical and scientific purposes, while the 1971 Convention on Psychotropic Substances and 1988 Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances, control tetrahydrocannabinol.

In some EU countries, there are authorized medicines with synthetic tetrahydrocannabinol and nabilone in capsules and standardized cannabis extract as a oromucosal spray. Some EU countries allow the use of *Cannabis flos* and its preparations in the form of magistral formulas. In contrast, no country authorizes the smoking of cannabis for medical purposes.

In Slovenia, the Regulation on the classification of illicit drugs allows tetrahydrocannabinol, nabilone and cannabis for medicinal use.

Keywords: cannabinoids, tetrahydrocannabinol, legislation, medicinal use

POVZETKI VABLJENIH PREDAVANJ

Varnost kanabinoidov pri medicinski uporabi

Interakcije kanabinoidov z zdravili

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Čeprav je bilo narejenih zelo malo specifičnih študij o interakcijah med kanabinoidi in drugimi učinkovinami, pa v zadnjem času število znanstvenih objav narašča, kar kaže na aktualnost in pomembnost problematike. Pri hkratnem uživanju pripravkov s THC in/ali CBD ter zdravili na recept lahko pride do medsebojnega delovanja (interakcij) med THC in/ali CBD ter učinkovinami. Lahko pride do farmakokinetičnih ali farmakodinamičnih interakcij. Pri farmakokinetičnih interakcijah kanabinoida neposredno vplivata na normalno presnovo druge učinkovine in na njeno koncentracijo v krvi ali obratno. Farmakodinamične interakcije pa se nanašajo predvsem na seštevanje zaviralnih učinkov THC in učinkovin na osrednji živčni sistem, pri učinkovinah, ki zaviralno delujejo na osrednje živčevje (benzodiazepini). THC in CBD sta substrata ali zaviralca citokrom P450 encimskih poti, ki so relevantne za presnovo pogosto predpisanih učinkovin. Obstaja potencial za klinično pomembno zvišanje koncentracij THC pri posameznikih, ki imajo zmanjšano CYP2C9 in/aliCYP3A4 funkcijo ter za klinično pomembno zvišanje koncentracij CBD pri posameznikih z zmanjšano CYP2C19 in/ali CYP3A4 funkcijo. Zaviralci CYP3A4 lahko rahlo zvišajo koncentracije THC, medtem ko induktorji CYP3A4 lahko zmanjšajo koncentracije THC in CBD. CBD zveča koncentracije klobazama pri otrocih zdravljenih za epilepsijo. THC je induktor CYP1A2, medtem ko je CBD zaviralec CYP3A4 in CYP2D6, kar lahko vpliva na presnovo številnih učinkovin. Pacienti bi morali biti seznanjeni, da istočasna izpostavljenost THC in/ali CBD z drugimi učinkovinami, lahko vodi do interakcij, ki imajo lahko vpliv na učinkovitost in varnost zdravljenja. Pri standardiziranem zdravilu s kanabisom (Sativex) so uspeli dokazati, da so koncentracije THC in CBD v krvi prenizke, da bi prišlo do relevantnih interakcij z drugimi učinkovinami. Pri višjih plazemskih koncentracijah CBD (kot je Epidiolex) so bile nekatere interakcije potrjene. V realnosti so interakcije med kanabinoidi in učinkovinami kompleksne in obstaja še veliko lukenj v razumevanju in klinični pomembnosti interakcij med kanabinoidi ter drugimi učinkovinami. Trenutno lahko podamo le nespecifična priporočila o previdnosti, vendar bolj specifičnih priporočil o klinično pomembnih interakcijah trenutno ni mogoče podati na osnovi podatkov, ki jih imamo.

Ključne besede: interakcije med THC in/ali CBD ter drugimi učinkovinami, farmakokinetične interakcije, farmakodinamične interakcije

Drug interactions of cannabinoids

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Many articles have covered this topic recently, which is good for awareness. Interactions between ethanol and drugs can occur with the concurrent use of ethanol and medicinal products. Concomitant use of THC and/or CBD and prescription drugs can result in pharmacokinetic or pharmacodynamic interactions. Pharmacokinetic interactions occur when ethanol directly affects the normal metabolism of the drug and its concentration in the blood. Pharmacodynamic interactions are primarily the result of additive effects of ethanol and drugs on the central nervous system. There is therefore a risk of excessive sedation when using drugs that act depressively on the central nervous system. THC and CBD are substrates and inhibitors of cytochrome P450 enzymatic pathways relevant to the biotransformation of commonly prescribed psychotropic agents. There is the potential for clinically meaningful elevations in Δ9-THC exposure in individuals with diminished CYP2C9 and/or CYP3A4 function, and in CBD exposure in individuals with diminished CYP2C19 and/or CYP3A4 function. CYP3A4 inhibitors slightly increase THC levels. CYP3A4 inducers slightly decrease THC and CBD levels. CBD increased clobazam levels in children treated with CBD for epilepsy. THC is a CYP1A2 inducer while CBD is a potent inhibitor of CYP3A4 and CYP2D6. Patients should be informed that the consumption of THC and/or CBD may result in interactions that are of consequence to the expected probability of the efficacy, tolerability and/or safety of their treatment. The company behind Sativex (a standardized cannabis extract medicine approved in some countries) was able to argue successfully that the plasma THC and CBD concentrations from their product were too low to cause any drug interactions. However, at higher CBD doses (such as with Epidiolex), some interactions have now been confirmed. In reality, interactions between canabinoids and drugs are often complex and there are some large gaps in knowledge. Non-specific statements that caution is warranted should indeed be expected, but more specific directives at this point would be more inferential than data driven.

