

International Association for Cannabis as Medicine

**IACM 5th Conference on
Cannabinoids in Medicine**

**2-3 October 2009
Cologne, Germany**

cannabis
medicille *International*
Association for Cannabis as Medicine

2009 Conference on Cannabinoids in Medicine

Place	Holiday Inn, Dürener Strasse 287, 50935 Cologne, Germany
Registration Fee	200 Euros for both days Students pay a reduced fee of 100 Euros for both days Members of the IACM pay a reduced fee of 150 Euros for both days The registration fees include a copy of the abstract book, daily rates (lunch for both days, coffee during the breaks) and an evening dinner on Friday.
Organizer	IACM Am Mildeweg 6 59602 Rütthen Germany Phone: +49-2952-9708571 Fax: +49-2952-902651 E-mail: info@cannabis-med.org Internet: http://www.cannabis-med.org http://www.icam2009.org
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Please note in your diary:

The **IACM 6th Conference on Cannabinoids in Medicine** together with the European Workshop on Cannabinoids is anticipated for the end of May 2011 in Cologne.

Friday, October 2

08:15 – 08:45 **Registration**

08:45 **Greetings**

09:00 – 10:30 **First Session (Workshop)**

Potential Therapeutic Applications for Cannabinoids I

Chairs: Willy Notcutt, Donald Abrams

09:00 Markus Leweke

Review: State of the art approaches for the development of cannabinoid compounds for the treatment of schizophrenia

09:25 Philip Robson, Geoffrey Guy

Review: Metabolic abnormalities, abnormal stress response and chronic inflammation in schizophrenia - potential targets for cannabinoid medicines?

09:50 Patrick Roser

Review: Anti-depressant effects of cannabinoids

10:15 – 10:30

Discussion

What is the therapeutic potential of cannabis-based medicines in psychiatric disorders?

10:30 – 11:00 **Break**

11:00 – 12:20 **Second session (Human Studies)**

Chairs: Phillip Robson, Markus Leweke

11:00 Mark Ware, Wang T, Shapiro S, Collet JP for the COMPASS study team

Cannabis for the management of pain: assessment of safety study (COMPASS)

11:20 Vincent Maida, Marguerite Ennis, Shiraz Irani, Mario Corbo, Michael Dolzhykov

Effectiveness of adjunctive nabilone in the pain and symptom management of advanced cancer patients: a prospective observational study using propensity scoring

11:40 Donald I. Abrams, P Couey, SB Shade, A Dhruva, ME Kelly, N Benowitz

Cannabinoid:opioid pharmacokinetic interaction in chronic pain

12:00 John Zajicek, Marcus Reif, Martin Schnelle, on behalf of the UK MUSEC Study Investigators

Cannabis extract in the treatment of muscle stiffness and other symptoms in Multiple Sclerosis – results of the MUSEC study

12:20 – 13:45	Lunch	
13:45 – 15:15	Third Session (Workshop)	
	Potential Therapeutic Applications for Cannabinoids II	
	Chairs: Roger Pertwee, Rudolf Brenneisen	
13:45	Manuel Guzman	<u>Review:</u> Recent advances on cannabinoids in glioma
14:05	Raquel Abalo	<u>Review:</u> Effects of cannabinoids on gastrointestinal motility
14:25	Istvan Katona	<u>Review:</u> Breakdown of the synaptic circuit-breaker: endocannabinoid signalling and its impairment in epilepsy
14:45 – 15:15	<u>Discussion</u>	What are promising future indications of cannabinoids in somatic diseases?
15:15 – 15:45	Break	
15:45 – 17:00	IACM General Meeting	
	with IACM Award Ceremony – Part I	
17:00 – 18:00	Poster session	
18:00 – 19:00	Film	
	"Waiting to Inhale" by Jed Riffe	
19:00	Evening Dinner	
	with IACM Award Ceremony – Part II	
	Buffet at the Holiday Inn	

Saturday, October 3

08:45 – 09:15 **Registration**

09:15 – 10:30 First Session (Workshop)

Activating Cannabinoid Receptors in the Clinic

Chairs: Raphael Mechoulam, Andreas Zimmer

09:15	Roger Pertwee	<u>Review:</u> Some potential strategies for targeting cannabinoid receptors in the clinic
09:40	Raphael Mechoulam	<u>Review:</u> Endocannabinoids - the way ahead
10:05 – 10:30	<u>Discussion</u>	What is the best way to activate cannabinoid receptors in the clinic?

10:30 – 11:00 **Break**

11:00 – 12:15 Second session (Reviews)

Chairs: Arno Hazekamp, Manuel Guzman

11:00	Andreas Zimmer, Ildiko Racz, Anna-Lena Klauke, Astrid Markert, Jürg Gertsch	<u>Review:</u> Beta-Caryophyllene: A phyto-cannabinoid acting on CB2 receptors
11:25	Emmanuel Onaivi	<u>Review:</u> Genetic basis of marijuana use
11:50	Javier Fernandez-Ruiz	<u>Review:</u> The neuroprotective potential of cannabinoids in basal ganglia disorders

12:15 – 13:45 **Lunch**

13:45 – 15:05 Third session (Miscellaneous)

Chairs: Emmanuel Onaivi, Kirsten Müller-Vahl

13:45	Ethan Russo	Ancient cannabis from Xinjiang revisited
14:00	Jörg Fachner	Cannabis, music and altered temporality
14:20	Michelle Sexton, Eric	Effects of cannabinoids on human monocyte migration: implications for multiple sclerosis

	Zimmerman, Nephi Stella	
14:35	Rudolf Brenneisen, Pascale Meyer, Haithem Chtioui, Martial Saugy, Carine Schweizer, Matthias Kamber	Smoked cannabis and doping control: looking for the wrong analyte?
14:50 – 15:05	Gianpaolo Grassi, Salvatore Casano, Marco Michelozzi, Valentina Martini, Diaz Kroeze	Effect of salinity in nutrient solution on yield of cannabis growing indoors
15:05 – 15:30	Break	
15:30 – 17:15	Fourth Session (Workshop)	
	Problems Faced by Patients	
	Chairs: Ethan Russo, Mark Ware	
15:30	Ethan Russo	What are the main problems faced by patients who take cannabis-based medicines?
15:50	Franjo Grotenhermen	Side effects: The case of cannabis-associated arteriitis
16:05	Marco van de Velde	Experiences and current status of Dutch Office of Medicinal Cannabis
16:20	Alexandre Jeffrey	The patient perspective (France)
16:35	Kristen N. Peskuski	The patient perspective (USA)
16:50 – 17:15	<u>Discussion</u>	What are the main problems faced by patients who use cannabis or cannabinoids or want to use them?
17:15	End of the Meeting	

Posters

Poster Session on Friday, 17:00 – 18:00

Akihito Watanabe, Yuki Yamasakia, Naoki Amada, Serena Deiana, Tetsuro Kikuchi, Gernot Riedel	Evaluation of the purified cannabigerol (CBG) and extracts rich in CBG in the mouse irwin test
Cathrin Jöpen, Franziska Pahlisch, Heike Endepols, F. Markus Leweke	Behavioural and metabolic effects of chronic cannabidiol and [3-(3-carbamoylphenyl)phenyl] n-cyclohexylcarbamate (urb 597) administration in adult lister hooded rats (<i>rattus norvegicus</i>)
Franziska Pahlisch, Cathrin Joepen, Carola Schaefer, Heike Endepols, F. Markus Leweke	Effects of chronic cannabidiol and urb 597 administration on endocannabinoids and related lipids in different brain regions in adult lister hooded rats (<i>rattus norvegicus</i>)
J. Fishedick, F. Van der Kooy & R. Verpoorte	Quantitative and qualitative analysis of Cannabis sativa smoke and vapor constituents and CB1 binding activity
Naoki Amada, Akihito Watanabea, Yuki Yamasakia, Serena Deianaa, Tetsuro Kikuchi, Gernot Riedel	Effects of cannabigerol purified from cannabis extracts on stereotyped behaviour induced by apomorphine
Salvatore Casano, Giovanni Appendino	Development of Cannabis inbred lines with high proportions of non psychotropic propyl cannabinoids
Salvatore Casano, Roberta Biadolla, Giampaolo Grassi	Cryopreservation of hemp (<i>Cannabis sativa</i> L.) genetic resources
Serena Deiana, Akihito Watanabea, Yuki Yamasakia, Naoki Amadaa, Tetsuro Kikuchi, Gernot Riedel	Effects of cannabis extracts rich in cannabichromene (CBC), cannabidiol (CBD), cannabigerol (CBG) or tetrahydrocannabivarinic acid (THCVA) on locomotor activity in mice
Tara Halpern, Mersiha Mehanovic, Norman Schanz, Robert Benno, Emmanuel Onaivi	Cannabinoid-induced behavioral effects in a mouse model of autism spectrum disorders
Yuki Yamasaki, Naoki Amada, Akihito Watanabe, Serena Deiana, Tetsuro Kikuchi, Gernot Riedel	Effects of tetrahydrocannabivarin (THCV) on conditioned avoidance responding in rats
Zlatko Mehmedic, Suman Chandra, Hemant Lata, Ikhlās A. Khan, Mahmoud A. ElSohly	Effect of light intensity on photosynthetic characteristics of four high Δ^9 -THC yielding varieties of Cannabis sativa
K. Schoedel, N Chen, E Sellers, CG Stott	Abuse Potential of Nabiximols Oromucosal Spray (Sativex®) Compared With Dronabinol and Placebo in Recreational Marijuana Users

Guy GW, Wright S, Stott CG, White L, Russo E

Abuse potential of Sativex® oromucosal spray (nabiximols) compared with dronabinol and placebo in recreational marijuana users

Notcutt W, Davies P, Langford R, Ratcliffe S

Results of a randomised withdrawal study of subjects with spasticity due to multiple sclerosis who were receiving long term Sativex®

Notcutt W, Z Ambler, P Davies, C Gasperini, J Haas, A Klimek & X Montalbán

A two-phase study of Sativex® in the relief of spasticity due to multiple sclerosis: phase a single-blind response assessment followed by phase b double-blind, randomised, placebo-controlled, parallel-group study

Oral Presentations

METABOLIC ABNORMALITIES, ABNORMAL STRESS RESPONSE AND CHRONIC INFLAMMATION IN SCHIZOPHRENIA – POTENTIAL TARGETS FOR CANNABINOID MEDICINES?

Robson PJ^{1,2}, Guy GW²

¹ Oxford University Dept of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK

² GW Pharmaceuticals, Porton Down Science Park, Salisbury SP4 0JQ, UK

Schizophrenia is a devastating mental disorder with a worldwide lifetime prevalence of approximately 1%, and a peak incidence between the ages of 15 – 25 years for males and 25 – 35 for women. It typically manifests through a mixture of positive symptoms (hallucinations, delusions, thought disorder), negative symptoms (loss of motivation, social withdrawal, lack of affect, anhedonia), and cognitive deficits. This diversity has led to calls to move away from a concept of monotherapy and instead to target narrower ranges of symptoms with separate drugs alongside psychological and social interventions.

Schizophrenia is not simply a brain disease. Evidence summarised in this review will demonstrate that in at least a proportion of patients it is also linked to both natural and iatrogenic metabolic abnormalities, hyperadrenalism and an exaggerated HPA response to stress, and chronic systemic inflammation. Alongside the expected emotional, perceptual and behavioural problems, patients may present with obesity, dyslipidaemia, impaired glucose tolerance or diabetes mellitus; symptoms and signs of an abnormal stress reaction; susceptibility to certain infections and haematological evidence of chronic systemic inflammation. Symptomatic diversity may thus be an even bigger challenge than hitherto appreciated.

In recent years much concern has arisen over the possibility that cannabis smoking in adolescence may be a risk factor for schizophrenia in adult life, although this remains a controversial issue. In contrast, considerable interest in the potential role of the non-psychoactive naturally occurring cannabinoid cannabidiol (CBD) as an anti-psychotic medicine has also developed.

The anti-inflammatory and immunomodulatory effects of both THC and CBD are well established. A systematic literature review has suggested the intriguing possibility that habitual cannabis use may protect cognitive function in schizophrenia patients, and CBD has been shown to improve a marker of this in healthy subjects. There are preliminary data to suggest that cannabinoids may have beneficial effects on abnormal stress reaction, metabolic dysfunction and dyslipidaemia. Since the mechanism of action for the anti-psychotic effects of CBD and other cannabinoids almost certainly differs from all existing agents, synergistic combinations with both typical and atypical antipsychotics are a possibility.

Taken overall, these observations lead to the hypothesis that an appropriately formulated medicine containing a combination of selected cannabinoids may have the potential to target all the major components of the schizophrenia syndrome and thereby significantly reduce the need for polypharmacy.

