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ORIGINAL INVESTIGATION

Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour

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Abstract
Rationale Phytocannabinoids are useful therapeutics for multiple applications including treatments of constipation, malaria, rheumatism, alleviation of intraocular pressure, emesis, anxiety and some neurological and neurodegenerative disorders. Consistent with these medicinal properties, extracted cannabinoids have recently gained much interest in research, and some are currently in advanced stages of clinical testing. Other constituents of *Cannabis sativa*, the hemp plant, however, remain relatively unexplored in vivo. These include cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (Δ^9-THCV) and cannabigerol (CBG).
Objectives and methods We here determined pharmacokinetic profiles of the above phytocannabinoids after acute single-dose intraperitoneal and oral administration in mice and rats. The pharmacodynamic–pharmacokinetic relationship of CBD (120 mg/kg, ip and oral) was further assessed using a marble burying test in mice.
Results All phytocannabinoids readily penetrated the blood–brain barrier and solutol, despite producing moderate behavioural anomalies, led to higher brain penetration than cremophor after oral, but not intraperitoneal exposure. In mice, cremophor-based intraperitoneal administration always attained higher plasma and brain concentrations, independent of substance given. In rats, oral administration offered higher brain concentrations for CBD (120 mg/kg) and CBDV (60 mg/kg), but not for Δ^9-THCV (30 mg/kg) and CBG (120 mg/kg), for which the intraperitoneal route was more effective. CBD inhibited obsessive–compulsive behaviour in a time-dependent manner matching its pharmacokinetic profile.
Conclusions These data provide important information on the brain and plasma exposure of new phytocannabinoids and guidance for the most efficacious administration route and time points for determination of drug effects under in vivo conditions.

Keywords Phytocannabinoids · Systemic administration · Bioavailability · Area under curve · Elimination half-life · Pharmacokinetic · Pharmacodynamic · Rats · Mice

Introduction

Phytocannabinoids have been used as medicines for thousands of years, and their use for the treatment of

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constipation, malaria, rheumatism and menstrual pain has been first published around 2800 B.C. in the Chinese compendium of herbal medicines (Iversen. 2007). Nowadays, cannabinoids (CB) are known to possess several effects including catalepsy, hypothermia, anti-inflammation, antinociception (Pertwee 2008) and control of cell survival, particularly in cells of the central nervous system (CNS) (Guzman et al. 2002). Moreover, a number of therapeutic applications have been proposed, including the alleviation of intraocular pressure (Carlini. 2004), emesis (Soderpalm et al. 2001), anxiety (Sethi et al. 1986), multiple sclerosis (Rog. 2010), pain (Turcotte et al. 2010), nausea associated with cancer chemotherapy (Carey et al. 1983; Voth and Schwartz. 1997) and mood disorders (Bambico et al. 2009). Since the discovery of the main psychoactive constituent of *Cannabis sativa* L. Δ^9-tetrahydrocannabinol (Δ^9-THC) (Gaoni and Mechoulam 1964), research confirmed the presence of about seventy phytocannabinoids (Elsohly and Slade. 2005). Indeed, besides Δ^9-THC, other cannabinoids are produced in relatively high concentrations; amongst these is cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (Δ^9-THCV) and cannabigerol (CBG) (Fig. 1). They are present in the plant at different proportions depending on the specific variety.

With respect to these minor phytocannabinoids, research has concentrated on CBD, the second most abundant constituent of the hemp plant. In contrast to Δ^9-THC, it is not psychoactive and with K_i values of >2,300 nM has relatively low affinity for CB receptors (Pertwee 1997; Pertwee et al. 2010). Recently, it has emerged that CBD can antagonize CB_1/CB_2 receptor agonists and may behave as a CB_1 partial antagonist and CB_2 receptor 'inverse agonist' (Thomas et al. 2007). It also blocks the deorphanised receptor GPR55 (Pertwee 2007; Ryberg et al. 2007), activates both TRPV1 channel (Bisogno et al. 2001) and 5-HT_{1A} receptors (Russo et al. 2005; Pertwee 2008) and inhibits the enzymatic activity of fatty acid amide hydrolase as well as the reuptake of the endocannabinoid anandamide (Ligresti et al. 2006). Given this complex pharmacological profile, research suggests that CBD is protective in several animal models of neurodegeneration and mental disorders, and clinical trials have confirmed potential benefits in the management of Alzheimer's disease, multiple sclerosis (MS), Parkinson's disease and amyotrophic lateral sclerosis (Iuvone et al. 2009). CBD also ameliorated positive and negative symptoms of schizophrenia (Zuardi et al. 2006; Zuardi. 2008; Leweke et al. 2009) and was shown to be anxiolytic (Pertwee 2004; Zuardi 2008). Finally, CBD induced a small non-significant reduction of food intake (Riedel et al. 2009), and it exerted beneficial effects on bone formation and fracture healing (Whyte et al. 2009).

Another phytocannabinoid that has attracted some recent interest is CBDV, the content of which is higher in plants of *Cannabis indica* than in plants of *C. sativa* (Hillig and