Keywords: interactions between THC and/or CBD and prescription drugs, pharmacokinetic interactions, pharmacodynamic interactions

Toksični učinki kanabinoidov v srčno-žilnem sistemu

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Kanabinoidi so ena najbolj razširjenih prepovedanih skupin drog in najbolj priljubljenih zaradi legalizacije ter dekriminalizacije. Kljub široki uporabi se pojavljajo novi dokazi o škodljivih učinkih na zdravje srčno-žilnega in možgansko-žilnega sistema zaradi vseprisotne uporabe kanabinoidov. Z njimi so povezane ishemične in hemoragične kapi ter ostali spremljajoči zdravstveni zapleti, kot so hipertenzija in interakcije med zdravili.

Redni uporabniki kanabinoidov imajo višjo stopnjo kapi ali prehodnega ishemičnega napada kot ne-uporabniki. Nenadno srčno smrt in druge žilne dogodke, aritmije in stresne kardiomiopatije so ugotovili tudi pri osebah brez predhodnega srčno-žilnega tveganja. Pri osebah s tipičnimi srčno-žilnimi tveganji ali boleznimi je nevarnost kapi večja.

O hemoragičnih kapeh, povezanih z uporabo psihostimulantov, delujočimi na simpatični sistem, so poročali tudi pri uporabnikih kanabinoidov. Nenadna krvavitev po trombolitični terapiji po ishemični kapi je bila opisana pri bolnici s prekomernim uživanjem kanabinoidov. Med terapijo z aktivatorji plazminogena je bolnica izgubila možganske funkcije in kasneje so možgani izgubili vso odzivnost. Nekateri avtorji so poročali o več sočasnih možgansko-žilnih stenozah pri mlajših uporabnikih. Eden od predlaganih mehanizmov za to patologijo je prekomerna proizvodnja reaktivnih kisikovih vrst zaradi kanabinoidov, ki poruši oksidacijsko-redoks ravnotežje in vodi do oksidativne stresne kapi. Citokrom CYP2J2, primarni CYP v kardiomiocitih, je odgovoren za presnovo kanabinoidov v kardioprotektivne epokside, ki pri rednih uporabnikih ne nastanejo.

Toksično delovanje Δ^9-tetrahidrokanabinola je pripisana porušenemu delovanju endo-kanabinoidnega in avtonomnega živčnega sistema, receptorskim in nereceptorsko posredovanim potem. Kanabinoidi lahko vplivajo na možgansko avtoregulacijo, endotelijsko disfunkcijo in žilni tonus, kar vodi do vazokonstrikcije in akutne ishemične kapi. Poleg tega se lahko učinki konoplje povečajo ali zmanjšajo s sočasno uporabo drugih zdravil ali prepovedanih drog.

Ključne besede: prekomerno uživanje kanabinoidov, hipertenzija, tahikardija, miokardni infarkt, ishemična kap

The toxic effects of cannabinoids on the cardiovascular system

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Cannabinoids are among the most widespread illicit drugs and have recently become the focus of attention for their legalization and decriminalization. Despite being widely used, there is new evidence of adverse health effects on the cardiovascular and cerebrovascular systems from heavy usage of cannabinoids. Ischemic and haemorrhagic strokes are associated with it, as well as related accompanying health risks such as hypertension and drug interactions.

Heavy cannabinoid users have a higher rate of non-fatal stroke or transient ischemic attack than non-cannabis users. Sudden cardiac death and other vascular events, arrhythmias and stress cardiomyopathy are found even in subjects with no previous history of cardiovascular risk. Individuals with typical cardiovascular risks or disease are at much higher risk.