ANTIDEPRESSANT EFFECTS OF CANNABINOIDS

Patrik Roser

Department of Psychiatry, Ruhr-University Bochum, 44791 Bochum, Germany

There is increasing evidence that cannabis modulates affective and emotional regulation. Acute effects of cannabis include feelings of 'high', euphoria and contentment, whereas chronic cannabis use has been found to increase the rates of depressive symptoms. Animal and human experimental studies demonstrated a relationship between the endogenous cannabinoid system and the pathogenesis of major depression. It is suggested that a hypofunction of the endogenous cannabinoid system, particularly in the prefrontal cortex, may be a causal factor for the development of depressive disorders. Central cannabinoid (CB₁) receptor agonists (e.g., HU210, WIN55,212-2), selective inhibitors of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) (e.g., URB597), as well as the endocannabinoid reuptake inhibitor AM404 were found to have antidepressant properties in established animal models of depression, such as the forced swim test or the tail suspension test. As these effects could be blocked by selective CB₁ receptor antagonists (e.g., SR141716), a CB₁ receptor-mediated mechanism may underlie these findings. Moreover, chronic stress revealed a significant reduction of the CB₁ receptor density in the hippocampus and of the concentration of the endocannabinoid 2-arachidonylglycerol (2-AG) in the hippocampus and in the thalamus in rats. Similarly, 2-AG serum concentrations in patients with major depression were significantly reduced compared to healthy controls, and were negatively correlated with the duration of the depressive episodes. On the other hand, a post-mortem study showed an up-regulation of the CB₁ receptor density in the dorsolateral prefrontal cortex of depressive suicide victims probably as a consequence of the depressive disorder to increase the sensitivity of the CB₁ receptors towards CB₁-agonistic endocannabinoids. Besides the behavioral cannabinoid effects in animal models of depression, there are animal studies showing antidepressant effects on the molecular basis as well. According to the monoamine hypothesis, it is suggested that hypofunction of monoaminergic neurotransmission, particularly of serotonergic and noradrenergic transmission, is a principal causal factor for the pathogenesis of major depression. The CB₁ receptor agonist WIN55,212-2 as well as the selective FAAH inhibitor URB597 were found to increase the firing activity of serotonergic neurons in the rat dorsal raphe nucleus, probably due to a disinhibition of excitatory projections from the medial prefrontal cortex. Moreover, URB597 increased the firing activity of noradrenergic neurons in the locus coeruleus and CB₁ mRNA expression in serotonergic neurons in the dorsal raphe nucleus. Based on these findings, the endogenous cannabinoid system may offer a novel target for the treatment of depressive disorders.

CANNABIS FOR THE MANAGEMENT OF PAIN: ASSESSMENT OF SAFETY STUDY (COMPASS)

Ware MA, Wang T, Shapiro S, Collet JP for the COMPASS study team

Introduction

An estimated 10-15% of patients with chronic noncancer pain self-administer cannabis to manage symptoms. Little is known of the long-term safety of such use, including adverse events (AEs), endocrine, pulmonary and cognitive function.

Methods

We conducted a cohort study to compare patients with chronic pain using cannabis (cases) with those who were not cannabis users (controls). Standardized herbal cannabis was dispensed for one year. Adverse events were reported monthly. A subset of patients underwent cognitive, pulmonary, hematological, biochemical, liver, renal and endocrine testing.

Results

A total of 215 cases and 216 controls were recruited from 7 clinics across Canada; 67 cases and 35 controls dropped out. Cases had higher pain, were younger, more likely to be male, disabled and to have used tobacco compare to controls. The average cannabis dose was 1.89g/day. No difference in serious AEs was noted [Crude IRR: 0.78 (0.44, 1.39)], though there were more non-serious AEs among cases than controls [Crude IRR: 1.64 (1.36, 1.98)]. Neurocognitive testing improved for both groups over one year with no differences between groups. A median of 30-mL decrease in FEV1 ($p=0.02$) with a median 1% decrease in FEV1/FVC ratio was observed ($p=0.01$) among cases. No differences were noted for other parameters.

Discussion

Cannabis use for chronic pain over one year is not associated with major changes in lung, endocrine, cognitive function or serious adverse events. The increase in non-serious adverse events is consistent with those for pharmaceutical cannabinoids. Longer-term studies are required to determine effects over more than one year.

**EFFECTIVENESS OF ADJUNCTIVE NABILONE IN THE PAIN AND SYMPTOM
MANAGEMENT OF ADVANCED CANCER PATIENTS:
A PROSPECTIVE OBSERVATIONAL STUDY USING PROPENSITY SCORING**

Vincent Maida,¹ MD, BSc, ABHPM, Marguerite Ennis², PhD, Shiraz Irani,³ RN,MSN,FNP,
Mario Corbo,⁴ BHSc, Michael Dolzhykov,⁵ BSc

1. Assistant Professor, University of Toronto, Division of Palliative Medicine, William Osler Health Centre, Toronto, Canada
2. Applied Statistician, Markham, Canada
3. CNS, Hope Health Care, Sydney, Australia
4. Faculty of Health Sciences, McMaster University, Hamilton, Canada
5. Faculty of Science & Engineering, York University, Toronto, Canada

A prospective observational study was carried out to assess the effectiveness of adjuvant nabilone (Cesamet®) therapy in the pain and symptom management of advanced cancer patients. The primary outcomes of the study were the difference between treated and untreated patients at 30 days follow-up, in Edmonton Symptom Assessment System (ESAS) pain scores and in total morphine sulfate equivalent (MSE) use, after adjusting for baseline differences. To adjust for baseline differences between the nabilone and non-nabilone group, the propensity score method was employed. Secondary outcomes included other ESAS parameters and frequency of use of other drug therapies. Data from 112 patients (47 nabilone, 65 non-nabilone treated) met criteria for analyses. The propensity-adjusted pain scores and total MSE use in nabilone treated patients were significantly reduced compared with patients who did not receive nabilone (both $P < .0001$). Other ESAS parameters that improved significantly in patients receiving nabilone were nausea ($P < .0001$), anxiety ($P = 0.0284$) and overall distress (total ESAS score) ($P = 0.0208$). Appetite was borderline improved ($P = 0.0516$). Nabilone therapy also resulted in a lower initiation rate or a higher tendency to discontinue nonsteroidal anti-inflammatory agents, tricyclic antidepressants, gabapentin, dexamethasone, metoclopramide, and ondansetron.

CANNABINOID:OPIOID PHARMACOKINETIC INTERACTION IN CHRONIC PAIN

Abrams DI, Couey P, Shade SB, Dhruva A, Kelly ME, and Benowitz N.

San Francisco General Hospital, University of California San Francisco
San Francisco, California USA

Background: Cannabinoids and opioids share several pharmacologic properties, including antinociception, hypothermia, sedation, hypotension and inhibition of intestinal mobility and locomotor activity. Data suggest the existence of independent but related analgesic pathways for cannabinoids and opioids such that the two may be synergistic. Cannabinoids may also ameliorate opioid side effects, particularly nausea and vomiting.

Methods: Participants on stable doses of long-acting morphine or long-acting oxycodone were admitted to the Clinical Research Center at San Francisco General Hospital for a 5-day inpatient stay. Evaluation of opioid disposition kinetics was obtained on day 1. Beginning on the evening after the baseline pharmacokinetic blood collection, participants were exposed to vaporized cannabis three times daily for the next 3 days. Following a final dose in the morning on day 5, cannabis pharmacokinetic sampling was performed and opioid pharmacokinetics were repeated to determine the effect of concomitant cannabinoids on the opioid kinetics. Chronic pain was assessed daily.

Results: 21 participants (10 morphine, 11 oxycodone) completed the trial. The participants included 11 men and 10 women. Sixteen were white, 2 African American, 2 multiracial and 1 Latino. The mean age was 45 years. The majority of the participants had chronic non-malignant pain of musculoskeletal origin. The results of the pharmacokinetic assays which were batched and run together after all participants completed the trial will be available for presentation. Most patients reported further relief of their chronic pain with the addition of vaporized cannabis; these results will also be fully analyzed and presented.

Conclusions: The complete pharmacokinetic and pharmacodynamic results will be analyzed and presented. Cannabinoids may augment the analgesic effects of opioids, allowing longer treatment at lower doses with fewer side effects.

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CANNABIS EXTRACT IN THE TREATMENT OF MUSCLE STIFFNESS AND OTHER SYMPTOMS IN MULTIPLE SCLEROSIS – RESULTS OF THE MUSEC STUDY

John Zajicek+, Marcus Reif*, Martin Schnelle*, on behalf of the UK MUSEC Study

Investigators

+Peninsula College of Medicine and Dentistry, Plymouth, PL6 8BX, UK;

* IKF, Berlin

Background The 'Cannabinoids in Multiple Sclerosis' (CAMS) study assessed the value of cannabinoids in the treatment of MS symptoms such as spasticity, pain, and sleep quality. Although in the primary analysis the Ashworth score did not show a significant benefit, the patients themselves rated cannabinoids clearly helpful. Here we report the results of the 'Multiple Sclerosis and Extract of Cannabis' (MUSEC) study conducted from 06/2006 to 09/2008, aiming at confirming the patient-based findings of the CAMS study.

Methods Across 22 UK centres 279 patients were randomised to oral cannabis extract or placebo. The study consisted of a 1- to 2-week screening period, a 2-week dose titration phase from 5 to 25 mg THC daily (final dose based on tolerability), and a 10-week maintenance phase. Main in-/exclusion criteria were definite MS disease stable for the previous 6 months, troublesome muscle stiffness, with stable antispastic medication and physiotherapy. Primary outcome measure was the patients' assessment of change from baseline regarding muscle stiffness. Beneficial response ('relief') was defined as marking categories 0 to 3 on an 11-point category rating scale (CRS). Further measures included CRS for body pain, spasms and sleep quality, and the MS Spasticity Scale (MSSS-88), MS Impact Scale (MSIS-29), and MS Walking Scale (MSWS-12). At an interim analysis an Independent Data Review Board recommended reducing the final sample size for statistical and ethical reasons.

Results The rate of relief in muscle stiffness after 12 weeks was almost twice as large under cannabis extract compared to placebo (29.4% vs 15.7%, $p=0.004$ one-sided). Equivalent results were found in sensitivity analyses, after 4 and 8 weeks, and also in rates of relief for body pain, spasms and sleep quality at all time points. Corresponding scales of the questionnaires corroborated these findings. Most frequent AEs were urinary tract infections, dizziness, dry mouth, and headache. Pronounced differences in the frequency of adverse drug reactions were mainly seen during the 2-week dose escalation phase.

Conclusion The study met its primary objective to show superiority of cannabis extract over placebo in the treatment of muscle stiffness in MS. Secondary efficacy parameters corroborated these results. The profile of AEs in patients treated with cannabis extract was consistent with the known side effects of cannabinoids. No new safety concerns were raised.

RECENT ADVANCES ON CANNABINOIDS IN GLIOMA

Manuel Guzmán

Department of Biochemistry and Molecular Biology I, School of Biology, Complutense University, 28040 Madrid, Spain

Δ^9 -Tetrahydrocannabinol (THC) and other cannabinoids inhibit the growth of glioma cells both in vitro and in various rodent xenograft models. This anticancer activity is dependent on the modulation of key signalling pathways that trigger cancer cell death as well as other events such as inhibition of tumour angiogenesis, cell proliferation and cell invasion. We have recently observed that THC induces glioma cell death through stimulation of autophagy and have unraveled the mechanism underlying this action by showing that THC, via ceramide accumulation, activates an endoplasmic reticulum stress response that promotes autophagy via inhibition of the Akt/mammalian target of rapamycin complex 1 (mTORC1) axis. We have also shown that autophagy is upstream of apoptosis in THC-induced cancer cell death and that activation of this pathway is necessary for THC anticancer action in mice. On the other hand, we have tested the combined effect of cannabinoids, alone or in combination with chemotherapeutic drugs, on glioma cell growth in vitro and in mice, and have sought for potential molecular markers of resistance of glioma cells to cannabinoid growth-inhibiting action. Thus, we have analysed the gene expression profile of a large series of glioma cell lines, and have found a subset of genes with a marked differential expression in THC-sensitive vs. THC-resistant cells. Furthermore, we have identified growth factors such as amphiregulin as likely candidates to mediate the resistance of glioma cells to cannabinoid-induced apoptosis, thereby supporting the emerging notion that targeted inhibition of growth factor-evoked pro-survival signals can improve the efficacy of anticancer therapies. Altogether, these findings may set the basis for future clinical trials aimed at evaluating the potential activity of cannabinoids as anticancer agents.

EFFECTS OF CANNABINOIDS ON GASTROINTESTINAL MOTILITY

Raquel Abalo

Departamento de Farmacología y Nutrición, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Alcorcón, Madrid, Spain

Preparations from *Cannabis sativa*, derivatives and synthetic analogues (cannabinoids) exert potent effects on gastrointestinal (GI) motility. These effects might be useful for the treatment of several GI diseases, but could also be considered as adverse effects when non-GI pathologies are to be treated.