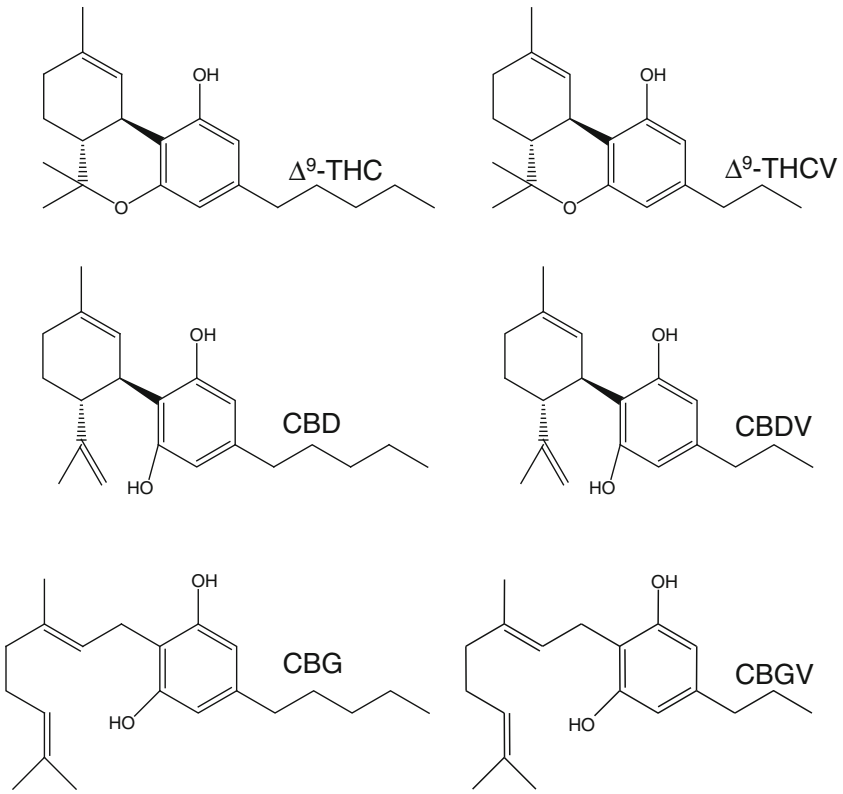

Fig. 1 Chemical structures of phytocannabinoids used in this study and their sister compounds. Both Δ^9-THC and CBGV are drawn for comparison

Mahlberg 2004). Under acidic conditions, it isomerizes into Δ^9-THCV and it is the biosynthetic precursor of Δ^9-THCV in the plant. It is not known whether CBDV would isomerise in vivo at stomach pH levels. To date, very little research has been performed on CBDV, with the majority of papers reporting chemical or botanical studies. Recent work, however, reported on its anti-inflammatory action (Tubaro et al. 2010) and its beneficial effects on bone formation and fracture healing by stimulating the recruitment of quiescent mesenchymal stem cells present in bone marrow (Scutt and Williamson. 2007).

Early experiments using Δ^9-THCV indicated its Δ^9-THC-like pharmacology by inducing catalepsy in mice (Gill et al. 1970) and in humans (Hollister. 1974), but with much lower potency. More recently, in vitro studies confirmed that Δ^9-THCV acts as a CB_1 and CB_2 antagonist and also as a CB_2 receptor partial agonist, whereas in vivo, depending on the dose, it can behave as a CB_1 antagonist (at doses <3 mg kg^{-1} i.v.) or as a CB_1 agonist (at doses >10 mg kg^{-1} i.v.) (Thomas et al. 2005). Importantly, Δ^9-THCV at low doses is devoid of inverse agonism at both CB_1 and CB_2 receptors, making it an interesting candidate for therapeutic use as it may not lead to depression and suicidal ideation, which have been widely reported for other CB_1 antagonists/inverse agonists (de Mattos Viana et al. 2009; Despres 2009). Potential benefits in terms of clinical use may include the reduction of seizures (Hill et al. 2010), pain relief in inflammatory models in mice (Bolognini et al. 2010) and weight loss due to hypophagic properties (Riedel et al. 2009).

Of recent scientific interest is CBG, which has been found to activate alpha(2)-adrenoceptors, to block 5-HT_{1A} and CB_1 receptors and bind to CB_2 receptors (Cascio et al. 2010). It possesses antibacterial (Appendino et al. 2008) and antitumoural (Baek et al. 1998) activity and may serve as a treatment for glaucoma (Colasanti et al. 1984; Colasanti 1990). Data on neuronal activity are currently lacking.

Given the multitude of actions of these phytocannabinoids, it is suggested that they may have a potential medicinal value for the treatment of a variety of conditions, once future research has unravelled their toxicology, pharmacology and molecular mechanisms. In order to perform comprehensive pre-clinical research on therapeutic effects of these phytocannabinoids knowledge of their pharmacokinetic (PK) and pharmacodynamic properties would be useful. Their lipophilicity and multitude of neurological actions makes them particularly interesting candidates as medicines to cure CNS disorders. Differences in the pharmacodynamics and potency of cannabinoids may depend upon their penetrability and disposition in the brain. Notably, some of the difficulties with cannabinoids arise from the choice of vehicle, the route of administration and species-specific differences in absorption, pharmacokinetics and tissue distribution. This has become clear from the pharmacokinetic profiling of Δ^9-THC (Mantilla-Plata and Harbison. 1975; Alozie et al. 1980; Agurell et al. 1986; Goulle et al. 2008), CBD (Alozie et al. 1980; Agurell et al. 1986) and cannabichromene (Alozie et al. 1980; Agurell et al. 1986). By contrast, there are no reports on CBG, CBDV and Δ^9-THCV. Most PK studies are conducted in humans, and following inhalation of cannabis, obviously no data on brain penetration can be reported in these studies, whereas little is published in animals including alternative administration routes such as intraperitoneal and oral.