Haemorrhagic strokes, which are often linked to the use of psychostimulants affecting the sympathetic system, have also been reported in cannabinoid users. A rapid development of haemorrhage following thrombolytic therapy has been described in a patient with heavy cannabinoids use history after an ischemic stroke. During plasminogen activator therapy, the patient lost brain functions and was later declared brain dead. Several authors have reported cerebrovascular stenosis in younger users. One of the proposed mechanisms for this pathology is considered an overproduction of reactive oxygen species due to cannabinoids disrupting the oxidative-redox balance, leading to an oxidative stress related stroke. Cytochrome CYP2J2, the primary CYP in cardiomyocytes, is responsible for the metabolism of cannabinoids into cardioprotective epoxides, a mechanism that is disturbed in heavy cannabinoids users.

Toxic interaction of Δ^9-tetrahydrocannabinol is attributed to the endo-cannabinoid and autonomic system, receptor and non-receptor mediated pathways. Cannabinoids can affect cerebral auto-regulation, endothelial dysfunction and vascular tone, leading to vasoconstriction and acute ischemic stroke. Moreover, the effects of cannabinoids may be enhanced or deprived by concomitant use of medications or other illicit drugs.

Keywords: cannabinoids, hypertension, tachycardia, myocardial infarction, ischemic stroke.

Izkušnje glede uporabe kanabinoidov pri zdravljenju trdovratnih epilepsij otrok

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Epilepsija (sinonim tudi božjast) prizadene približno en odstotek prebivalstva po vsem svetu. Otroška epilepsija, ki se pojavi pred tretjim letom starosti in ima trdovratne epileptične napade (napadi odporni na najmanj dve protiepileptični zdravili – PEZ), je lahko v mnogih primerih povezana z zmanjšanim inteligenčnim količnikom v poznejšem otroškem in mladostniškem obdobju. Taka epilepsija ne botruje zgolj zmanjšanju kognitivnih funkcij pač pa tudi pogostim vedenjskim in psihiatričnim motnjam, ki se lahko pokažejo šele v mladostniškem obdobju. Zato so v tem zgodnjem in tako ranljivem obdobju otroštva (sprva zlasti glede gibalnega razvoja samega, nato pa predvsem razvoja socialnih in mentalnih veščin) tako pomembni zgodnja prepoznava, pravilno ukrepanje in zdravljenje epileptičnih napadov, ki so odporni na različna PEZ (farmakorezistentni). Končni cilj celotne obravnave otroške epilepsije mora zato vselej biti popolna odsotnost epileptičnih napadov, oziroma čim boljša kontrola epileptičnih napadov. Pri tem pa nas tudi tedaj, ko kot otroški nevrologi menimo, da je možnost ozdravitve majhna, takšno stališče ne sme privesti do tega, da ne poskusimo uporabiti prav vseh načinov zdravljenja, ki so danes na voljo. Z zdravljenjem s kanabidiolom (CBD), ne-psihotropno substanco v konoplji, smo začeli v začetku leta 2015, in sicer smo uvozili v ta namen sintetični produkt, kasneje pa ga zamenjali z naravnim. Klinična študija (odobritev Komisije za medicinsko etiko) sicer ni imela primerjalne skupine, vendar je bil njen poglavitni namen določiti pogostnost epileptičnih napadov med uvajanjem CBD in po njegovi uvedbi v primerjavi s pogostnostjo epileptičnih napadov pred vstopom v raziskavo, določiti tisti odmerek CBD, ki ga bodo otroci dobro prenašali in bo učinkovit (vsaj 50 % zmanjšanje pogostnosti epileptičnih napadov) ter spremljanje morebitnih nezaželenih učinkov. Rezultati so bili naslednji in zelo primerljivi z drugimi podobnimi študijami: od 66 otrok, jih je 32 (48,5 %) imelo več kot 50-odstotno zmanjšanje napadov in od teh je bilo 14 (21,2 %) povsem brez napadov, pri 15 otrocih (22,7 %) pa ni bilo učinka. Samo pri petih otrocih smo zabeležili blage neželene učinke. CBD ima potencialne učinke v dopolnilnem zdravljenju trdovratnih epilepsij pri otrocih.

Ključne besede: otroška epilepsija, konoplja, kanabnoidi

Experiences of cannabinoids use in resistant childhood epilepsies

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Epilepsy is a condition that affects about one percent of the worldwide population. Childhood epilepsy that occurs before the age of three and involves persistent seizures (seizures resistant to at least two antiepileptic drugs - AED), can be associated in many cases with reduced IQ in later childhood and adolescence. Such epilepsy not only causes a reduction of cognitive functions but is also frequently associated with behavioral and psychiatric disorders, which can also first appear in adolescence. In this early and vulnerable childhood period (initially in particular in regard to motor development itself, and later especially considering the development of social and mental skills) early recognition is therefore very important, followed by correct action and proper treatment of epileptic seizures that are resistant to AED (pharmacoresistant). The ultimate goal of the entire treatment of childhood epilepsy must therefore always be the complete absence of seizures, or good control of seizures. Even in cases when we pediatric neurologists think that the possibility of cure is small, such a position must not discourage us from trying all the treatments that are available today. Our study of the use of cannabidiol (CBD) started at the beginning of 2015, with the approval of the National Ethics Board. The study was not a randomized trial but we were interested in seeing the efficacy of CBD, adapting the correct dosage and monitoring the side-effects. Sixty-six children were included in the analysis, 32 (48.5%) had more than 50% improvement in terms of seizure burden, 14 (21.2%) became seizure-free. CBD had no effect in 15 (22.7%), adverse effects were reported in 5/66 children. CBD has potential benefits as add-on therapy for refractory childhood epilepsies.