Cannabinoid agonists reduce GI motor function. Although a central site of action can not be discarded, this effect is mainly due to the activation of prejunctional CB1 receptors, located on the myenteric neurones, and subsequent reduction of neurotransmitter release. This has been demonstrated to occur at all levels of the GI system using *in vitro* and *in vivo* assays, under both control and inflammatory conditions. The role of CB2 receptors on motility is still controversial, but could be relevant in the stomach or under inflammation.

Due to these effects, cannabinoid agonists have therapeutic potential for the treatment of a variety of alterations involving GI motility, such as nausea and emesis (which are associated to gastric dysmotility) or diarrheas (which generally involve accelerated transit of the lower GI tract). Whereas the effect on diarrheas might be due to reduced motility after activation of CB1/CB2 receptors in the intestinal myenteric neurons, the mechanisms involved in the reported suppression of emesis are not so clear, probably because in this case a central component plays also an important role. On the other hand, new therapeutic strategies using cannabinoid antagonists are also being explored. Thus, paralytic or postoperative ileus might benefit from their “prokinetic” effects.

Although cannabinoids are being proposed for the treatment of an increasing number of chronic diseases, their effects on GI motility upon chronic administration have received little attention. We have shown in the rat that, upon daily administration, cannabinoid-induced delayed gastric emptying was resistant to the development of tolerance, whereas delayed intestinal transit was not. In comparison, upon weekly administration, the cannabinoid effect, at least on gastric emptying, was enhanced.

Due to their acute and chronic effects on GI motility, the development of adverse effects of GI origin upon chronic treatment with cannabinoid drugs should be expected to occur. For example, cannabinoids might be useful to treat neuropathic pain induced by antitumoral drugs. However, these drugs are also capable of altering GI motility. When WIN was administered in a weekly fashion to rats that received also cisplatin, neuropathic pain did not develop, but GI motility alterations were aggravated.

In conclusion, experimental data demonstrate that cannabinoid agonists and antagonists have therapeutic potential to treat GI and non-GI diseases. However, it is necessary to bear in mind the possible development of adverse effects not only in the central nervous system, but also in the periphery, including the GI tract.

Supported by: SAF2006-13391-C03-01; URJC-CM-2006-BIO-0604; S-SAL/0261/2006.

SOME POTENTIAL STRATEGIES FOR TARGETING CANNABINOID RECEPTORS IN THE CLINIC

Roger G. Pertwee

School of Medical Sciences, Institute of Medical Sciences,
University of Aberdeen, Aberdeen, Scotland, UK

It is now generally accepted that the endocannabinoid system adopts an “autoprotective” role in certain disorders. This discovery has provided additional rationale for the accepted applications of cannabinoid receptor agonists as medicines and encouraged a search for new therapeutic uses for these agonists. It has also prompted a search for strategies that would allow the endocannabinoid system to be activated in the clinic with an improved benefit-to-risk ratio. These include “direct” strategies that rely on the selective activation (i) of cannabinoid CB₂ receptors or (ii) of cannabinoid CB₁/CB₂ receptors that are located outside the blood-brain barrier or are expressed by a particular tissue. They also include “indirect” strategies that rely on the ability of an administered compound to increase cannabinoid receptor activation by endogenously released endocannabinoids when these are inducing sought-after effects. These are strategies that involve (i) increasing endogenous levels of the endocannabinoids, anandamide and/or 2-arachidonoylglycerol, by inhibiting their cellular uptake or intracellular metabolism or (ii) increasing endocannabinoid-induced activation of the CB₁ receptor through allosteric enhancement. One other potential strategy is "multi-targeting". Thus, there have been reports that certain cannabinoid receptor agonists can interact in an additive or synergistic manner at relatively low doses either with a non-cannabinoid or, intriguingly, with a cannabinoid receptor antagonist, to ameliorate unwanted symptoms such as pain, anxiety, depression and emesis.

The endocannabinoid system also appears to mediate harmful effects in some disorders, prompting the development of medicines that will reduce the activity of this system. Initial attention focused particularly on selective cannabinoid CB₁ receptor antagonists and this led to the development of rimonabant as a medicine for treating obesity/metabolic syndrome. However, although this compound is a proven anti-obesity agent, it also appears to trigger severe depression/suicidality in some patients. There is, therefore, now a need for a pharmacological strategy that would allow all or just some cannabinoid CB₁ receptors to be blocked in the clinic in a manner that would still be effective against disorders such as obesity/metabolic syndrome but that would not induce such serious adverse effects. Some strategies worth exploring include (i) blocking the CB₁ receptor with a selective neutral antagonist rather than with a rimonabant-like mixed antagonist/inverse agonist, (ii) administering an allosteric CB₁ receptor antagonist that selectively blocks the CB₁-mediated actions of only anandamide or only 2-arachidonoylglycerol, (iii) blocking CB₁ receptors with a bioavailable antagonist that does not readily penetrate the blood-brain barrier or (iv) "multi-targeting", for example, by administering a CB₁ receptor antagonist at a low dose in combination with another type of anti-obesity agent.

Finally, it is important to bear in mind that some plant, endogenous or synthetic cannabinoids may act beyond the endocannabinoid system to produce beneficial effects. Recent research at Aberdeen has yielded data suggesting that one little-investigated plant cannabinoid, cannabigerol, interacts potently with a therapeutically relevant established receptor that does not form part of the endocannabinoid system. Some of these data will be presented.

Supported by GW Pharmaceuticals and NIH grant DA03672.

ENDOCANNABINOIDS – THE WAY AHEAD

Raphael Mechoulam

Hebrew University of Jerusalem

In the proceedings of a symposium aimed at delineating future research we must try to put down some of the developments we hope to see. I expect that the following three areas will become topics of very active research.

1. Identification of novel endocannabinoid-like compounds as active molecular species.

In addition to 2-AG and anandamide, numerous endocannabinoid-like compounds are known to play important biological roles. I shall describe our recent work on oleoyl serine, an endogenous material involved in bone formation and on arachidonoyl serine, a neuroprotective endogenous endocannabinoid-like material. These two compounds do not bind to either the CB₁ or the CB₂ receptor.

2. Profiling of endocannabinoid changes.

Over the last decade numerous examples have been reported of significant changes in the levels of endocannabinoids and endocannabinoid-like lipids (2-AG, anandamide, arachidonoyl serine etc) due to a specific disease state. We have seen that such compounds are involved in the metabolic changes taking place. As about 60 such compounds have been identified in mammalian tissues it seems reasonable to expect that we shall see extensive research along these lines. Results may be of major importance in the establishment of novel diagnostic procedures for a variety of diseases and possibly for treatment. I shall try to summarize the work on such endocannabinoid-like materials.

3. Novel therapeutics.

The recent demise of SR141716 as an anti-obesity drug probably indicates that CB₁ antagonists that penetrate the blood brain barrier will not open a new therapeutic area, due to their side effects. However we can expect to see such compounds with specific peripheral action, possibly as new drugs in liver, gastrointestinal and lung diseases. CB₁ agonists are already used in several types of pathological conditions and we can expect their use in post trauma and pain.

CB₂ agonists should lead to new drugs in pain, inflammation and neurological diseases. A long list of additional approaches can be visualized – from gastrointestinal to bone diseases.

And we should not forget cannabidiol, which seems to act on too many disease states (anxiety, inflammation, cardiac conditions, schizophrenia etc) and yet has no major side effects.

BETA-CARYOPHYLLEN: A PHYTO-CANNABINOID ACTING ON CB2 RECEPTORS

Andreas Zimmer¹, Ildiko Racz¹, Anna-Lena Klauke¹, Astrid Markert¹, Jürg Gertsch²

¹Institute of Molecular Psychiatry, University of Bonn, 53125 Bonn, Germany

²Institute of Biochemistry and Molecular Medicine, University of Bern, CH-3012 Bern, Switzerland

The sesquiterpene beta-caryophyllene is a plant volatile found in large amounts in the essential oils of many different spice and food plants, such as avocado (*Persea americana* MILL.), hop (*Humulus lupulus* L.), lemon (*Citrus* spp.), oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.), clove (*Syzygium aromaticum*), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus serpyllum*), sage (*Salvia officinalis* L.), black pepper (*Piper nigrum* L.) and others. Due to its weak aromatic taste, it is also commercially used as a food additive and in cosmetics. Several health effects have been attributed to beta-caryophyllene, including anti-inflammatory, local anaesthetic and anti-carcinogenic activity. Recently, we have discovered that beta-caryophyllene specifically binds to and activates the predominantly non-neuronal, non-psychoactive CB2 cannabinoid receptor, which is present in immune and bone cells. Hence, beta-caryophyllene has no psychoactive effects, unlike the classical phytocannabinoid Δ^9 -tetrahydrocannabinol (THC) from the plant *Cannabis sativa*, which activates the neuronal CB1 cannabinoid receptor. It is estimated that the realistic, estimated daily intake of 10 to 200 mg beta-caryophyllene from vegetable food is sufficient for significant CB2 cannabinoid receptor activation. CB2 receptor signaling has an important role in key physiological and pathophysiological processes, particularly those involving inflammatory and immune responses. Agonists of CB2 receptors may thus be efficacious for the treatment of a spectrum of inflammatory disorders, including neuropathic pain conditions. We have demonstrated that oral administration of beta-caryophyllene reduces inflammatory responses in animal models at doses that are comparable to the nutritional intake. We shall now present data demonstrating that the efficacy of beta-caryophyllene in neuropathic pain models equals the efficacy of CB2-selective synthetic compounds. Considering these facts, it is likely that beta-caryophyllene belongs to a group of common plant bioactives with a major potential impact on human health.

GENETIC BASIS OF MARIJUANA USE

Emmanuel Onaivi

Biology Department, William Paterson University, Wayne, USA.

A major breakthrough in marijuana-cannabinoid research has been the discovery of a previously unknown but elaborate endogenous endocannabinoid system (ECS) composed of endocannabinoids and the enzymes for their biosynthesis and degradation with genes encoding two distinct cannabinoid (CB1 and CB2) receptors (CBRs) that are activated by endocannabinoids, cannabinoids and marijuana use. Physical and genetic localization of the cannabinoid receptor *CNR1* and *CNR2* genes have been mapped to human chromosome 6 and 1 respectively. A number of variations in CBR genes have been associated with human disorders including osteoporosis, ADHD, PTSD, drug dependency, obesity and depression. The ubiquitous abundance and differential distribution of the ECS in the human body and brain along with the coupling to many signal transduction pathways may explain the effects in most biological system and the myriad behavioral effects associated with smoking marijuana. The remarkable progress in understanding the biological actions of marijuana and cannabinoids have provided a much richer than previously appreciated cannabinoid genomics and raised a number of critical issues on the molecular mechanisms of cannabinoid induced behavioral and biochemical alterations.

Methods: Multidisciplinary approaches involving RT-PCR, immunoblotting, genotyping and analysis of human CBR gene structures and isoform specific expression patterns, behavioral modification in mouse models of emotionality and motor function tests were utilized to determine the genetic basis of marijuana use.

Results: The results focuses on cannabinoid genomics and the surprising new fundamental roles that the ECS plays in signaling associated with cannabinoid inhibition of neurotransmitter release to the genetic basis of marijuana use. Our data on the cloning of mouse CB1 cDNA and the chromosomal localization of mouse *CB1* and *CB2* genes is syntenic with the human chromosomal *CNR1* and *CNR2* genes. Additional evidence is provided for the complex *CNR1* and *CNR2* gene structures and their associated regulatory elements and variants. Our discovery of post-synaptic localization of neuronal CB2-Rs warrants a re-evaluation of the role of CB2-Rs and their interaction with CB1-Rs in the mammalian brain. Our data also indicate a number of polymorphisms and sub-type cannabinoid receptor specificity indicating that marijuana use may not only be coded in our genes but may be exploited in disorders associated with disturbances of endocannabinoid system.

Conclusions: It is concluded that in the coming era of personalized medicine, genetic variants and haplotypes in *CNR1* and *CNR2* genes associated with obesity or addiction phenotypes may help identify specific targets in conditions of endocannabinoid dysfunction. Thus, understanding the ECS in the human body and brain will contribute to elucidating this natural regulatory mechanism in health and disease.