Our test cannabinoids were extracted from *C. sativa* and purified. We aimed at establishing the systematic route-specific (oral, intraperitoneal) pharmacokinetic profile of four phytocannabinoids, CBD, CBDV, THCV and CBG, in plasma and brain of rats and mice after acute single-dose administration. An initial pilot study also explored the use of two vehicles (cremophor or solutol) to evaluate possible advantages in cannabinoid absorption/bioavailability, but in the following, we opted for cremophor as solvent. In a further step towards confirmation of pharmacodynamic drug efficacy, we explored the action of CBD accordingly to its pharmacokinetic profile. We tested mice injected with CBD in the marble burying test (MBT), a behavioural assay sensitive to CBD (Casarotto et al. 2010). MBT measures obsessive–compulsive behaviour (Thomas et al. 2009) and is sensitive to antidepressants (Takeuchi et al. 2002; Li et al. 2006; Nicolas et al. 2006), anxiolytics (Njung'e and Handley 1991; Borsini et al. 2002; Li et al. 2006; Nicolas et al. 2006) and typical antipsychotics (Broekkamp et al. 1986). Recently, the involvement of CB_1 cannabinoid receptors in MBT was also confirmed (Gomes et al. 2011).

Materials and methods

Drugs

CBD, Δ^9-THCV, CBG and CBDV, extracted from *Cannabis* plants, were subsequently purified and provided by GW Pharmaceuticals (Porton Down, Wiltshire, UK). Purity for all extracted phytocannabinoids was above 95%; HPLC traces are provided as Supplementary Fig. 1a–d. Compounds were stored in a freezer (at approximately −20°C), protected from light and freshly prepared immediately prior to injection. Drugs were suspended in cremophor EL/ethanol/saline in a ratio of 1:1:18 and mixed by Vortex shaker followed by sonication. Cannabinoids were injected intraperitoneally (ip) and orally (po) at the dose of 120 mg/kg (CBD and CBG), 60 mg/kg (CBDV) and 30 mg/kg (Δ^9-THCV) in 10 (mouse) or 5 (rat)ml/kg injection volumes. Doses were based on maximal concentration to be diluted in solvent. In a separate

experiment performed in rats, CBD was also suspended in a different vehicle—30% Solutol® HS 15 (BASF, Ludwigshafen, Germany). In the Marble burying test, CBD was administered to mice at the dose of 120 mg/kg either orally or ip 2 and 6 h prior to test.

Animals

Young adult male Wistar rats (200–250 g) and male Swiss mice (25–30 g) (Harlan UK) were allowed food and water ad libitum and maintained under standard conditions (temperature 20−22°C; 60−65% relative humidity) on a 12-h light/dark cycle (light on at 07:00). Communities of five (rats) or ten (mice) animals were housed in groups until the beginning of the study, when they were individually caged 24 h prior to drug administration. Subjects receiving oral injection were food-deprived over night.

Experimental design

Marble burying test

Pure CBD or vehicle (cremophor-based) was administered intraperitoneally or orally 2 h (vehicle and CBD) or 6 h (CBD) prior to test. The time point of 2 h was selected based on T_{max} for plasma at which time point maximal behavioural effects were predicted. At 6 h post-injection, the concentration of CBD in plasma and brain was substantially reduced; consequently, we did not observe altered burying behaviour. No tests at later time points were conducted as a result. Fifteen mice (Swiss) per group were used. Mice were habituated to the test room for 1 h and were thereafter exposed to the experimental cage (Plexiglass, 45×25.5×11 cm) filled with 5 cm sawdust for 5 min, in the absence of marbles. Thirty minutes later, mice were tested for their marble burying activity. Twenty black glass marbles (16 mm diameter) were equally spaced in the cage as shown in Fig. 6c and considered as buried when it was sawdust-covered by two third of its volume. Displacement of non-buried marbles and burying of marbles was noted as dependent variables.

Statistics

Behavioural data were expressed as group mean±SEM and analysed by conventional statistics (GraphPad Prism 5.02) using unpaired Student *t* tests. Alpha level was set to 0.05.

Pharmacokinetic study

A comparative rat and mouse pharmacokinetic study of four phytocannabinoids was conducted using oral and intraperitoneal administration routes. For each route, four subjects were sacrificed at 0.5, 1, 2, 4, 6 and 24 h after drug administration by injection of an overdose of anaesthetic (10 ml/kg Avertin; tribromoethanol). Control animals were administered vehicle and sacrificed 1 h later. After terminal anaesthesia, blood and brain samples were harvested. Blood was taken via cardiac puncture using heparinized plastic syringes and was stored in glass tubes, kept refrigerated in dry ice for a maximum of 30 min and thereafter was centrifuged for 10 min at 3,000 RPM. Finally, plasma was frozen at −80°C until analysis.

After cardiac puncture, 0.9% saline was infused into the animal's circulatory system to clear the brain tissues of blood. Whole brain was collected and stored at −80°C in plastic universal vials for analysis. Brains were homogenized in a mixture of 90% acetonitrile with 10% water; the water contained 0.1% (*w*/*v*) ascorbic acid prior to mixing. The ratio of brain tissue to extraction solvent was 1.0:1.5 (*w*/*v*). Each individual brain was weighed and 1.5× (*w*/*v*) homogenate cold solvent (90% acetonitrile+10% water containing 0.1% ascorbic acid) was added. Each brain sample was thereafter homogenized for ~1 min.

Analytical methods

Mass spectrometer instrument parameters Sample analysis was performed commercially by Biofocus (Chesterford Research Park, Chesterford Pk, Little Chesterford, Saffron Walden, UK). Analytical conditions were optimized on a Quattro Micro mass spectrometer (S/N: QAA028, Waters Ltd, 730-740 Centennial Court, Centennial Park, Hertfordshire, UK). The settings of the electrospray ion source used for method development and subsequent data acquisition are detailed in Table 1 for all four cannabinoids. Multiple daughter ions were observed and the multiple reaction monitoring listed in Table 2 was selected as giving the best instrument response.

Detection limits The limit of detection was determined to be 3 ng/ml for CBD, 1 ng/ml for CBDV and 1 and 2 ng/ml for Δ^9-THCV and CBG in plasma and brain homogenate. The limit of quantification for both matrices was then set at 25 (mice) or 10 (rats)ng/ml for CBD and 5 ng/ml for CBDV, Δ^9-THCV and CBG.