Keywords: childhood epilepsy, cannabis, cannabinoids

Razpoložljivost in priporočila za uporabo kanabinoidov za zdravljenje bolečine v Evropi

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Konoplja in njeni ekstrakti se od pamtiveka uporabljajo za zdravstvene težave in rekreacijo. Slednje je pripeljalo do zakonskih omejitev in je vplivalo na razpoložljivost ter prisotnost zdravil iz konoplje v medicini. V zadnjih desetletjih se krepi pritisk javnosti za legalizacijo uporabe konoplje in kanabinoidov, kar je pripeljalo do uporabe teh učinkovin za številna medicinska stanja, trenutno najpogosteje kot del zdravljenja bolečine in mišičnih spazmov pri multipli sklerozi in simptomov bolezni v paliativni oskrbi. V številnih evropskih deželah se kanabinoidi in medicinska konoplja uporabljajo kot uradna zdravila na osnovi spremenjene zakonodaje o uporabi konoplje.

Nasproti splošnemu mnenju o zdravilnem učinku sestavin konoplje na številne bolezni je o tem zelo malo znanstveno utemeljenih podatkov ali sploh niso dokazani. Ob tem pa se premalo govori in ve koliko škode takšno zdravljenje lahko povzroči.

Še so prisotne negotovosti in nasprotja o vlogi ter pravilni uporabi konoplje in kanabinoidov za lajšanje kronične bolečine. Na osnovi do sedaj dostopnih podatkov je skupina evropskih strokovnjakov objavila priporočila za uporabo zdravil na bazi konoplje. Priporoča se, da ta zdravila lahko svetuje izkušen zdravnik kot del celostnega zdravljenja in prvenstveno kot dodatek, če osnovna priporočana zdravila niso zadostno učinkovita ali povzročajo neobvladljive neželene učinke. Na osnovi do sedaj utemeljenih podatkov je razumno pretehtati o uporabi kanabinoidov za lajšanje kronične nevropatske bolečine. Za vse druge kronične bolečine se kanabinoidi lahko priporočajo le kot individualni terapevtski preizkus. Cilji zdravljenja morajo biti realno zastavljeni , bolniki pa morajo biti pod strogim kliničnim nadzorom. Zdravljenje se prekine če ni učinkovito in dodatno obremenjuje bolnika ali ga spremljajo neželeni učinki in znaki zasvojenosti ter nepravilne uporabe zdravila.

Trenutno stanje utemeljenosti uporabe kanabinoidov za kronično bolečino v Evropi ni zadostno. Naraščanje števila evropskih držav, ki legalizirajo zdravila na bazi konoplje za medicinsko uporabo podaja možnosti nadaljnjega raziskovanja kanabinoidov in vrednotenja kliničnih izkušenj kar bo omogočilo boljšo utemeljenost ter posodabljanje priporočil za medicinsko prakso.

Ključne besede: kanabinoidi, razpoložljivost, zdravljenje bolečine, priporočila

Availability and position paper on appropriate use of cannabinoids for pain management in Europe

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Cannabis and its extracts have long been used for both health problems and recreation. The last led to the prohibition of the herb and affected the availability and presence of cannabis-based medicines in healthcare. In recent decades, there has been strengthened public pressure for the legalization of cannabis and cannabinoids, which has enabled their use in a number of medical conditions, currently most commonly as a part of pain and muscle spasms alleviation in MS, and symptom control in palliative care. In some European countries, legislation has allowed the official use of cannabinoids and medical cannabis in medicine.

In contrast to the public view of the efficacy of cannabis and cannabinoids for many medical diseases, the evidence is weak or negative. In addition, there is insufficient information about the harm of such treatment.

There are uncertainties and controversies on the role and appropriate use of cannabis and cannabinoids for the management of pain. A European group of experts has formulated recommendations for the appropriate use of cannabis-based medicines on the basis of a review of literature and clinical experience. Therapy with cannabis–based medicines should only be considered by experienced clinicians as part of multidisciplinary treatment, and preferably as adjunctive medication if guideline-recommended first- and second-line therapies have not provided sufficient efficacy or tolerability. The quantity and quality of evidence are such that cannabis-based medicines may be reasonably considered for chronic neuropathic pain. For all other chronic pain conditions, the use of cannabis-based medicines should be regarded as an individual therapeutic trial. Realistic goals of therapy must be defined. All patients must be kept under close clinical surveillance. As with any other medical therapy, if the treatment fails to achieve the predefined goals and/or the patient is additionally burdened by an unacceptable level of adverse effects and/or there are signs of abuse or misuse of the drug by the patient, therapy with cannabis-based medicines should be terminated.