THE NEUROPROTECTIVE POTENTIAL OF CANNABINOIDS IN BASAL GANGLIA DISORDERS

Javier Fernández-Ruiz

Departamento de Bioquímica y Biología Molecular and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Facultad de Medicina, Universidad Complutense, 28040-Madrid (Spain); e-mail: jjfr@med.ucm.es

Cannabinoids have been proposed as promising medicines for the treatment of Parkinson's disease (PD), Huntington's disease (HD) and other basal ganglia disorders, given the abundance of elements of the cannabinoid signaling system in these structures and the relevance of their effects in the healthy basal ganglia but also in conditions of malfunctioning and/or degeneration (Fernández-Ruiz, *Brit. J. Pharmacol.* 156, 1029-1040, 2009). These benefits include first the alleviation of specific motor symptoms, e.g. choreic movements in HD with CB₁/TRPV₁ receptor agonists (Lastres-Becker et al., *J. Neurochem.* 84, 1097-1109, 2003) and bradykinesia in PD with selective CB₁ receptor antagonists (González et al., *Brain Res.* 1073/74, 209-219, 2006). However, the most important challenge is the possibility that cannabinoids may serve to delay/arrest disease progression based on their neuroprotective and/or neuroregenerative properties. This would include the use of **CB₁ receptor agonists** to attenuate excitotoxic events that frequently operate in neurodegenerative disorders. In this respect, CB₁ receptors experience an early down-regulatory response in HD and PD, which may be associated with early pathogenic events and play an instrumental role in triggering excitotoxicity (García-Arencibia, *J. Neural Transm. Suppl.* 73, 269-275, 2009). By contrast, the activation of these receptors might provide neuroprotection by reducing glutamate release and the concomitant activation of NMDA receptors and associated intracellular events (e.g. activation of calcium-dependent destructive pathways). **CB₂ receptor agonists** may also exert a protective effect by limiting the toxicity of reactive microglia for neurons typical of most neurodegenerative disorders. Numerous studies have demonstrated that CB₂ receptors are induced/up-regulated in glial elements in response to brain injury, whereas compounds targeting selectively this receptor are able to reduce cytokine generation, particularly in HD (Sagredo et al., *Glia* 57, 1154-1167, 2009). In relation with this disorder, the generation of double mutants, by crossing R6/2 mice (a well-characterized genetic model of HD) with CB₂ knockout mice, aggravated the striatal pathology and enhanced inflammatory events (Palazuelos et al., *Brain*, in press, 2009). Lastly, **antioxidant cannabinoids** may also serve to attenuate oxidative damage, another important cytotoxic event involved in the pathogenesis of most neurodegenerative disorders. This antioxidant effect may be exerted by acting as mere scavengers of reactive oxygen species, but recent evidence suggest that antioxidant cannabinoids may also activate several intracellular signals linked to the induction/activation of endogenous antioxidant defenses (Sagredo et al., *Eur. J. Neurosci.* 26, 843-851, 2007; García-Arencibia et al., *Brain Res.* 1134, 162-170, 2007; Lastres-Becker et al., *Neurobiol. Dis.* 19, 96-107, 2005). The present lecture will review the anatomical, neurochemical, electrophysiological and pharmacological bases that sustain the importance of the cannabinoid system in basal ganglia function, trying to collect the present information and the future lines for research on the therapeutic potential of this system in PD, HD and other basal ganglia disorders.

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ANCIENT CANNABIS FROM XINJIANG REVISITED

Ethan Russo^{1,2}, Arieh Moussaief³, Sergey Malitsky³, Asaph Aharoni³, Raphael Mechoulam⁴,
Hong-En Jiang⁵, Xiao Li⁶, En-Guo Lü⁷, & Cheng-Sen Li⁵

¹20402 81st Avenue SW, Vashon Island, WA 98070 USA, ²GW Pharmaceuticals, Porton Down Science Park, Salisbury SP4 0JQ UK, ³Department for Plant Sciences, Weizmann Institute of Science, Rehovot, Israel, ⁴Medicinal Chemistry and Natural Products Dept, Pharmacy School, Ein-Kerem Medical Campus, the Hebrew University of Jerusalem, Israel, ⁵Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, ⁶Bureau of Cultural Relics of Turpan Prefecture, Turpan 838000, Xinjiang, China, ⁷Xinjiang Institute of Archaeology, 4-5 South Beijing Road, Ürümqi, Xinjiang 830011, China

Introduction: Excavations of the Yanghai Tombs near Turpan, Xinjiang-Uighur Autonomous Region, China, revealed two graves of Caucasoid shamans of 2500 and 2700 years' antiquity. One grave included a large cache of cannabis, superbly preserved by climatic and burial conditions. A previously reported study demonstrated through botanical and phytochemical investigations and genetic deoxyribonucleic acid analysis by polymerase chain reaction that this material contained tetrahydrocannabinol, the psychoactive component of cannabis, its oxidative degradation product, cannabinol, other metabolites, and its synthetic enzyme, tetrahydrocannabinolic acid synthase, as well as a novel genetic variant of the enzyme with two single nucleotide polymorphisms (Russo et al. *Journal of Experimental Botany* 59(15):4171-4182, 2008). The cannabis was presumably employed by this culture as a medicinal or psychoactive agent, or an aid to divination, but there was no specific evidence to indicate techniques of administration. The current study examined proximal and distal head hair samples from these two shamans to ascertain whether any trace of phytocannabinoids or their metabolites might be demonstrated.

Methods: Negative control hair samples from two volunteers at the Weizmann Institute were analyzed along with five hair samples from Xinjiang. Samples were washed twice with neutral liquid soap and six times with distilled water under mechanical stirring for 45 min. Each sample was dried at 38°C for 24 h. Dried samples of 70 mg each were frozen in liquid nitrogen and ground to powder with a boll mill machine. Hydrolysis and extraction of cannabinoids followed the technique of Villamor et al. 2004. Cannabinol, cannabidiol and tetrahydrocannabinol standards were utilized for GC/MS analysis with a Thermo Trace GC Ultra coupled to a DSQ2 Detector and Xcalibur 1.4 SRI software.

Results: Unfortunately, these studies were negative, providing no evidence of THC, CBD or CBN in the hair samples.

Conclusion: These negative findings could alternatively be explained by 3 possibilities: a) These individuals did not employ cannabis themselves or not in sufficient quantity to be assayed, or b) These individuals only administered cannabis to others, or, the most likely explanation, c) The extreme antiquity of these archeological samples did not allow preservation of phytocannabinoid metabolites above the limits of detection. Additional research in the Yanghai Tombs may provide additional clues to this enduring mystery.

CANNABIS, MUSIC AND ALTERED TEMPORALITY

Fachner, Jörg

Finnish Centre of Excellence in Interdisciplinary Music Research
University of Jyväskylä, Finland

The contradiction between a subjectively as extended perceived temporal duration of exciting and meaningful or stressful and frightening moments, and their real chronological extend is a known experience (Eagleman & Holcombe, 2002). Attention focus and according working memory load seem to modify the experience of temporality (Pöppel, 2000). This may reflect the consciousness state of a subject and its cognitive and attentional behaviour during state-related timing processes (J. Fachner, 2009).

This paper focuses on cannabis and its action on timing and aims to discuss selected scientific streams of research on the neurophysiological and neuropharmacological base of timing mechanisms.

Drug-induced altered temporality is a well-known effect of cannabis action that is utilised from musicians and music listeners for music appreciation since the early days of jazz (J. Fachner, 2000). Music occurs in time and in particular rhythm refers to short time intervals, whereby for exact performance timing processes the millisecond range is of importance. Cannabis has an influence on timing processes at short time scales of hundreds of milliseconds as O'Leary et al (2003) have shown in their tapping studies, proving evidence of an altered cerebellar functioning. Clock speed (pacemaker) can be influenced by dopaminergic manipulations whereas memory processes (reference) can be influenced by cholinergic manipulations (Buhusi & Meck, 2005). Lieving explains the role of THC in timing as an acceleration of clockspeed mediated via an increase in activity of dopaminergic neurons, while the anticholinergic action of THC expands the duration of a remembered event. „The more acetylcholine is present, the shorter the remembered duration of events.“ (Lieving, Lane, Cherek, & Tcheremissine, 2006, p. 182).

The change of the scalar property of auditory events may lead to a change in the metric frame of reference when perceiving acoustic and spatial relations (J. Fachner, 2000). It influences perceiving the musical time-space and seem to have inspired sound design in popular music (J. Fachner, 2002a). Cannabis induced reframing of acoustic events may be of benefit for people with hearing impairment (J. Fachner, 2002b).

EFFECTS OF CANNABINOIDS ON HUMAN MONOCYTE MIGRATION: IMPLICATIONS FOR MULTIPLE SCLEROSIS

Michelle Sexton¹, Eric Zimmerman², Nephi Stella^{1,3}

¹Department of Pharmacology, ²Undergraduate Program in Neurobiology, ³Department of Psychiatry and Behavioral Science, University of Washington, Seattle WA 98195 USA

The non-psychotropic Cannabinoid 2 (CB2) receptor is known to modulate immune cell migration. Under conditions of neuroinflammation, as observed in patients with multiple sclerosis (MS), immune cells migrate to the brain. Lymphocyte migration is a therapeutic target in MS, but monocytes have yet to be a pharmacologic target. Migration of monocytes into the brain, where they become microglia-like, either contribute to or counteract neuroinflammation. Because endocannabinoid (eCB) levels are dramatically increased in mouse models of MS, and patients with MS report symptom relief with clinical Cannabis use, pharmacological manipulation of the endocannabinoid signaling system (eCBSS) is a promising therapeutic avenue. We sought to determine the role of cannabinoids in monocyte recruitment using a novel modified Boyden chamber assay. We measured the response to a variety of cannabinoids and show how basal migration is mitigated or enhanced.

Methods: HL60 cells or human peripheral blood mononuclear cells (PBMC) were used in migration experiments. Monocytes were isolated from PBMCs using the Miltenyi Monocyte Isolation kit II. Cells were stained with 700 nM DRAQ5 for fluorescence quantification, and then loaded into the top of a 96-well chemotaxis chamber (Neuroprobe, Cabin John, MD) using a fibronectin-coated filter with 5µm pore diameter. Migration toward chemoattractants was measured by scanning the bottom of the filter using the Odyssey Infrared Imaging System (LI-COR, Lincoln, NE) in the 700nm channel. After subtracting out background fluorescence, results are expressed as percent of basal migration.

Results: Quantifying cell migration using near-infrared fluorescence, we tested several chemoattractants on human monocyte migration. We first established basic parameters to optimize the migration assay for human cells by determining cell density, kinetics and basal migration conditions and a positive control. We then tested a variety of compounds for their effect on basal migration. Anandamide (arachidonylethanolamine:AEA) increased migration over basal levels, a result that was mitigated by palmitoylethanolamine (PEA).

Conclusion: Peripheral immune cell migration plays a role in the pathogenesis of brain lesions associated with MS. In our system, AEA was chemoattractant for monocytes, while the additive effect of PEA to AEA reduced this response. Characterization of the interplay between the eCBSS and specific neuroinflammatory processes, particularly effects on monocyte recruitment to the CNS, may provide implication for MS therapy. Boosting the eCBs in patients with MS, therefore may provide a new avenue of therapy in this and other neuroinflammatory and neurodegenerative diseases.

SMOKED CANNABIS AND DOPING CONTROL: LOOKING FOR THE WRONG ANALYTE?

Rudolf Brenneisen¹, Pascale Meyer¹, Haithem Chtioui², Martial Saugy³, Carine Schweizer³, and Matthias Kamber⁴

¹Dept. Clinical Research, University of Berne, Murtenstr. 35, 3010 Berne; ²Clinical Investigation Unit, University Hospital of Berne, 3010 Berne; ³Swiss Laboratory for Doping Analyses, 1066 Epalinges; ⁴Antidoping Switzerland, P.O. Box 606, 3000 Berne 22, Switzerland

Introduction: Since 2004, Cannabis is prohibited by the World Anti-Doping Agency (WADA) for all sports in competition. In the years since then, about half of all positive doping cases in Switzerland have been related to Cannabis consumption. In most cases, the athletes plausibly claim to have consumed Cannabis several days or even weeks before competition and only for recreational purposes not related to competition. In doping analysis, the target analyte in urine samples is 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the reporting threshold for laboratories is 15 ng/mL. However, the wide detection window of this long-term THC metabolite in urine does not allow a conclusion concerning the time of consumption or the impact on the physical performance.

Aim: The purpose of the present pharmacokinetic study on volunteers was to evaluate target analytes with shorter urinary excretion time. Subsequently, urines from athletes tested positive for Cannabis should be reanalyzed including these analytes.

Methods: In an one-session clinical trial (approved by IRB, Swissmedic, and Federal Office of Public Health), 12 healthy, male volunteers (age 26 ± 3 yrs, BMI 24 ± 2 kg/m²) with Cannabis experience (\leq once/month) smoked a Cannabis cigarette standardized to 70 mg THC/cigarette (Bedrobinol[®] 7%, Dutch Office for Medicinal Cannabis) following a paced-puffing procedure. Plasma and urine was collected up to 8 h and 11 days, respectively. Total THC, 11-hydroxy-THC (THC-OH), and THC-COOH were determined after enzymatic hydrolyzation followed by SPE and GC/MS-SIM. The limit of quantitation (LOQ) for all analytes was 0.1 ng/mL. Visual Analog Scales (VAS) and vital functions were used for monitoring psychological and somatic side-effects at every timepoint of specimen collection (up to 480 min).