Sample analysis

Quantification of cannabinoids in plasma Plasma concentrations of compounds in study samples were quantified using appropriate calibration standards prepared in blank rat and mouse plasma. Plasma proteins were precipitated and test compounds extracted using three volumes of acetoni-

Table 1 Instrument settings for mass spectrometer to detect cannabinoids in biological tissues

Parameter setting	Compounds			
	CBD	CBDV	Δ^9-THCV	CBG
Capillary voltage (kV)	3	3.5	3.5	3.5
Extractor cone voltage (V)	3	40	40	40
Source temp (°C)	120	150	150	150
Desolvation gas temp (°C)	400	500	500	500
Desolvation gas flow (L/h)	650	1,000	1,000	1,000
Cone gas flow (L/h)	100	100	100	100

Note that for CBDV, Δ^9-THCV and CBG, the sensitivity was similar and identical settings were applied. Also, no adjustments were required for different samples or species

trile containing internal standard. Extracted samples were stabilized with 50% aqueous acetonitrile containing 0.1% (*w*/*v*) ascorbic acid prior to injection. For CBD, two calibration ranges were used: a higher range of 100–50,000 ng/ml and a lower range of 10–2,000 (rat) and 25–7,500 (mice)ng/ml for confirmation of results at sample concentrations below 100 ng/ml, whereas a calibration range of 5–2,000 ng/ml, 5–4,000 ng/ml and 5–5,000 ng/ml was selected for quantification of CBDV, Δ^9-THCV and CBG, respectively. Settings and materials of HPLC are illustrated in Table 3.

Brain sample analysis Brain homogenates were centrifuged and aliquots of supernatant mixed 1:4 (CBD), or 1:7 (CBDV) and 1:10 (Δ^9-THCV and CBG) with internal standard solution, prepared in 50% aqueous acetonitrile containing 0.1% ascorbic acid. Study samples with concentrations above the calibration curve were re-analysed with appropriate dilution with blank brain homogenate prior to centrifugation.

Pharmacokinetic analysis Pharmacokinetic parameters were determined by non-compartmental analysis using the software package, PK Solutions 2.0 (Summit Research Services). Area under the curve (AUC) values were calculated by the trapezoidal method. Maximal drug concentration in tissue (C_{max}), time to reach maximal tissue levels (T_{max}), apparent elimination half-life (2–6 h—po; 4–24 h—ip), AUC and brain/plasma ratios based on AUC (0–6 h) were evaluated for plasma and brain samples at six time points following ip and oral administration of the tested cannabinoids.

Results

Pharmacokinetic study

Recovery of each phytocannabinoid in LC/MS–MS was converted into time–concentration plots, and averages for each group are presented. Specific emphasis was placed on comparisons between administration routes within and between rodent species. The extended gap between the 6- and 24-h time points makes extrapolation somewhat unpredictable; thus, values for AUC are provided for the period 0–6 and 0–24 h as well as the extrapolated values for 0–∞. When data are available, in the text we only refer to 0–∞; otherwise, we use AUC (0–6 h). Since intravenous administration was not part of this study, it was not possible to determine absolute exposure and true elimination half-life. Hence, it remains unclear whether low circulating concentrations, relative to dose level, reflect slow absorption from the site of administration or extensive distribution and elimination.

Cannabidiol

Administration of 120 mg/kg CBD ip to mice leads to much higher brain and plasma concentrations than oral dosing (Fig. 2a, b; Table 4). Following ip administration, T_{max} occurred between 60 and 120 min in brain and plasma, with a considerable, yet unexplained variability of results at 2 h. Oral dosing induced a faster peak in plasma (60 min) compared to brain (6 h). Drugs were below detection threshold at 24 h post-dose. Overall brain exposure (T_{max}=360 min) was low after oral drug administration with $AUC_{0–6\ h}$ at 319 μg/g min relative to 1,229 μg/g min following ip treatment. Brain/plasma ratios

Table 2 Multiple reaction parameters tuned for detection of daughter ions of cannabinoids

Compound	Transition	Ionisation mode	Cone voltage (V)	Collision energy (eV)	Dwell (ms)
CBD	313.3−245.2	Negative	26	25	300
CBDV	287.23−165.16	ESP+	30	22	200
Δ^9-THCV	287.23>165.11	ESP+	32	24	150
CBG	317.27−193.14	ESP+	20	20	150

Despite some minor adjustments, settings were surprisingly similar

Table 3 Settings and materials of HPLC used for the detection of cannabinoids in rodent brain and plasma

Parameter	Setting									
Drug	CBD		CBDV		Δ9-THCV			CBG		
Column	Luna C18, 50×2.0 mm, 5 μm		Acquity UPLC® BEH C18, 50×2.1 mm, 1.7 μm		Acquity UPLC® BEH C18, 50×2.1 mm, 1.7 μm			Acquity UPLC® BEH C18, 50×2.1 mm, 1.7 μm		
Flow rate	0.35 ml/min		0.6 ml/min		0.6 ml/min			0.6 ml/min		
Injection volume	5 μl		5 μl		2 μl			4 μl		
Mobile phase	A—1 mM ammonium formate in water		A—0.01% formic acid in water		A—0.01% formic acid in water			A—0.01% formic acid in water		
	B—acetonitrile		B—0.01% formic acid in methanol		B—0.01% formic acid in methanol			B—0.01% formic acid in methanol		
Gradient profile	Time (min)	% mobile phase B	Time (min)	% mobile phase B	Time (min)	% mobile phase B		Time (min)	% mobile phase B	
	0	25	0	15	0	15		0	50	
	0.2	25	0.2	15	0.2	15		0.2	80	
	4	100	2.2	98	1.7	98		4.2	80	
	4.7	100	2.7	98	2.2	98		4.7	50	
	4.8	25	2.8	15	2.3	15		4.8	50	
	6	25	3	15	2.4	15		5	50	

For details, see "Materials and methods"

based on $AUC_{0–6\ h}$ were 0.84 (po) and 0.51 (ip), suggesting a similar brain penetration following the two administration routes. With 280 (plasma) and 289 min (brain), the apparent elimination half-life was similar in both tissues after ip injection, but not detectable for the oral route.