The current status of evidence and use of cannabis-based medicines for chronic pain in Europe is insufficient. The increase in the number of countries that have moved towards authorization of medical cannabis and cannabis-based medicines for chronic pain will afford the opportunity for research and analysis of clinical experience and provide better evidence and an update of recommendations for clinical practice.

Keywords: cannabinoids, availability, pain treatment, recommendations

Kanabinoidi v onkologiji

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Učinke konoplje se je v medicinske namene uporabljalo že pred več tisoč leti. Njena uvrstitev na seznam prepovedanih drog je raziskovanje njene uporabe v medicini prehodno upočasnilo, v zadnjih letih pa ponovno opažamo večje zanimanje. Zato danes beležimo porast v številu raziskav in tudi v obsegu novih dognanj na temu področju. Konoplja vsebuje preko 100 različnih kanabinoidov in drugih aktivnih substanc. Najbolj raziskana kanabinoida sta tetrahidrokanabinol (THC) in kanabidiol (CBD). Njuno delovanje se izraža preko več kanabinoidnih receptorjev. Človekovo telo tudi samo po sebi proizvaja endokanabinoide, ki prav tako delujejo preko istih receptorjev. Receptorji CB1 se nahajajo večinoma v možganih in vplivajo predvsem na njihovo delovanje, receptorje CB2 pa najdemo večinoma na celicah imunskega sistema in sodelujejo pri modulaciji imunskega odziva. V onkolgiji najpogosteje uporabljamo pripravke s kombinacijo kanabinoidov THC in CBD. S takimi pripravki izkoriščamo dobre lastnosti obeh komponent in medsebojno delovanje. THC lajša nekatere simptome raka oziroma stranske učinke specifičnega zdravljenja, CBD pa deluje protivnetno in blaži psihotropne učinke THC. Uradne, strokovno priznane, indikacije za predpis kanabinoidov v onkolgiji so: s standardnimi zdravili slabo nadzorovana slabost, inapetenca in kompleksna bolečina. Kanabinoidov z namenom zdravljenja raka se v redni praksi ne uporablja, ker zaenkrat ni ustreznih dokazov o taki učinkovitosti. Zdravljenje s kanabinoidi mora potekati pod strokovnim nadzorom, saj se kot pri vsakem zdravilu, tudi ob uporabi kanabinoidov pojavljajo stranski učinki in zapleti zaradi interakcij. Odmerek je potrebno strokovno titirati do želenega učinka in ga prilagoditi metabolizmu posameznika. Glede na dosedanje dokaze raziskav je danes uporaba kanabinoidov v onkologiji v vsakodnevni praksi zelo jasna. Ostajajo pa nekatera vprašanja, ki bi potrebovale dodatne dobro zasnovane razsikave. Rezultat raziskav na področju uporabe kanabinoidov onkologi redno spremljamo.V primeru novih odkritij in dokazov bomo sproti prilagajali našo vsakdanjo prakso.

Ključne besede: kanabinoidi, onkologija, slabost, inapetenca, bolečina

Cannabinoids in oncology

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Cannabis has been used for thousands of years for medical purposes. Since its classification as a prohibited drug, research into it and its use in medicine have been slowed down, but increased interest can be observed in recent years. An increase in the amount of research and number of new discoveries in the field can be seen. Cannabis contains over 100 different cannabinoids and other active substances. The most researched cannabinoids are tetrahydrocannabinol (THC) and cannabidiol (CBD). Their action is expressed through cannabinoid receptors. The human body also produces endocannabinoids, which act via the same receptors. CB1 receptors are found mostly in the brain and primarily affect its functioning. CB2 receptors are mainly found in cells of the immune system and are involved in the modulation of the immune response. Oncology generally uses a combination of the cannabinoids THC and CBD, thus taking advantage of the good properties of both components and their interaction. THC is known to alleviate some of the symptoms of cancer or some of the side effects of specific treatments, while CBD has an anti-inflammatory effect and eases the psychotropic effects of THC. Official indications for cannabinoids use in oncology include: poorly controlled nausea, anorexia and complex pain despite standard treatment. Cannabinoids for the treatment of cancer are not used in regular practice because there is currently no relevant evidence of their efficacy. Treatment with cannabinoids should be carried out under expert supervision since, as with any medical product, including cannabinoids, side effects and interaction complications occur. The dose should be carefully titrated to the desired effect and adjusted to the metabolism of the individual. According to all research completed to date, the present use of cannabinoids in oncology in everyday practice is fairly clear. However, there are some questions that still need additional clarification and well-designed research needs to be carried out. Oncologists regularly monitor the results of such research. In the case of new discoveries and strong evidence, we will adjust our daily practice accordingly.