Results: Eight puffs delivered a mean THC dose of 45 mg. Mean plasma levels of total THC, THC-OH and THC-COOH were measured in the range of 0.1-20.9, 0.1-1.8, and 1.8-7.5 ng/mL, respectively. Peak concentrations were observed at 5, 10, and 90 min. Mean urine levels were measured in the range of 0.1-0.7, 0.10-6.2, and 0.1-13.4 ng/mL, respectively. The detection windows were 2-8, 2-96, and 2-120 h. No or only mild effects were observed, such as dry mouth, sedation, and tachycardia. Besides high to very high THC-COOH levels (0-978 ng/mL), THC (0.1-24 ng/mL) and THC-OH (1-234 ng/mL) were found in 90 and 96% of the Cannabis-positive urines from athletes.

Conclusion: Instead of or in addition to THC-COOH, the pharmacologically active THC and THC-OH should be the target analytes for doping urine analysis. This would allow the estimation of more recent Cannabis consumption, probably influencing performance during competition.

EFFECT OF SALINITY IN NUTRIENT SOLUTION ON YIELD OF *CANNABIS* IN INDOOR CONDITION

Gianpaolo Grassi¹, Salvatore Casano^{1,2}, Marco Michelozzi³, Valentina Martini³ and Diaz Kroeze⁴

¹CRA-CIN, Rovigo branch section, Italy

²DAAT, Faculty of Agricultural Sciences, University of Palermo, Italy

³Institute of Plant Genetics, National Research Council, Sesto Fiorentino, Italy

⁴PlantResearch International BV, Oosterhout nb, The Netherlands

Recent cultivation tests showed that in rock wool cultivation the fertiliser level, generally expressed as Electrical Conductivity (EC), affects yield and quality of *Cannabis sativa* L. The precise effects differ between varieties. However, the interaction between EC and active constituents (cannabinoids and terpenoids) remains poorly understood. So as to increase the EC level of the nutrient solution without causing phytotoxicity, agronomically optimised formulas of fertilizers were designed by PlantResearch BV to be used for this trial.

Methods: Four levels of EC (1, 2, 3 and 4 dS m⁻¹) have been applied with an automatic system of irrigation starting from the generative phase of two different chemotypes: Δ9-THC (genotype 13) and Δ9-THC/CBG (genotype 16). Two female (pistillate) plants were used as sources for cuttings. Three-week-old cuttings were placed in 5-litre pots with rock wool as substrate, with a 6 plants/m² density. Two groups of 6 tables (1 m x 2 m) were used, resulting in 72 pots for each genotype. The light was supplied by 12 lamps of 600 watts fixed on the ceiling at 160 cm from the plants that produced about 24'000 Lux on a growing area of 24 m². After harvest, the chlorophyll content, plant height and stem weight were measured. Furthermore, the leaf-flower production was determined and cannabinoid content was analysed on GC. Terpenes were identified by comparison of retention times with those of standards under the same conditions. The relative amount (proportion of profile) of each monoterpene was expressed as a percentage of total monoterpenes.

Results: The EC levels affected plant growth differently and the different parameters were evaluated. The portable chlorophyll meter (SPAD-502, Konica Minolta) showed that chlorophyll content in the leaves increased by increasing EC levels. The measured parameter at the lowest EC value is half of the value recorded on the plants fertilised with the highest EC. The plant height of both genotypes and EC level was correlated, and the highest plants were produced when levels EC 3 and EC 4 were used.

The effect of EC was positive on plant weight when the total and the leaf-flower portion were considered. Cannabinoid content, tested by cannabidiol (CBD), cannabigerol (CBG) and delta-9-tetrahydrocannabinol (Δ9-THC), was modified by the EC level of the fertiliser solution. The high level of EC tended to reduce the concentration of the three cannabinoids, although not significantly. The optimal value of EC was close to 3 dS m⁻¹ because this showed a positive effect on the total production of the three cannabinoids' yield per square meter when genotype 16 is considered, while this trend was the same only for CBG and Δ9-THC when genotype 13 is considered.

No significant changes in proportions of the main monoterpene constituents were detected for any of the treatments. These results agree with data in the literature showing that the relative proportions (percentages) of constitutive monoterpenes are under strong genetic control and little affected by abiotic factors.

Conclusions: The salinity level has a major effect on *Cannabis* chlorophyll content and plant mass production. However, cannabinoid content in the flower and leaves changed only slightly, with an inverse tendency to the increase of EC level. At the optimal fertilizer concentration, the leaf-flower yields were 330 gr/m² and 437 gr/m² respectively using chemotype Δ9-THC/CBG and Δ9-THC.

WHAT ARE THE MAIN PROBLEMS FACED BY PATIENTS WHO TAKE CANNABIS-BASED MEDICINES?

Ethan Russo^{1,2}

¹20402 81st Avenue SW, Vashon Island, WA 98070 USA

²GW Pharmaceuticals, Porton Down Science Park, Salisbury SP4 0JQ UK

Introduction: Risks of cannabis usage have been debated for centuries, but despite such claims, it has been used therapeutically by humans for all of recorded time. Common concerns include pulmonary damage, increased cancer risk, dependency, cognitive changes, and mental health sequelae.

Methods: The author's files and current literature were reviewed.

Results: Chronic use studies in recreational users in Jamaica, Greece, and Costa Rica in the 1970s-1980s documented minor pulmonary changes without significant neuropsychological or other sequelae. The Chronic Use Study of 4 subjects employing high daily intake of cannabis therapeutically over a long interval in the USA Compassionate Use Investigational New Drug Program similarly demonstrated slight pulmonary function changes, with minimal executive function effects, but no endocrine, immunological, neurophysiological or anatomical changes. Surveys of therapeutic cannabis usage and adverse event recording from clinical trials of cannabis and cannabis-based medicine document pulmonary complaints (from smoking) and primarily CNS events such as nausea, dizziness, somnolence, or intoxication-type symptoms, often of a transient nature. Problems claimed in relation to recreational cannabis usage, such as induction of cancer, depression, psychosis, suicidal ideation, addiction, etc., have not been reported to any significant degree in a therapeutic context to this point in time. Sativex was tested as having a drug abuse liability equal to, or less than that of Marinol.

Conclusion: The pre-eminent risk of smoked cannabis in a therapeutic context is pulmonary, including chronic cough and bronchitis. These problems can likely be minimized or eliminated by alternative delivery systems such as vaporization, oral, or oro-mucosal administration. There is no current compelling evidence to support that cannabis increases risks of carcinogenesis, and it may induce a protective effect for some cancers (lung, head and neck). Long-term cognitive effects of cannabis in therapeutic applications seem to be within acceptable limits as compared to other medications employed to treat serious diseases and symptoms. Similarly, the drug abuse liability of cannabis-medicines seems relatively benign, especially when extra-pulmonary administration is utilized. Cannabis usage remains subject to criminal sanction in many jurisdictions, but apart from this, medical risks associated with such usage appear quite low. Suitable caution is advised in pediatric and adolescent populations.

REVIEW: CANNABIS-ASSOCIATED ARTERITIS

Franjo Grotenhermen

Nova-Institut, Hürth, Germany, e-mail: franjo.grotenhermen@nova-institut.de

Purpose: To investigate the hypothesis that cases of arteritis similar to thromboangiitis obliterans (TAO) and associated with the use of cannabis were caused by cannabis or THC (dronabinol), or that cannabis use is a co-factor of TAO. TAO, or Buerger's disease, is an occlusive, inflammatory, non-atherosclerotic disease of the small and medium-sized arteries and veins of the upper and lower limbs, which usually affects young, male, heavy tobacco smokers.

Methods: A systematic review on case reports and the literature on so-called cannabis arteritis, TAO, and cardiovascular effects of cannabinoids was conducted.

Results: Fifteen reports with 57 cases of an arteritis associated with the use of cannabis and two additional case series of TAO, in which some patients also used cannabis, were identified. Clinical and pathological features of cannabis-associated arteritis do not differ from TAO and the major risk factor of TAO, tobacco use, was present in most, if not in all of these cases. The proposed pathophysiological mechanisms for the development of an arteritis by cannabis use are not substantiated.

Conclusion: This review examines the question of whether it is justified to use the term cannabis arteritis and to claim that cannabis is a cause of an arteritis, which has similarities or is identical to thromboangiitis obliterans (TAO). There is little reason to justify this term. First, there is no difference between clinical and pathological features of TAO and cases of arteritis, which were attributed to the use of cannabis. Second, in nearly all or all cases of so-called cannabis arteritis there was additional tobacco use, and moderate use of tobacco has been reported in a considerable number of TAO cases. Even cases attributed or similar to TAO with no tobacco use have been reported. Third, there is no established pathophysiological mechanism that would explain the development of an arteritis by the use of cannabis. Fourth, supporters of the cannabis arteritis hypothesis offer many disclaimers that point out the uncertainty that cannabis compounds are indeed responsible for the observed effects.

In the future, it will be of interest if a cannabis associated arteritis is observed in countries where cannabis is customarily used alone, such as in the USA and Canada, since the mixture with tobacco, which is the usual way to consume cannabis in Morocco and many European countries, may obscure a hypothetical arteritis caused solely by cannabis. Before better evidence is available, it is reasonable to avoid the term cannabis arteritis, which suggests a causal association.

This review has been accepted for publication in VASA.

EXPERIENCES AND CURRENT STATUS OF DUTCH OFFICE OF MEDICINAL CANNABIS

Marco van de Velde, Kathrin Höner-Snoeken, Catherine Sandvos, Ellen Koster

Office of Medicinal Cannabis, Ministry of Health Welfare and Sport, P.O. Box 16114,
2500 BC The Hague, The Netherlands

The Office of Medicinal Cannabis (OMC) was established in 2000 by the Dutch government. The government wanted to meet patients and patients' associations wishes to make cannabis with a pharmaceutical quality legally available for patients' use. The OMC acts as wholesaler and assigns third parties to cultivate cannabis, to perform quality control and arrange logistic.

Over the years the OMC dealt with a lot of issues concerning the organisation, quality aspects and financial affairs which have been solved. The OMC has been realized from the beginning that cannabis is mainly known as an addictive drug abroad as well in the Netherlands. To prevent stigmatization of patients as drug users the policy of medicinal cannabis became part of the pharmaceutical affairs unit. This unit is strongly separated from the policy of drug addiction / use. In addition the OMC which is product responsible strongly focus on quality aspects. The products which are available have to comply with the EU quality requirements e.g. free of pesticides, micro-organisms. The OMC have experienced that product standardization often requires a lot of difficulty for the growers. On that account a contract was broken of one of the two growers in the past causing an unwanted interdependence.

In 2006 the OMC experienced that stakeholders like physicians, health insurance companies and politicians were rather unacquainted with the therapeutic properties of cannabis. Health care professionals were poor informed about the position of cannabis as part of the therapeutic regimen for symptoms like neuropathic pain or appetite loss. Dutch physicians act in conformity with given guidelines. At the moment medicinal cannabis is not included in any guideline or part of university training. Therefore OMC have been consulting patient-organisations and associations of medical professionals to inform them and to debate with them.

Besides physicians also the medical advisors of health insurance companies do not have an actual overview about the therapeutic value of medicinal cannabis. Medical advisors as well as physicians have pointed to the lack of well performed clinical studies (randomised, placebo-controlled, etc) which must show therapeutic value with respect to registered pharmaceutical products. A present survey organized by the OMC has been shown that only 60% of health insurers in the Netherlands (partly) reimburse cannabis due to this lack of evidence. As health insurers are susceptible for cost containment pharmaco-economic data about patient's use of cannabis versus other medication, research on this topic would be of general interest.

info@cannabisbureau.nl
www.cannabisoffice.nl

Posters

EVALUATION OF THE PURIFIED CANNABIGEROL(CBG) AND EXTRACTS RICH IN CBG IN THE MOUSE IRWIN TEST

Akihito Watanabe^{a,b}, Yuki Yamasaki^{a,b}, Naoki Amada^{a,b}, Serena Deiana^a, Tetsuro Kikuchi^b, Gernot Riedel^a

^a *Institute of Medical Sciences, University of Aberdeen, Foresterhill, AB25 2ZD, UK*

^b *Qs' Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, 771-0192, Japan*

Introduction: The cannabis plant has attracted much interest over the last years, not only because of problems associated with its abuse, but also because of the therapeutic potential of many of its constituents (phytocannabinoids). However, systematic observational analysis of these components are widely lacking. Thus, we here examined the effects of a phytocannabinoid, purified Cannabigerol (Pure CBG) and its extract from the plant containing CBG, on the central and the peripheral nervous system.