In rats, the pharmacokinetic plasma profile was similar for both administration routes (Fig. 2c, d; Table 5). Maximal plasma concentrations peaked at 2 h post-injection, and the highest concentrations were similar (2.6 and 2 μg/ml for ip and po, respectively), as were AUCs (1,778 μg/ml min for ip and 1,175 μg/ml min for po). By contrast, brain level and exposure were slightly higher after oral dosing with C_{max} and AUC values in favour of gavaging (8.6 μg/g and 4,941 μg/g min, respectively, versus 6.8 μg/g and 4,532 μg/g min after ip administration). Interestingly, CBD levels in brain continued to rise progressively over 4 h and were higher after oral administration (brain/plasma ratios 2.64 (ip) and 3.54 (po)). The apparent elimination half-live was similar between tissues but differed according to administration routes (Table 5). As already seen for mice, the elimination appeared to be complete after 24 h.

These cremophor-based results were compared with the vehicle solutol (Supplementary Fig. 2; Table 5). When CBD was administered ip, the two solvents produced similar brain penetration (brain/plasma ratios 2.64 for cremophor and 2.42 for solutol), as well as maximal tissue levels (C_{max} 2.4–2.6 μg/ml in plasma; 5.2–6.8 μg/ml in brain), and these were relatively stable over 6 h. By contrast, C_{max} after oral gavaging was considerably higher in solutol and reached maximal levels at 4–6 h. This was due to a prolonged and continuous absorption of CBD in plasma and brain possibly owing to limited metabolism of CBD in this vehicle. Nevertheless, elimination was complete at 24 h. Interestingly, oral administration led to higher brain concentrations in the presence of solutol (C_{max}=12.6 μg/ml) relative to cremophor (C_{max}=8.6 μg/ml), in line with higher plasma levels that increase absorption into brain. Brain/plasma ratios were similar for both solvents (3.66 for solutol and 3.54 for cremophor). Unfortunately, animals receiving solutol-based CBD showed adverse effects, such as drowsiness and piloerection, rendering solutol a non-suitable vehicle for in vivo tests, at least at the concentration used in this study.

Relative to rats, mice showed much higher CBD plasma exposure following ip administration, whereas oral administration resulted in a similar plasma C_{max} in both species, with mice presenting with an earlier peak time and faster elimination. Analysis of brain PK showed that rats had a slower absorption but longer-lasting CBD exposure, with similar C_{max} between species following intraperitoneal administration. By contrast, the two species responded dramatically differently after gavaging in that CBD concentrations in rats attained a six times higher C_{max} in brain.

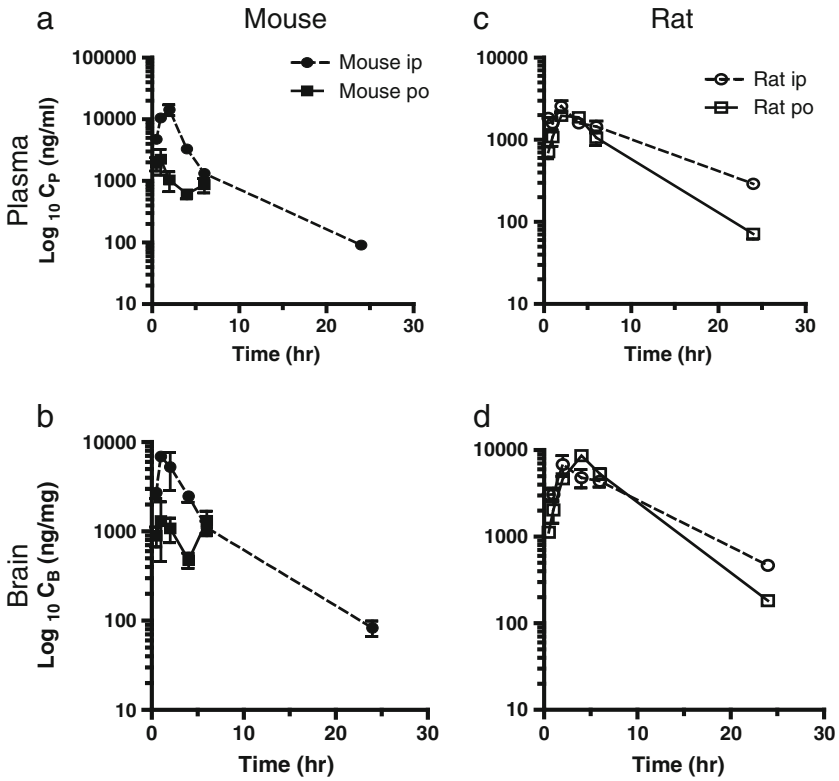

Fig. 2 Pharmacokinetic analysis of CBD in mouse and rat. CBD (120 mg/kg) was administered at time point 0 via ip injection or oral gavage and tissue harvested at six time points within 24 h post-dosing. Drug levels are shown in *top row* (**a**, **c**) as log 10 of plasma concentration (C_P) and in **b** and **d** as log 10 of brain concentration (C_B). Mean±SEM. Note that values at 24 h were below detection threshold for oral administration