Keywords: cannabinoids, oncology, nausea, anorexia, pain

POVZETKI POSTERJEV

In silico toxicological and pharmacokinetic profiling of cannabinoids

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The use of cannabinoids for medical and recreational purposes has significantly increased in recent decades. Various medical investigations are therefore in progress in the search for cannabinoids that could be used as therapeutics in various diseases. On the other hand, it is of real concern that most synthetic cannabinoids are continuously produced as designer drugs and the toxic and other side effects of most of them are even not evaluated. Using *in silico* approaches, virtual profiling for many toxicological and pharmacokinetic effects can be adequately implemented. Predictions for 120 cannabinoids were made with quantitative structure-activity relationship (QSAR) models, based on their 2D chemical structures. The dataset included 70 natural and 50 synthetic cannabinoids. Several models for prediction of various health and environmental toxicological endpoints available online were used for toxicological profiling. In addition, pharmacokinetic profiling was performed based on QSAR model predictions of the inhibition of major membrane transporters. Altogether, predictions with over twenty models were made for each cannabinoid. The results were carefully analysed and discussed. The compendium of predictive QSAR models used in this study can be implemented in virtual profiling of various drug candidates or food supplements.

Keywords: natural and synthetic cannabinoids, QSAR models, membrane transporters, toxicological endpoints, virtual drug profiling

Masnospektrometrična analiza kanabinoidov v izdelkih iz konoplje

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Kanabinoidi v industrijski konoplji se veliko uporablajo pri zdravljenju bolečine, multiple skleroze, pediatrične epilepsije, kapi in predvsem pri onkoloških bolnikih. V konoplji je bilo poleg osnovnih tetrahidrokanabinol (THC), kanabidiol (CBD) in kanabinol (CBN) določeno že več kot 80 kanabinoidov, večinoma v nizkih koncentracijah. Pri kemijski analizi izdelkov iz konoplje smo za spektrometrično karakterizacijo kanabinoidov uporabili tekočinsko kromatografijo v povezavi z masno spektrometrijo (LC-MS). Razvili smo UPLC-MS metodo za hitro ločevanje, identifikacijo in kvantifikacijo kanabinoidov v izdelkih iz konoplje. Določanje kanabinoidov in razmerje THC/CBD smo izvedli z uporabo tandemskega masnega spektrometra visoke ločljivosti Q-Tof Premier (Waters). Na ta način smo lahko identificirali in določili strukturo tudi drugih kanabinoidov ter razgradnih produktov v rastlinskem materialu konoplje, npr. THC in CBD kisline (THCA in CBDA), ki so termično labilne in se pretvorijo v THC ali CBD; kanabikromen (CBC) in kanabigenol (CBG) ipd. Poznavanje razmerja THC/CBD je pomembno za zdravnike pri predpisovanju izdelkov iz konoplje. Za medicinsko konopljo je značilna visoka koncentracija CBD in nizke koncentracije THC. Terapevtski CBD je zaželen zaradi njegovega zdravilnega učinka, medtem ko je potrebno prisotnost psihoaktivnega THC pri pediatričnih bolnikih preprečiti.

Ključne besede: določanje kanabinoidov, masna spektrometrija, medicinska uporaba izdelkov iz konoplje

Mass spectrometric analysis of cannabinoids in hemp products

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Cannabinoids in industrial hemp show potential for the treatment of pain, multiple sclerosis, pediatric pharmacoresistant epilepsy and for stroke. Over 40 cannabinoids have been identified, most in trace amounts, with the primary cannabinoids (which determine potency) being tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). In this study, liquid chromatography coupled with mass spectrometry (LC-MS) was used for the spectrometric characterisation of cannabinoids. An UPLC-MS method was developed for their fast separation, identification and quantification. Determination of cannabinoids and the THC/CBD ratio was performed using a high-resolution tandem mass spectrometer Q-Tof Premier (Waters). This was further used to identify and elucidate the structure of other cannabinoids and degradation products in cannabis plant material, e.g., THC acid (THCA) and CBD acid (CBDA), which are thermally labile and convert to THC or CBD, respectively; cannabichromene (CBC), and cannabigerol (CBG). Knowing the THC/CBD ratio is of importance to medical practitioners when prescribing cannabis products. Medical hemp is characterized by having high-levels of CBD and low-levels of THC. The therapeutic CBD is desirable for its medicinal effect while psychoactive THC must be avoided for child patients.