Methods: Pure CBG and CBG extract were evaluated in the neurobehavioural observation battery at 3, 10, 30, 100 mg/kg doses after *intraperitoneal (i.p.)* administration, using ICR mice aged 6-9 weeks (Harlan UK). Animals were examined in a modified Irwin test battery (Irwin S., *Psychopharmacologia* 1968;13:222-257), including observations in home and novel cages. They were assessed at several time points of up to 25hrs after administration. At each time point, the observations started in the home cage, then mice were transferred into a novel cage, where they were evaluated for signs of distress, which did not require any interaction with the experimenter (e.g., reactivity, locomotor activity). Then additional abnormalities were assessed by handling the mice (e.g., startle response, grip strength, reflexes).

Results: Neither cannabinoid caused serious toxicity such as epilepsy even at doses of 100 mg/kg. Although both of them caused changes in several parameters (e.g.,spontaneous activity, body posture), most of the observed changes were not reliable.

Conclusion: Behavioural analysis utilizing Pure CBG and CBG extracts can readily assess doses up to 100mg/kg. However, careful attention is required to dissociate side effects from direct effects on animal models.

BEHAVIOURAL AND METABOLIC EFFECTS OF CHRONIC CANNABIDIOL AND [3-(3-CARBAMOYLPHENYL)PHENYL] N-CYCLOHEXYLCARBAMATE (URB 597) ADMINISTRATION IN ADULT LISTER HOODED RATS (*RATTUS NORVEGICUS*)

Cathrin Jöpen¹, Franziska Pahlisch¹, Heike Endepols², F. Markus Leweke³

¹University of Cologne, Department of Psychiatry and Psychotherapy, Kerpener Str. 62, 50924 Cologne, Germany, ²Max Planck Institute for Neurological Research, Multimodal Imaging, Gleueler Str. 50, 50931 Cologne, Germany, ³Central Institute of Mental Health, Department of Psychiatry and Psychotherapy, J5, 68159 Mannheim, Germany

Delta-9-tetrahydrocannabinol (Δ^9 -THC) and Cannabidiol (CBD) are the most important compounds of *Cannabis sativa*. Δ^9 -THC has been identified as the major psychoactive compound and is held responsible for the twofold increase in the relative risk for schizophrenia in adolescent vulnerable cannabis users. In contrast, CBD appears to act non-psychoactively and might be used as a potential antipsychotic substance. In a first clinical trial in schizophrenia we showed that CBD decreases psychotic symptoms and induces fewer side effects compared to the antipsychotic drug amisulpride. By 1H nuclear magnetic resonance spectroscopy we found an elevation of the glucose concentrations in cerebrospinal fluid from acute naive first episode schizophrenic patients that indicates a disturbance of glucose metabolism (*Holmes et al., PloS Medicine, 2006, 3, 1420-1428*).

Otherwise [¹⁴C] 2-deoxyglucose imaging refers to an increase of local cerebral glucose use in some brain regions of rats by CBD (*Brett et al., SFN Atlanta, 2006*).

To investigate more precisely behavioural and metabolic effects of CBD, we conducted an animal study targeting the endocannabinoid system.

Methods: We compared effects of CBD with URB 597, the selective inhibitor of the fatty acid amide hydrolase (FAAH). Adult Lister hooded rats were chronically treated with either CBD (n=5), URB 597 (n=5), CBD+URB 597 (n=5) or vehicle (n=4) and underwent behavioural tests and 2-[¹⁸F] fluoro-2-deoxyglucose (FDG) μ PET analysis. The behavioural tests were analogous to those used to estimate negative symptoms, cognitive dysfunctions and anxiety of schizophrenic patients and measure general activity (open field), object-related working memory (object recognition task), social behaviour (social interaction task), anxiety (elevated plus maze) and sensori-motor gating mechanisms (prepulse inhibition).

Results: 1) In general, chronic administration of the mentioned substances had no behavioural effect on adult Lister hooded rats. None of the substances had a sedative or stimulating effect. Object recognition and the entire social interaction behaviour were not influenced. Just the time spent on non-anogenital investigation of foreign rats was longer after treatment with CBD compared to URB 597 and CBD+URB 597. Furthermore, no alteration in anxiety behaviour was observed and the substances did not influence prepulse inhibition.

2) CBD and URB as well as a combination of both seems to decrease the relative metabolic brain activity in awake, behaving rats in some regions, e.g. the hippocampus and parts of the limbic system (nucleus accumbens) as well as the anterior olfactory nucleus.

Conclusions: Chronic administration of CBD, URB 597 and CBD+URB 597 had no behavioural side effects in healthy adult rats, although the relative metabolic activity of some brain regions was unexpectedly decreased. Differences to the results of Brett et al. may be due to the chronic CBD administration instead of a single CBD injection. Nevertheless this study confirms clinical data of the few side effects of CBD and provides a basis for the upcoming study that will analyse possible antipsychotic effects of CBD in an animal model for schizophrenia.

EFFECTS OF CHRONIC CANNABIDIOL AND URB 597 ADMINISTRATION ON ENDOCANNABINOIDS AND RELATED LIPIDS IN DIFFERENT BRAIN REGIONS IN ADULT LISTER HOODED RATS (*RATTUS NORVEGICUS*)

Franziska Pahlisch¹, Cathrin Joepen¹, Carola Schaefer¹, Heike Endepols²,
F. Markus Leweke³

¹University of Cologne, Department of Psychiatry and Psychotherapy, Kerpener Str. 62, 50924 Cologne, Germany, ²Max Planck Institute for Neurological Research, Multimodal Imaging, Gleueler Str. 50, 50931 Cologne, Germany, ³Central Institute of Mental Health, Department of Psychiatry and Psychotherapy, J5, 68159 Mannheim, Germany

In the pathophysiology of psychiatric disorders the endocannabinoid system seems to play an important role besides the known dopaminergic and serotonergic disturbances. We have shown that cerebrospinal fluid (CSF) from schizophrenic patients contains significantly higher levels of the endocannabinoid anandamide than CSF from healthy volunteers. Moreover, CSF anandamide levels correlate inversely with psychotic symptoms, suggesting that anandamide release in the brain may serve as an adaptive mechanism countering neurotransmitter abnormalities in acute psychoses (Giuffrida et al., 2004, *Neuropsychopharmacology*, 2004, 29, 2108-14). Furthermore in a first clinical trial in schizophrenia the non-psychoactive cannabinoid Cannabidiol (CBD) - important ingredient of *Cannabis sativa* - was already shown to offer antipsychotic effects which were comparable to Amisulpride. It is very likely that in its mode of action the endocannabinoid system and other related lipids are involved.

In previous in-vitro assays we found first evidence that CBD inhibits the fatty acid amide hydrolase (FAAH). This enzyme is mainly responsible for the degradation of anandamide and other related fatty acid ethanolamides. In FAAH expressing rat brain membrane preparations CBD exhibits an IC₅₀ of 8,7µM for the inhibition of 3H-AEA hydrolysis. These data are consistent with the results of Bisogno et al. (*Br J Pharmacol*, 2001, 134, 845-52.), who found a slightly higher IC₅₀ of 27,5µM.

To illuminate the possible mechanism of CBD as an antipsychotic drug we conducted an animal study which included behavioural and metabolic testings.

Methods: Adult Lister hooded rats were chronically treated with either CBD, URB 597, the selective inhibitor of FAAH, CBD + URB 597 or vehicle. Beside the liver, the brains of the tested rats were removed and dissected in different regions. After lipid extraction of these tissues with methanol-chloroform the organic layer was further purified as described before (Fegley et al., *J Pharmacol Exp Ther*, 2005, 313, 352-8). Subsequent, the analytes anandamide (AEA), the further endocannabinoid 2-arachidonoylglycerol and the structural related derivatives oleoylethanolamide and palmitoylethanolamide as well as CBD were measured by isotope dilution LC-MS/MS (Giuffrida et al., *Anal Biochem*, 2000, 280, 87-93).

Results: 1.) FAAH-Inhibitor URB597 increased significantly (p<0.001) the amount of AEA, OEA and PEA in all brain areas except for the orbitofrontal cortex, compared to the vehicle whereas 2-AG concentration is unaltered both in the URB and in the CBD+URB group. The CBD treatment group did not differ from the vehicle group.

2.) Nearly the same results for all treatment groups were found in the liver.

Conclusions: In contrast to URB 597 CBD seems to act not as a FAAH-Inhibitor in this conducted study. A reason could be the used CBD concentration. It cannot be ruled out that the selected CBD dosage for injections was insufficient to block the FAAH enzyme.

Furthermore, the treated rats were healthy rats without any impairment and are therefore not comparable to schizophrenic patients. It is possible that CBD acts differently on the levels of the analytes dependent on the subjects conditions.

QUANTITATIVE AND QUALITATIVE ANALYSIS OF *CANNABIS SATIVA* SMOKE AND VAPOR CONSTITUENTS AND CB1 BINDING ACTIVITY

Justin Fishedick, Frank van der Kooy & Rob Verpoorte

*Division of Pharmacognosy, Section of Metabolomics, Institute of Biology, Leiden University,
PO Box 9502, 2300RA Leiden, The Netherlands*

Smoking cannabis is undesirable for medical purposes due to the inhalation of hazardous combustion products however vaporizing cannabis plant material seems to offer a viable safe alternative to smoking. Although much research has been done in the past to identify compounds found in cannabis smoke and even vapor most studies lack quantitative information about non-cannabinoid compounds and even minor cannabinoids present in cannabis smoke and vapor. Therefore it was the goal of this study to identify and quantify the main components found in the smoke and vapor of 3 different medicinal cannabis varieties. A secondary goal of this study was to see if there are any compounds besides Δ^9 -tetrahydrocannabinol (Δ^9 -THC) that contribute significantly to binding activity at CB1 receptor in the concentrations encountered in cannabis smoke and vapor.

Methods: Cannabis smoke was collected by burning 2 1 g cannabis joints per sample at a regular puff interval and sucking the smoke through two 50 mL solvent traps containing 1:1 ethanol: hexane with the use of a vacuum. Cannabis vapor was collected by vaporizing in the Volcano[®] in 250 mg portions 5 times per sample. Each bag of vapor was sucked through the solvent trap completely with the use of a vacuum. Cannabis smoke, vapor, and unprocessed extracts were analyzed by GC-FID, GC-MS, and HPLC. A radioactive displacement assay for CB1 binding was performed on all samples which were diluted to contain equimolar (10 nM) Δ^9 -THC levels and were compared with pure Δ^9 -THC at 10 nM concentration.

Results: Cannabis extracts contained cannabinoids, mono-terpenoids, and sesquiterpenoids with differences observed for all 3 varieties. Many of the same components found in cannabis extracts were also observed in cannabis vapor. Cannabis smoke contained many of the same compounds identified in cannabis extracts and vapor plus a host of other compounds which are formed or released from combustion of the plant material. There was no significant difference between CB1 binding of pure Δ^9 -THC when compared to cannabis smoke and vapor samples containing equimolar concentrations of Δ^9 -THC.

Discussion: Our results show that cannabis vapor contains many of the same compounds observed in cannabis extracts without the combustion by products observed in cannabis smoke. Therefore vaporizing cannabis may be a safer alternative to smoking that retains the pharmacokinetic advantages of inhalation. Furthermore the results of our CB1 binding assay indicate that only Δ^9 -THC is responsible for CB1 binding in cannabis smoke or vapor. Other components may contribute to the overall effects of medicinal cannabis however it is unlikely that such effects are due to binding at the CB1 receptor. Our quantitative analysis of cannabis smoke and vapor should serve as a guide for other researchers who wish to understand what components present in what concentrations could contribute to the medical effects of cannabis other than Δ^9 -THC.

EFFECTS OF CANNABIGEROL PURIFIED FROM CANNABIS EXTRACTS ON STEREOTYPED BEHAVIOUR INDUCED BY APOMORPHINE

Naoki Amada^{a,b}, Akihito Watanabe^{a,b}, Yuki Yamasaki^{a,b}, Serena Deiana^a,
Tetsuro Kikuchi^b, Gernot Riedel^a

^a *Institute of Medical Sciences, University of Aberdeen, Foresterhill, AB25 2ZD, UK*

^b *Os' Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, 771-0192, Japan*

Introduction: Understanding the pharmacology of the different cannabinoids and terpenoids of the hemp plant has received renewed interest. Potentially, these constituents, although available only in small amounts in the extract, may be of therapeutic use. Some may have been beneficial in the treatment of psychiatric disorders such as schizophrenia, anxiety, depression etc. In an attempt to assess the cannabis extract cannabigerol (CBG), which was chemically purified to >99% purity, we used apomorphine hydrochloride to induce stereotyped behaviour. Suppression of stereotyped behaviour is a characteristic of many antipsychotics and the model enables determination of the D₂ receptor antagonistic action of the compounds. At the same time, we set up the apomorphine protocol and validated the method by establishment of a dose-response profile.