Cannabidivarine

Rapid absorption was observed for CBDV in mice, and this was independent of tissue analysed and administration route (Fig. 3a, b; Table 4). Consequently, plasma and brain T_{max} was recorded for 30 min post-ip injection and 30 (plasma) and 60 (brain)min following oral gavaging. Clearly, brain C_{max} was higher after ip dosing (three to five times higher) leading to greater AUC values. Contrary to CBD, however, the concentration in brain and plasma was not different at any time point for CBDV and continuously declined until reaching values close to the detection threshold

Table 4 Pharmacokinetic parameters for CBD, CBDV, Δ^9-THCV and CBG in mouse plasma (a) and brain (b) following oral (po) or intraperitoneal (ip) administration

PK parameter	CBD (120 mg/kg)		CBDV (60 mg/kg)		Δ9-THCV (30 mg/kg)		CBG (120 mg/kg)	
	po	ip	po	ip	po	ip	po	ip
Plasma								
C_{max} (μg/ml)	2.2	14.3	0.47	4	0.24	0.88	0.67	40.8
T_{max} (minutes)	60	120	30	30	30	30	30	120
Apparent elimination half-life (min)	IS	280	222	280	87	345	193	176
$AUC_{0–6\ h}$ (μg/ml min)	378	2,381	74	393	≤22	78	57	4,324
$AUC_{0–24\ h}$ (μg/ml min)	IS	3,144	IS	523	IS	116	IS	5,563
$AUC_{0–\infty}$ (μg/ml min)	IS	3,181	109	529	≤23	120	67	5,571
Brain								
C_{max} (μg/g)	1.3	6.9	0.94	3.57	0.43	1.69	0.42	3.48
T_{max} (min)	360	60	60	30	30	30	60	120
Apparent elimination half-life (min)	IS	289	204	96	80	385	≤121	252
$AUC_{0–6\ h}$ (μg/g min)	319	1,229	152	454	≤49	177	≤44	679
$AUC_{0–24\ h}$ (μg/g min)	IS	1,868	IS	IS	IS	275	≤65	1,103
$AUC_{0–\infty}$ (μg/g min)	IS	1,903	217	498	≤52	288	≤86	1,117

IS indicates insufficient data for calculation. Apparent elimination half-life (minutes) 2 to 6 h for po; 4 to 24 h for ip

Table 5 Pharmacokinetic parameters for CBD (in solutol and cremophor solvents), CBDV, Δ^9-THCV and CBG in rat plasma (a) and brain (b) following oral (po) and intraperitoneal (ip) administration. Apparent elimination half-life (minutes) 2 to 6 h for po and 4 to 24 h for ip

PK parameter	CBD (120 mg/kg)		CBD (120 mg/kg)		CBDV (60 mg/kg)		Δ9-THCV (30 mg/kg)		CBG (120 mg/kg)	
	Cremophor po	Cremophor ip	Solutol po	Solutol ip	po	ip	po	ip	po	ip
Plasma										
C_{max} (μg/ml)	2	2.6	3.2	2.4	2.2	1.3	0.21	0.4	1.05	0.81
T_{max} (min)	120	120	360	30	30	240	120	30	30	60
Apparent elimination half-life (min)	277	465	242	606	239	399[a]	89	601	100	~560
$AUC_{0–6\ h}$ (μg/ml min)	536	636	748	684	476	358	42	49	98	≤199
$AUC_{0–24\ h}$ (μg/ml min)	1,148	1,577	2,572	1,987	857	789[a]	IS	110	IS	≤514
$AUC_{0–\infty}$ (μg/ml min)	1,175	1,778	2,623	2,463	868	850[a]	46	131	106	≤617
Brain										
C_{max} (μg/ml)	8.6	6.8	12.6	5.2	6.3	3.9	0.3	1.62	0.97	1.23
T_{max} (min)	240	120	240	360	120	60	30	30	120	60
Apparent elimination half-life (min)	222	347	240	663	232	383	126	570	96	447
$AUC_{0–6\ h}$ (μg/g min)	1,900	1,683	2,742	1,656	1,509	906	97	162	181	335
$AUC_{0–24\ h}$ (μg/g min)	4,882	4,297	9,383	5,406	2,772	1,809	IS	353	IS	884
$AUC_{0–\infty}$ (μg/g min)	4,941	4,532	9,574	7,031	2,803	1,923	97	416	205	992

[a] Based on a mean value of 106 ng/ml at 24 h post-dose, which assumes a concentration at the LOQ (5 ng/ml) for one animal with measured value <LOQ

at 6 h. The brain/plasma ratios nevertheless suggest a somewhat better brain penetration following oral dosing (2.05) relative to ip (1.15). An interesting observation was the relatively short apparent brain elimination half-life of 96 min (Table 4) in the ip group compared to a slower elimination after oral treatment (204 min).

A similar rapid absorption was noted for rats (Fig. 3c, d; Table 5) with T_{max} appearing within the first 2 h after oral dosing. Drug concentrations in plasma and brain after po exceeded those recorded after ip administration. This was especially noteworthy for the first 3 h post-drug. Overall, plasma concentrations and AUC were lower than brain values and this was independent of administration route. Despite this difference, no differences occurred in elimination half-lives between tissues. Finally, brain penetration overall was slightly better following oral administration (3.17) compared with ip (2.53).

An important difference between the two species was the observation of higher drug concentrations in plasma and brain following oral treatment in rats, opposite to mice. Intraperitoneal CBDV administration resulted in a similar plasma PK profile between species; brain concentrations in rats were higher.

Δ^9-Tetrahydrocannabivarin

As with CBDV, Δ^9-THCV also was readily detectable in mouse plasma and brain as shown by a T_{max} of 30 min in both tissue samples independent of administration routes (Fig. 4a, b). However, ip injection led to higher exposure reflected in greater C_{max} and AUC (Table 4). Brain penetration was substantial and Δ^9-THCV brain concentrations exceeded plasma levels in both ip and po treatments (brain/plasma ratios ~2.2 for both ip and po). Δ^9-THCV elimination following ip administration was slow (>5 h for plasma and brain) but faster when orally administered (<1.5 h), the brain and plasma profiles for each route followed a similar profile.