Keywords: determination of cannabinoids, mass spectrometry, medical effects of hemp products

Detekcija kanabinoidnih receptorjev v celičnih linijah raka dojke z metodo točkovnega nanosa

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Uvod: Večina oblik raka dojke je pozitivnih za estrogenski receptor (ER). Uspešno je zdravljenje s tamoksifenom, ki z vezavo na hormonski receptor inhibira od estrogena odvisno transkripcijo genov. Zadnje študije so pokazale, da so poleg hormonskih receptorjev v patologijo številnih bolezenskih stanjih vpleteni tudi endokanabinoidi [1]. Endokanabinoidni receptorji so z G-proteinom sklopljeni receptorji, izraženi v centralnem živčnem sistemu (receptor tipa 1, CB1) in v celicah imunskega sistema (receptor tipa 2, CB2). Metaboliti in izomeri tamoksifena lahko delujejo kot inverzni agonisti CB in tako povzročajo toksičnost po od ER-neodvisni poti [2, 3]. Povezanost med zdravljenjem s tamoksifenskim in endokanabinoidnim sistemom bo v prihodnje tema številnih raziskav. Cilj naše študije je opredeliti raven izražanja CB1, CB2 in ERα v izbranih celičnih linijah raka dojke.
Metode: Podatki o izraženosti CB so bili pridobljeni s pregledom literature na PubMedu in podatkovne zbirke Cancer Cell Line Encyclopedia. Celokupni proteini so bili izolirani iz različnih celičnih linij raka dojke. Specifičnost primarnih in sekundarnih protiteles je bila preverjena na celičnih linijah človeškega izvora MCF7 in MDA-MB361 z metodo točkovnega nanosa.
Rezultati in zaključki: Specifičnost protiteles proti CB1 in ERα je bila zadovoljiva v širokem območju koncentracij. Pokazali smo, da so protitelesa proti CB1 visoko nespecifična. Pomanjkljiva specifičnost in učinkovitost predstavljata pogosto težavo pri komercialno dostopnih protitelesih. Izražanje CB1 receptorja bo ponovno testiran z uporabo novega primarnega protitelesa in ustreznih kontrol (pozitivnih in negativnih).
V nadaljevanju bodo protitelesa proti CB1, CB2 in ERα uporabljena za določanje izraženosti receptorjev v različnih celičnih linijah raka dojke in kontrolnih celičnih linijah z metodo prenosa Western in imunohistokemijsko metodo.

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Ključne besede: kanabinoidni receptorji, rak dojke, detekcija, metoda točkovnega nanosa

Detection of cannabinoid receptors in breast cancer cell lines

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Introduction: The vast majority of all known breast cancers are estrogen receptor (ER) positive. The best known treatment to-day, tamoxifen, binds to hormone receptors and inhibits estrogen dependent gene transcription. Apart from hormone receptors, recent research has discovered that endocannabinoids are involved in many disease states [1]. The endocannabinoid receptors are G-proteins found in the central nervous system (Type 1 receptor, CB1) and the immune system (Type 2 receptor, CB2). Recent studies have shown that tamoxifen metabolites and isomers display affinity and act as inverse agonists on CB, producing toxicity in an ER-independent mechanism [2, 3]. The interplay of tamoxifen treatment and the endocannabinoid system should carefully be investigated. The aim of our study was to characterize the expression of CB1, CB2 and ERα receptors in selected breast cancer cell lines.
Methods: Receptor expression data was obtained from PubMed and the Cancer Cell Line Encyclopaedia. Total proteins were isolated from several breast cancer cell lines. The specificity of primary and secondary antibodies was tested with the dot-blot method, using human cell line MCF-7 and MDA-MB 361.
Results and conclusions: We obtained satisfactory specificity for CB2 and ERα antibodies over various antibody concentrations. We showed that the CB1 antibody is highly unspecific. Lack of specificity and reproducibility are common problems among commercial antibodies. CB1 receptor expression will be re-tested using a new primary antibody and appropriate controls (positive and negative).
In addition, CB1/CB2/ERα antibodies will be used to determine the expression of receptors in various breast cancer cell lines and controls with the Western blot and immunocytochemistry methods.
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Keywords: cannabinoid receptors, breast cancer, detection, dot blot

Interactions between cannabinoids and anticancer drugs: an example of Δ^9-tetrahydrocannabinol and irinotecan

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The global use of preparations based on *Cannabis sativa*, both prescribed and illicit, is increasing among cancer patients. Generally, drug-drug or herb-drug interactions can result in therapeutic failure and it has been estimated to be the cause of death in approximately 4% of cancer patients. Interactions of cannabinoids with conventional chemotherapeutics are still poorly recognized. Irinotecan (IRI) is an anticancer drug whose use may lead to severe toxicities (predominantly neutropenia and diarrhoea) that require co-medications. The main active constituent of *C. sativa*, Δ^9-tetrahydrocannabinol (THC), possesses a wide range of pharmacologic effects that might be of therapeutic benefit during therapy with IRI but its adverse psychotropic effects, as well as unknown toxic effects, must be borne in mind. Furthermore, there is a complex overlapping between IRI and THC metabolic pathways: both compounds are subject to the first pass hepatic metabolism mediated by the CYP3A4 enzyme; their metabolites are subject to glucuronidation via the UGT1A1 enzyme; the transport of parent compounds and their metabolites depends on the same or related transporters; both compounds are subject to enterohepatic recirculation; both compounds bind to plasma proteins; both compounds affect mitochondrial oxidative phosphorylation and fatty acids metabolism.