Materials and Methods: Male Wistar Han rats (Harlan UK) were used in this study. Stereotyped behaviour in rats was induced by apomorphine at doses of 0.7, 2.0, 4.0 and 6.4 mg/kg subcutaneously; stereotypy was classified according to severity for up to 120 minutes. In addition, purified CBG (0.1, 1, 10 and 100 mg/kg i.p. 1 hour prior to apomorphine) was probed against 0.7 mg/kg apomorphine-induced stereotypy in rats.

Results: Apomorphine induced stereotyped behaviour at all doses but the length and severity of the effect was dose-dependent. While 0.7 mg/kg apomorphine lasted for 30-50 minutes, the highest dose 6.4mg/kg lasted for ~2hours. Other doses were intermediate. We used 0.7 mg/kg apomorphine as the weakest effective dose and evaluated pure CBG on stereotypy, but did not get suppression.

Discussion: This is the first report assessing possible dopaminergic activity of CBG. Although our results indicate that CBG, even at high doses, is devoid of an effect in this model, it at the same time revealed safety of the drug. A more detailed analysis of CBG also in relation to other doses of apomorphine may be warranted.

DEVELOPMENT OF *CANNABIS* INBRED LINES WITH HIGH PROPORTIONS OF NON PSYCHOTROPIC PROPYL CANNABINOIDS

Salvatore Casano^{1,2} and Giovanni Appendino³

¹CRA-CIN, Rovigo branch section, Italy

²DAAT, Faculty of Agricultural Sciences, University of Palermo, Italy

³DISCAFF, Faculty of Pharmacy, University of Piemonte Orientale (Novara), Italy

Hemp (*Cannabis sativa* L.), one of the oldest known medicinal plants, has yielded a host of pharmacologically important substances, exemplified by cannabinoids, a class of meroterpenoids currently investigated as a promising treatment for several pathological conditions, including multiple sclerosis.

In their neutral form, most phytocannabinoids are 21-carbon compounds that differ for their terpenoid moiety and its way of attachment to the pentyl resorcinyl core. However, a small number of phytocannabinoids deviate from this structural pattern, featuring a shorter (C-3 vs C-5) alkyl chain bound to the aromatic ring. The alkyl side chain is a critical element of the cannabinoid pharmacophore, affecting affinity, selectivity and pharmacological potency of these compounds. In the most common cannabinoids [cannabigerol (CBG-C5), Δ^9 -tetrahydrocannabinol (Δ^9 -THC-C5), cannabidiol (CBD-C5), cannabichromene (CBC-C5)] the alkyl group is a pentyl, while in their lower homologues (CBGV-C3, Δ^9 -THCV-C3, CBDV-C3, CBCV-C3, named using the suffix “varin” or “varol”), the pentyl chain is replaced by a propyl chain. The identification of traces amounts of methyl and butyl cannabinoids is also reported in literature, but propyl cannabinoids are by far the major group of “shortened” cannabinoids.

Δ^9 -THCV-C3 has psychotropic activity and can be detected in high proportions in a wide range of drug accessions. Lines with Δ^9 -THCV-C3 as predominant cannabinoid were obtained through selective inbreeding by GW Pharmaceuticals plc. No accessions of non-psychotropic propyl cannabinoids with a substantial proportion of other C3 cannabinoids has been reported so far.

In this study some individual plants were identified for the production of cannabinoids with a propyl side chain during a screening of germplasm. 65 pistillate plants of 21 drug-accessions, recreational strains and landraces, were analysed by gas chromatography and classified differently as: drug-, intermediate-, fibre- and propyl-chemotype.

Self-pollination of the single non psychotropic propyl-chemotype plant (CBD-C5/CBDV-C3 + CBC-C5/CBCV-C3), identified in a landrace from Sicily (Italy), permitted to develop inbred lines S₁ with various proportions of non psychotropic propyl cannabinoids, estimated from approximately 0.05 to 0.53 of the content of the respective pentyl cannabinoids. Preliminary data from gas chromatography analysis of S₂ plants obtained from two inbred lines S₁ different in the proportions of (CBDV-C3 + CBCV-C3), respectively high (0.47) and low (0.10), confirmed that the biosynthesis of propyl cannabinoids is genetically controlled, and that the trait can be fixed through inbreeding. The best performing inbred lines are currently exploited in a breeding program aiming at the creation and registration of a cultivar with high proportions of (CBDV-C3 + CBCV-C3), or, eventually, with these propyl cannabinoids as predominant.

Accessions rich in propyl cannabinoids are of chemotaxonomic significance and of relevant value for further breeding achievements, and their exploitation could become of economic relevance if the pharmacological interest for propyl cannabinoids is indeed confirmed.

CRYOPRESERVATION OF HEMP (*CANNABIS SATIVA* L.) GENETIC RESOURCES

Salvatore Casano^{1,2}, Roberta Biadolla¹ and Giampaolo Grassi¹

¹CRA-CIN, Rovigo branch section, Italy

²DAAT, Faculty of Agricultural Sciences, University of Palermo, Italy

An exceptionally high degree of genetic polymorphism has been reported for hemp (*Cannabis sativa* L.). The CRA-CIN maintains in Italy a wide diversity of its genetic resources. Seeds of industrial and pharmaceutical cultivars, accessions and breeding lines are stored at -20°C for long-term but germination percentages decline after a decade of conservation, then regeneration cycles should be ensured in order to save the genetic diversity. Unfortunately regeneration interval is not pre-fixed at our section because it depends on available funding.

Cannabis is an allogamous species and its pollen grains are disseminated by wind in large amounts and for long distances. Recommended isolation distances for the field regeneration of accessions must be guaranteed to avoid undesirable crosses and to maintain the genetic integrity of the accessions. The complexity and the costs of maintaining the genetic integrity of accessions are both elevated, consequently, the aim of the present study is to explore the feasibility of cryopreservation for seed and pollen long-term conservation in order to acquire preliminary data for further development of suitable cryoprotocols.

Cryopreservation for 1 hour of commercial seeds (cultivars Carmono, Felina 34, Finola and Uso 31) at three different cooling rates and their dehydration by exposure to Plant Vitrification Solution 2 (PVS2) (from 0 to 1440 minutes) were investigated. Farm-produced seeds (cultivars Carmono, Felina 34 and Uso 31) were dehydrated by exposure to air (from 5 to 15 days) before being immersed in liquid nitrogen for 1 hour or for 3 months. Viability of 10 years-old cryopreserved pollen was tested *in vivo* by evaluating seed set on cross-pollinated inflorescences. Opportunely, germination percentages of cryopreserved commercial seeds were not significantly different from germination percentage of seeds not immersed in liquid nitrogen (93.2%). ‘Slow’, ‘intermediate’ and ‘fast’ cooling rates did not significantly influence the germination percentages, respectively 92.8%, 93% and 92.8%, of seeds immersed in liquid nitrogen. A prolonged exposure to PVS2 (>60 minutes) caused a significant decrease on germination percentages. However, even after 1440 minutes of PVS2 dehydration, 64.9% of seeds germinated. It can be supposed that the optimal moisture content for long-term conservation of *Cannabis* seeds should be around 7-9%, that is the range of moisture contents at which seeds are normally commercialized.

Germination percentages of farm-produced seeds were significantly higher when air dehydrated for 15 days. Germination percentage of seeds immersed in liquid nitrogen for 1 hour (33.5%) was not significantly different from germination percentage of seeds not immersed in liquid nitrogen (31.3%). Interestingly, germination percentage of seeds immersed in liquid nitrogen for 3 months (41.2%) was significantly higher than germination percentages of seeds not immersed in liquid nitrogen and seeds immersed in liquid nitrogen for only one hour.

Additionally, four different batches of 10 years-old cryopreserved pollen (CBD chemotypes) successfully fertilized single individuals of two pistillate clonal progenies (Δ^9 -THC chemotypes). The viability of an undefined portion of pollen grains was confirmed by the obtainment of heterozygous chemotypes in the F₁ progenies (CBD: Δ^9 -THC chemotypes).

Cryopreservation of seed and pollen is the most effective strategy for long-term *ex situ* conservation of genetic resources, particularly for the simplicity of application and for the amount of diversity conserved. These results are the first report on cryopreservation of *Cannabis* genetic resources.

EFFECTS OF CANNABIS EXTRACTS RICH IN CANNABICHROMENE (CBC), CANNABIDIOL (CBD), CANNABIGEROL (CBG) OR TETRAHYDROCANNABIVARINIC ACID (THCVA) ON LOCOMOTOR ACTIVITY IN MICE

Serena Deiana^a, Akihito Watanabe^{a,b}, Yuki Yamasaki^{a,b}, Naoki Amada^{a,b}, Tetsuro Kikuchi^b, Gernot Riedel^a

^a Institute of Medical Sciences, University of Aberdeen, Foresterhill, AB25 2ZD, UK

^b Q's Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, 771-0192, Japan

Introduction: Cannabinoids have recently been on the focus of scientific attention for their therapeutic potential on several domains, such as cancer, obesity, psychosis, depression. The hemp plant *Cannabis sativa* contains at least 66 phytocannabinoid that have been isolated to date, and some of the few investigated so far proved to own therapeutic properties. Here, we explored the behavioural effects of some novel phytocannabinoids enriched in genetic variations of the cannabis plants. Cannabinoid agonists affect basal locomotor activity leading, at high doses, to catalepsy. This is due to CB1 receptor-mediated drug actions, which may ultimately modulate other transmitter systems such as serotonin, glutamate and/or dopamine. Severe effects on motor abilities may be a potential confounder in behavioural tests, when cannabinoids are investigated for their therapeutic potential.

Materials and methods: Plant extracts rich in Cannabichromene (CBC), Cannabidiol (CBD), Cannabigerol (CBG) and Tetrahydrocannabivarinic acid (THCVA) provided by GW Pharmaceuticals were administered (ip) at doses between 30-100mg/kg to male adult ICR mice (Harlan UK) 30 minutes prior to a 20 minutes open field test to assess effects on basal locomotor activity.

Results: CBD and CBC extracts decreased locomotor activity in a dose-dependent manner, with the lowest doses of 30 mg/kg showing only minor effects. By contrast, THCVA not only dramatically decreased locomotor activity in a dose-independent manner, but it also induced sedation and unspecific side-effects in animals even at the lowest dose. Administration of CBG had no effect on locomotion, even at 100 mg/kg.

Conclusions: The present study showed for the first time the effects on locomotor activity induced by the plant extracts enriched in the phytocannabinoids CBD, CBC, CBG or THCVA. Apart from CBG, all extracts induced hypo-motility in mice, with THCVA showing signs of gross side effects. Since the pharmacology of THCVA still remains elusive, it is difficult to speculate on its mechanistic actions. However, lower doses need to be considered and may prove therapeutically viable. As for CBC, CBG and CBD, effects on motor activity were small or absent. Again, whether these are due to CB1 mediated mechanisms remains to be determined.

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CANNABINOID-INDUCED BEHAVIORAL EFFECTS IN A MOUSE MODEL OF AUTISM SPECTRUM DISORDERS

Tara Halpern, Mersiha Mehanovic, Norman Schanz, Robert Benno and Emmanuel Onaivi.
Department of Biology, William Paterson University, Wayne, NJ 07470.

The goal of this research is to determine the role that the endocannabinoid system plays in the development of behavioral changes associated with autism spectrum disorders (ASD) in a mouse model. The working hypothesis is that the ubiquitous endocannabinoid system (eCBs) may be involved in the biological processes involved in the development of behavioral and biochemical changes associated with autism. Much progress in cannabinoid research has revealed an endocannabinoid system in animals and humans. The eCBs consists of genes encoding cannabinoid receptors (CB1-Rs and CB2-Rs), their endogenous ligands called endocannabinoids and proteins that synthesize and degrade these endogenous cannabinoid ligands. Both CB1-Rs and CB2-Rs are distributed in the brain and peripheral tissues and are activated by endocannabinoids, and also the active ingredient in cannabis, Δ^9 -THC. We recently observed that the basal level of CB2A gene expression in the BTBR T+tf/J mice was upregulated in the cerebellum compared to control mice.

Methods: In this study we used the BTBR T+tf/J mice that have been shown to exhibit autism-like behavioral phenotypes to evaluate cannabinoid-induced behavioral changes. The forced swim test (FST) that is a reliable model with predictive validity for screening anti-depressants and the wheel running activity that measured the basal locomotor activity of the animals were used to study the cannabinoid-induced behavioral effects. We first determined the basal behavioral effects of the BTBR mice in the forced swim test (FST) and spontaneous wheel running activity in comparison to those of C57BL/6Js and 129S1/SvImJ that are the background control mice. Then we evaluated the effects of Δ^9 -THC (1 and 10 mg/kg ip) following 20 mins of administration. For the FST, all animals were pre-exposed for 15 mins on day one prior to the 5 min- swim test on day 2. On test days animals were treated acutely for 20 mins and tested on the FST for 5 mins or 10 mins for the spontaneous wheel running behavior.