Δ^9-THCV absorption was also rapid in rats and peaked at 30 min post-treatment. As already revealed for mice, ip injection yielded higher maximal concentrations in both tissues (Fig. 4c, d), which readily declined within the first 2 h. Yet, elimination half-lives exceeded 8 h. By contrast, oral administration did not have a high yield in both tissues but levels remained stable for 2 h post-gavaging. At 24 h, no compound was detected in any condition. In agreement with mouse data is the observation that brain concentrations were higher than plasma levels and thus AUC values were two to four times greater for brain (Table 5). Consequently, brain/plasma ratios were above 1, being 2.3 for po and 3.3 for ip routes, clearly in favour of distribution in the brain, and these were highest after ip injection.

Mice and rats responded similarly to intraperitoneal administration of Δ^9-THCV, with slightly higher plasma C_{max} in mice, compared to rats. Oral gavaging also produced similar PK profiles in the two species, with a

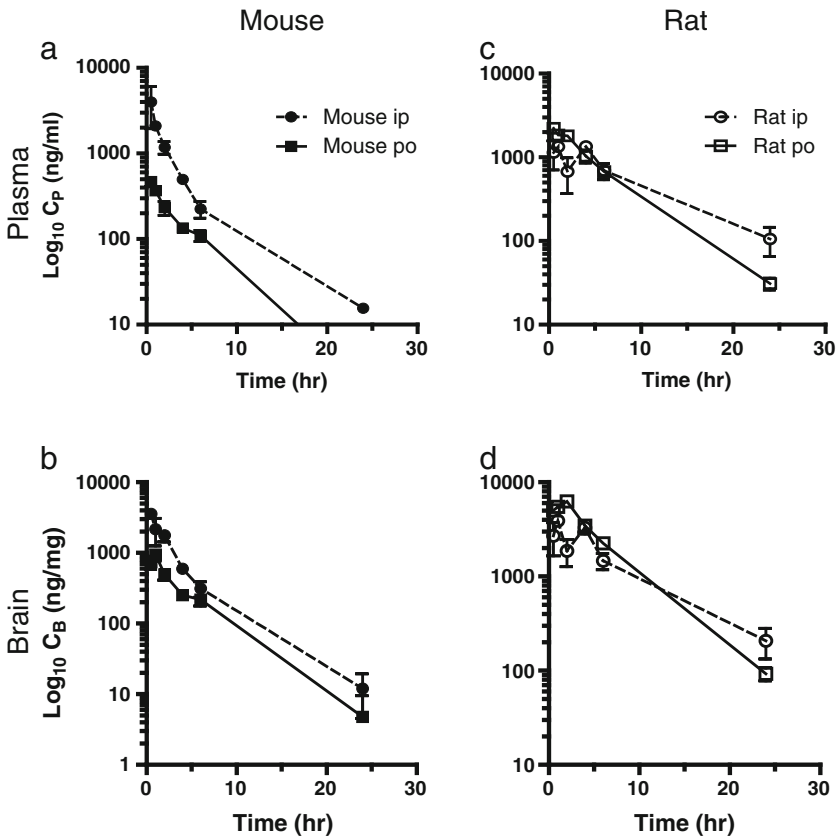

Fig. 3 Pharmacokinetic analysis of CBDV in mouse and rat. CBDV (60 mg/kg) was administered at time point 0 via ip injection or oral gavage and tissue harvested at six time points within 24 h post-dosing. Drug levels are shown in *top row* (**a**, **c**) as log 10 of plasma concentration (C_P) and in **b** and **d** as log 10 of brain concentration (C_B). Mean±SEM

slower and superior plasma, but not brain, concentration in rats.

Cannabigerol

Rapid absorption was also obtained for CBG (ip and po) into plasma and brain of mice (Fig. 5a, b). Plasma concentrations at 2 h showed two highly anomalous values (118,700 and 37,740 ng/ml); tissue samples were re-analysed and values confirmed; hence, these data were not omitted from the study (please note extreme variability in Fig. 5a).

In agreement with all other phytocannabinoids tested so far, ip injections yielded considerably higher plasma and brain concentrations compared with oral treatment so that T_{max} was observed at 30 min (plasma) and 2 h (brain) and AUCs were about 100-fold greater for ip (Table 4). However, ip concentrations in both tissues did not differ considerably over the time course recorded here; for po, concentrations were extremely low and close to detection threshold after 2 h. This was also reflected in the brain/plasma ratios which were 0.77 and 0.15 for po and ip administration routes, respectively.

CBG in rat was readily absorbed following ip and oral administration in both plasma and brain with T_{max} between 30 and 120 min (Fig. 5c, d). Again, ip administration gave better exposure, but overall concentrations were only marginally higher in brain (see AUCs in Table 5). Intriguingly, CBG was still above detection threshold 24 h after ip treatment. Lower concentrations of CBG were recorded after oral treatment (Fig. 5c, d), brain exposure was stable 2–4 h post-treatment and ip versus po brain/plasma ratios overlapped (1.84 po and 1.68 ip).

Mice and rats responded better to intraperitoneal administration of CBG, but the difference was only marginal in rats. Brain penetration was slower in mice, but lead to C_{max} twice as high as the one recorded in rats. By contrast, oral administration resulted in similar PK profiles between species.