We present the results of a pilot study conducted on healthy male Wistar rats as a preliminary assessment of IRI and THC interactions. The experimental schedule included a single intraperitoneal application of IRI (at 100 mg/kg), while THC was administered *per os* repeatedly for 1, 3, and 7 days (at 7 mg/kg). The concomitant use of THC with IRI caused a prominent body and liver weight reduction and affected several clinical chemistry parameters in rats. Enhanced urinary THC excretion in animals treated with THC and IRI was noticed compared to THC alone. THC intake caused a significant synergic enhancement of IRI genotoxicity and oxidative stress responses.

Keywords: Δ^9-tetrahydrocannabinol, irinotecan, interactions, rats

Vpliv kanabinoidnih substanc na glioblastom z različno izraženimi receptorji CB1 in CB2

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Glioblastom je najpogostejši, najagresivnejši in terapevtsko neodziven primarni možganski tumor pri človeku. Bolniki imajo s to obliko raka povprečno dobo preživetja po operaciji od 12 do 15 mesecev. Slaba prognoza bolezni, tudi ob konvencionalni obliki zdravljenja, kot je operativni poseg, obsevanje in kemoterapija ne preprečuje napredovanja raka, predvsem zaradi prisotnosti rakavih matičnih celic (GMC). Pomembno je razmišljati o novih načinih zdravljenja, ki so usmerjena v rakave matične celice. Obsežne predklinične raziskave so pokazale, da kanabinoidi, aktivne komponente *Cannabis sative,* sprožijo protitumorski učinek pri različnih vrstah raka. Večina študij je temeljila na uporabi sintetičnih komponent, v večini Δ^9-tetrahidrokanabinola (THC) in kanabidiola (CBD).

V naši študiji smo z imunohistološko/imunocitološko metodo določili stopnjo izražanja kanabinoidnih receptorjev CB1 in CB2 na tkivnih rezinah in celicah bolnikov ter z imunofluorescenco njihovo izražanje na GMC. Glede na različno stopnjo vsebnosti receptorjev CB1 in CB2, ki sta visoko izražena na površini GMC, smo proučevali protitumorski učinek komponent konoplje na viabilnost celic s testi MTT in MTS. Glioblastomske celice se dobro odzivajo na tretiranje s pripravki iz konoplje, kjer smo zviševali koncentracijo THC. Še posebej so na preparat odzivne glioblastomske matične celice. Učinek kanabinoidov smo preverili v kombinaciji z obsevanjem in kemoterapijo (temozolomid-TMZ) in pokazali, da je učinek kanabinoidov enak učinku standardne terapije.

Ključne besede: kanabinoidi, glioblastom, glioblastomske matične celice

Influence of cannabinoids on glioblastoma with differentially expressed receptors CB1 and CB2

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Glioblastoma is the most frequent, most aggressive and therapeutically non-responsive primary brain tumor in humans. Patients with this form of cancer have median survival after diagnosis from 12 to 15 months. There is poor disease prognosis, despite conventional treatments such as surgery, radiation and chemotherapy, which do not prevent cancer progression, mainly due to the presence of glioblastoma stem cells (GSC). It is important to consider new ways of treatment targeting cancer stem cells. Extensive pre-clinical studies have shown that cannabinoids, the active components of the hemp plant *Cannabis sativa,* produce antitumor responses in various types of cancer. Most of the studies have been based on the use of synthetic components, mainly Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD).

In the present study, we analysed the expression profile of CB1 and CB2 receptors on glioblastoma tissue sections and patient-derived glioblastoma cells, with immunohistology/immunocytology, and their expression on GSC with immunofluorescence. We studied the anti-tumor effect of cannabinoids on cell viability, depending on the different levels of cannabinoid receptors CB1 and CB2, which are highly expressed on the surface of GSC, using MTT and MTS assays. Glioblastoma cells responded well to treatment with cannabinoids, especially when THC concentrations were increased. The response was even higher on glioblastoma stem cells. The effect of cannabinoids in combination with irradiation and chemotherapy (temozolomide – TMZ) was examined and it was shown to be as effective as using conventional therapy.

Keywords: cannabinoids, glioblastoma, glioblastoma stem cells