Results: Our preliminary results indicate that the BTBR mice exhibited an enhanced basal spontaneous locomotor behavior in the spontaneous wheel running test and a reduced depressogenic profile in comparison to the control animals. The responses appeared to be enhanced by Δ^9 -THC.

Conclusions: In conclusion, these preliminary data provides a basis for further studies in evaluating the role of the various components of the endocannabinoid system in the etiology of autism spectrum disorders.

EFFECTS OF TETRAHYDROCANNABIVARIN (THCV) ON CONDITIONED AVOIDANCE RESPONDING IN RATS

Yuki Yamasaki^{a,b}, Naoki Amada^{a,b}, Akihito Watanabe^{a,b}, Serena Deiana^a,
Tetsuro Kikuchi^b, Gernot Riedel^a

^a Institute of Medical Sciences, University of Aberdeen, Foresterhill, AB25 2ZD, UK

^b Qs' Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, 771-0192, Japan

Introduction: The hemp plant *Cannabis sativa* contains a large number of phytocannabinoids, which can be extracted and may convey various benefits for human health. This includes the cannabinoid THCV, a putative CB1 receptor antagonist with no inverse agonism. Given that recent reports confirmed cannabinoids as potential antipsychotics in animal models like apomorphine-induced stereotyped behaviour, we reasoned that such examinations should be extended to other test. Here we selected the conditioned avoidance response (CAR) paradigm, which has been viewed traditionally as an animal model with high predictive validity for the screening of antipsychotic candidate compounds, since all clinically effective antipsychotics suppress the avoidance responding.

Materials and Methods: Male Wistar Han rats aged 7 weeks (Harlan UK) were used in this study. Animals were trained to criterion (3 successive days of 75% correct), matched according to performance for drug groups and tested on the following day. THCV was administered at doses between 0.03-60 mg/kg and tested against vehicle. As well as CAR, intertrial interval (ITI) crossing and avoidance latency, which indicated possibility of motor abnormality and lowered responding, were measured.

Results: Intraperitoneal treatment with THCV did not show any significant suppression on CAR at any dose tested. Moreover, no differences between vehicle- and drug-treated groups on ITI crossing and avoidance latency were found.

Discussion: This is the first report to analyse the effects of THCV on the suppression of CAR in rats. THCV has been shown to be active in doses administered here in tests of eating disorder (Riedel et al., 2009; *Br J Pharmacol.*). While these actions may be due to the putative CB1 antagonism of the drug, this suggests that CB1 blockade may not lead to alterations in avoidance responding.

EFFECT OF LIGHT INTENSITY ON PHOTOSYNTHETIC CHARACTERISTICS OF FOUR HIGH Δ^9 -THC YIELDING VARIETIES OF *CANNABIS SATIVA*

Zlatko Mehmedic¹, Suman Chandra¹, Hemant Lata¹, Ikhlas A. Khan^{1,2}
and Mahmoud A. ElSohly^{1,3}

¹National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA. ²Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA. ³Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, MS 38677, USA.

Cannabis sativa L. (Cannabaceae), an annual herb is the natural source of cannabinoids that mainly accumulates in glandular trichomes of the plant. Due to the allogamous (cross fertilization) nature of *Cannabis sativa* it is very difficult to maintain the efficacy of selected high THC yielding elite varieties if grown from seeds under field conditions. Thus, the indoor cultivation, under controlled environmental conditions, using vegetative propagation of selected high yielding female clones can be a better alternative for its mass propagation. In the present study, plants of four drug type *Cannabis* varieties namely HPM, MX, K2 and W1 were grown indoor, under controlled environmental conditions ($25 \pm 3^\circ\text{C}$, $55 \pm 5\%$ RH and $\sim 700 \pm 24 \mu\text{mol m}^{-2}\text{s}^{-1}$ light at plant canopy level). Gas and water vapour characteristics of these plants were studied at different Photosynthetic Photon Flux Density (PPFD; 000, 500, 1000, 1500 and 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$) for their efficient indoor cultivation. An increasing trend in photosynthesis (P_N), transpiration (Tr) and stomatal conductance (gCO_2) was observed with increase in PPFD up to 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$ in all the varieties at optimum growth temperature ($25 \pm 3^\circ\text{C}$). However, the magnitude of increase and maximum rate of P_N ($P_{N \text{ max}}$) varied with the varieties. Highest rate of photosynthesis was observed in W1 followed by MX, K2 and HPM. Water Use efficiency (WUE) in W1, MX and HPM increased with light up to highest level tested, whereas, in K2 highest WUE was observed at 1500 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Our results show that this species is able to use high level of PPFD for its P_N and therefore, may be cultivated in under bright indoor light (~ 1500 to 2000 $\mu\text{mol m}^{-2}\text{s}^{-1}$) for better growth and biomass. The strict control of other environmental factors however, should be maintained for the higher yield.

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ABUSE POTENTIAL OF NABIXIMOLS OROMUCOSAL SPRAY (SATIVEX[®]) COMPARED WITH DRONABINOL AND PLACEBO IN RECREATIONAL MARIJUANA USERS

Schoedel K¹, Chen N¹, Sellers E¹, Stott CG²

¹ Kendle Early Phase, Toronto, Canada, ²GW Pharma Ltd, Cambridgeshire, UK

Introduction: Nabiximols oromucosal spray (Sativex[®]) containing delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), is approved in Canada for neuropathic pain in multiple sclerosis and cancer pain. The active comparator, dronabinol (Marinol[®], synthetic THC), has some abuse potential but is not significantly abused.

Objective: Comparative evaluation of abuse potential of nabiximols versus dronabinol and placebo.

Methods: A randomized, double-blind, six-period crossover study with nabiximols four, eight and 16 sprays (containing 10.8, 21.6 and 43.2 mg THC), dronabinol 20 and 40 mg THC, and placebo in 23 active recreational marijuana users. The three primary endpoints were: Addiction Research Centre Inventory Morphine Benzodrine Group (ARCI-MBG), Drug Liking Visual Analogue Scale (DL-VAS), and Subjective Drug Value (SDV). Secondary endpoints included other VASs and ARCI subscales; all measures were tested over 24h following drug intake.

Results: Four and eight sprays nabiximols were not different from placebo on the ARCI-MGB scale, while the 16 spray dose was similar to 20mg dronabinol. For the DL-VAS and SDV scales, four sprays of nabiximols were not significantly different from placebo while eight and 16 sprays were similar to dronabinol 20 and 40 mg, respectively. In general eight sprays nabiximols had lower effects than dronabinol 20mg, while the 16 sprays dose was intermediate between 20 and 40 mg dronabinol.

Nabiximols eight sprays produced significantly lower scores than dronabinol 20mg on the following secondary endpoints: Overall Drug Liking, High, Stoned & Drowsiness VASs and on ARCI Marijuana & LSD scales.

The adverse event (AE) profile was similar between nabiximols and dronabinol, the majority of AEs were mild and there were no serious AEs.

Conclusions: In an abuse-prone population, nabiximols showed no greater abuse potential than dronabinol, with similar abuse potential to placebo for the primary endpoints at the low dose (four sprays). On a dose-per-dose basis, nabiximols had lower effects than dronabinol, suggesting these drugs are subjectively different.

ABUSE POTENTIAL OF SATIVEX® OROMUCOSAL SPRAY (NABIXIMOLS) COMPARED WITH DRONABINOL AND PLACEBO IN RECREATIONAL MARIJUANA USERS

Guy GW, Wright S, Stott CG, White L, Russo E

GW Pharma Ltd, Cambridgeshire, UK

Introduction: Sativex® oromucosal spray (nabiximols) containing delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), is approved in Canada for neuropathic pain in multiple sclerosis and cancer pain. The active comparator, dronabinol (Marinol®, synthetic THC), has some abuse potential but is not significantly abused.

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RESULTS OF A RANDOMISED WITHDRAWAL STUDY OF SUBJECTS WITH SPASTICITY DUE TO MULTIPLE SCLEROSIS WHO WERE RECEIVING LONG TERM SATIVEX[®]

Notcutt W, Davies P, Langford R, Ratcliffe S

GW Pharma Ltd, Cambridgeshire, UK

Introduction: Sativex[®] (nabiximols), an endocannabinoid system modulator, is approved in Canada for the relief of central neuropathic pain in multiple sclerosis (MS) and cancer pain.

Objective: To assess the maintenance of efficacy after long-term treatment with Sativex for spasticity in MS.

Methods: Eligible patients entered this five-week placebo-controlled, parallel-group, randomised withdrawal study. Patients were required to continue taking the medication at their current effective and tolerated dose. Patients could drop out at any time and return to their own supplies of prescribed Sativex.

The primary endpoint was time to treatment failure (defined as: withdrawal without completing the 28-day randomised treatment period, worsening of spasticity, or an increase in anti-spasticity/disease-modifying medication after randomisation). Secondary endpoints included: spasticity severity 0-10 Numeric Rating Scale (0-10 NRS), Sleep Disruption 0-10 NRS, Modified Ashworth Scale (MAS), Timed 10m walk, Motricity Index, and Carer and Subject's Global Impression of Change (CGIC & SGIC).

Results: 36 patients were enrolled (n=18 per group). Mean duration of MS and spasticity were 16.4 and 12.7 years respectively, with a mean of 3.6 years of Sativex use. Mean doses of study medication were 7.3 and 9.2 sprays per day respectively for Sativex and placebo. Three patients (16.7%) on Sativex withdrew from the study whilst 16 (88.9%) withdrew on placebo. Eight Sativex patients (44.4%) were treatment failures compared with 17 (94.4%) on placebo. Time to treatment failure was significantly in favour of Sativex (p=0.013; Hazard ratio=0.335; 90% CI: 0.162, 0.691). All secondary endpoints (except the MAS) showed trends in favour of Sativex, reaching statistical significance in the SGIC and CGIC (functional ability) scales (i.e. a greater worsening on placebo; p=0.017 and 0.001 respectively).

Conclusions: Withdrawal of Sativex treatment precipitated significant worsening in spasticity in a significant number of patients switched onto placebo. Maintenance of long-term efficacy was demonstrated with Sativex compared with placebo in this randomised-withdrawal setting.

A TWO-PHASE STUDY OF SATIVEX[®] IN THE RELIEF OF SPASTICITY DUE TO MULTIPLE SCLEROSIS: PHASE A SINGLE-BLIND RESPONSE ASSESSMENT FOLLOWED BY PHASE B DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED, PARALLEL-GROUP STUDY

Notcutt W, Z Ambler, P Davies, C Gasperini, J Haas, A Klimek & X Montalbán

GW Pharma Ltd, Cambridgeshire, UK

Introduction: Cannabinoids can ameliorate pain, spasticity and muscle spasms in multiple sclerosis (MS).

Objective: To assess the efficacy and safety of Sativex[®] (delta-9-tetrahydrocannabinol [THC]: cannabidiol [CBD] oromucosal spray) in MS spasticity using an enriched study design.

Methods: A 4-week single-blind treatment period (Phase A) was performed to identify initial responders to Sativex ($\geq 20\%$ reduction in spasticity score on a 0-10 numerical rating scale [NRS]). Responders then entered a 12-week double-blind, randomised, placebo-controlled, parallel-group study (Phase B). The primary endpoint was the mean change from baseline in spasticity severity NRS score. Daily dose, spasm frequency, sleep disruption, Modified Ashworth Score, quality of life, mood and safety measures were recorded throughout. Barthel Activities of Daily Living (ADL) Index and Timed Ten Metre Walk (TTMW) and Global Impression of Change were also assessed.

Results: 572 patients enrolled into Phase A: 272 (48%) were identified as initial responders, of whom 241 continued into Phase B.

In Phase B, the primary endpoint was significantly in favour of Sativex ($p=0.0002$), with an improvement of -0.04 in spasticity NRS compared with a 0.81 deterioration in placebo patients. 74% of Phase B Sativex-treated patients improved 30% or more from Phase A baseline ($p=0.0003$). Spasm frequency, sleep disruption NRS, and Physician, Carer and Subject Global Impression of Change and Barthel ADL were all significantly in favour of Sativex ($p=0.0046$, $p<0.0001$, $p=0.0045$, $p=0.0053$, $p=0.0234$ and $p=0.0067$ respectively). The mean dose of Sativex in Phase B was 8.3 sprays/day.

In Phase A, 268 of 572 patients (47%) reported at least one AE within the first four weeks of treatment, including the 10-day up-titration period, the most common being dizziness (14%). Of the 241 Phase B patients, 66 (53%) on Sativex and 57 (49%) on placebo reported at least one AE, most commonly urinary tract infection (Sativex 7%, placebo 10%). The majority of AEs were mild or moderate (92%).

Conclusions

Sativex[®] demonstrated a statistically significant and clinically relevant improvement in spasticity and was well tolerated in MS patients.

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Program and Abstracts

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