Marble burying test

As expected, CBD reduced marble burying behaviour (Fig. 6a). This effect was only observed at 2 h post-injection (p values <0.05 versus vehicle for ip and po routes), when plasma and brain concentrations were maximal. By contrast, no effect was noted after 6 h (p's> 0.05) independently of the administration route. Similar results were obtained for the displacement of marbles (Fig. 6b), but this endpoint did not meet reliability for the oral treatment. Representative photographs (Fig. 6c, d) illustrate marble burying for each drug treatment, route and time point, clearly indicating reduced marble burying at 2 h.

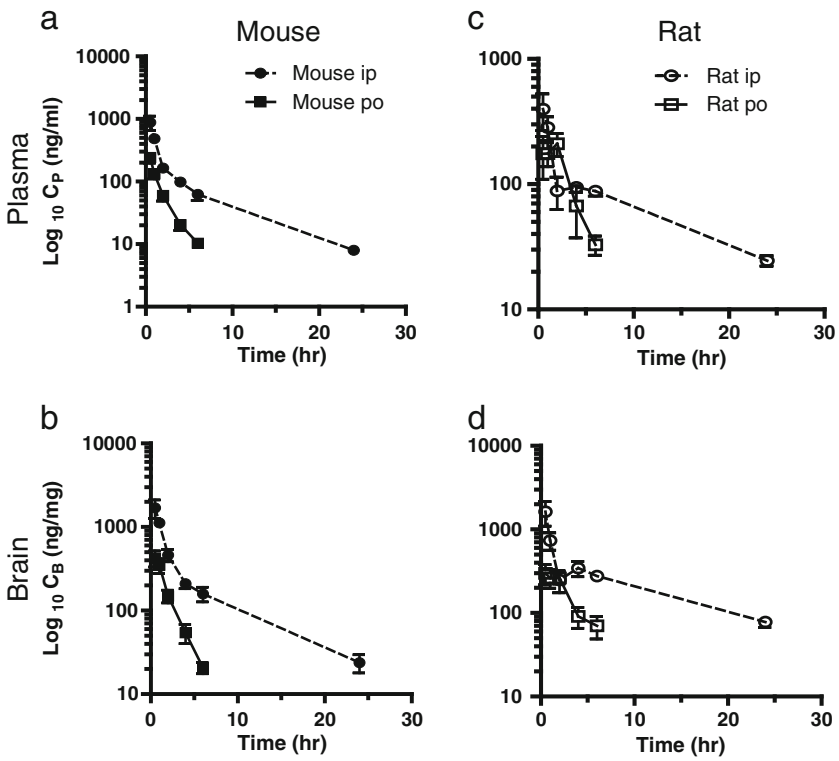

Fig. 4 Pharmacokinetic analysis of Δ^9-THCV in mouse and rat. Δ^9-THCV (30 mg/kg) was administered at time point 0 via ip injection or oral gavage and tissue harvested at six time points within 24 h post-dosing. Drug levels are shown in *top row* (**a** and **c**) as log 10 of plasma concentration (C_P) and in **b** and **d** as log 10 of brain concentration (C_B). Mean±SEM

Discussion

The primary objective of the present study was to define the pharmacokinetic profile of four phytocannabinoids, CBD, CBDV, Δ^9-THCV and CBG in terms of species- and administration route-specific differences. Moreover, we were particularly interested in acute exposure uncontaminated by any repeated or chronic loading of tissue with the com-

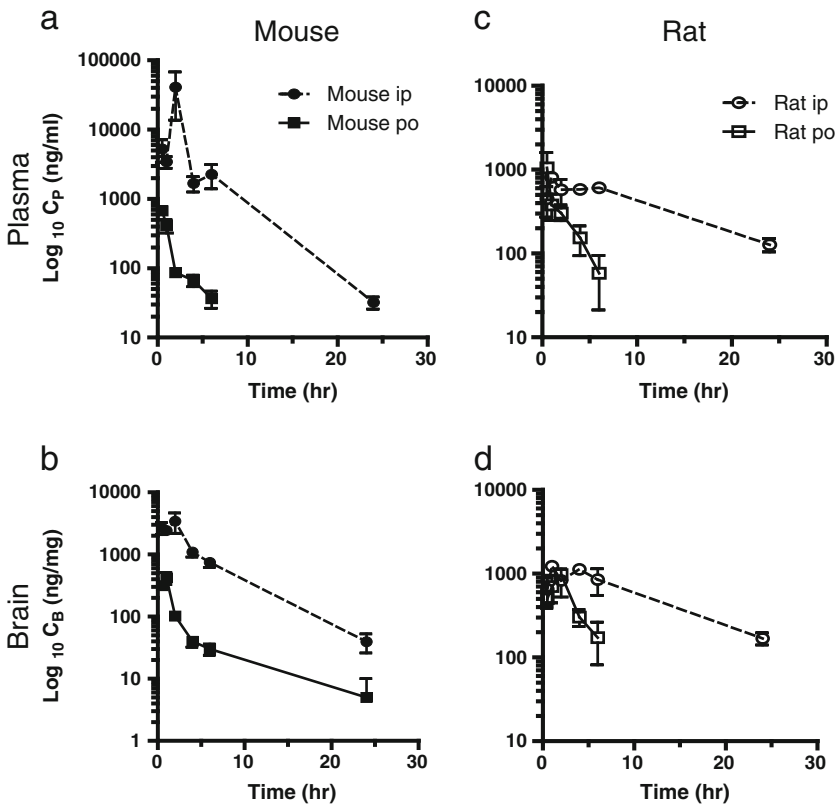

Fig. 5 Pharmacokinetic analysis of CBG in mouse and rat. CBG (120 mg/kg) was administered at time point 0 via ip injection or oral gavage and tissue harvested at six time points within 24 h post-dosing. Drug levels are shown in *top row* (**a**, **c**) as log 10 of plasma concentration (C_P) and in **b** and **d** as log 10 of brain concentration (C_B). Mean±SEM

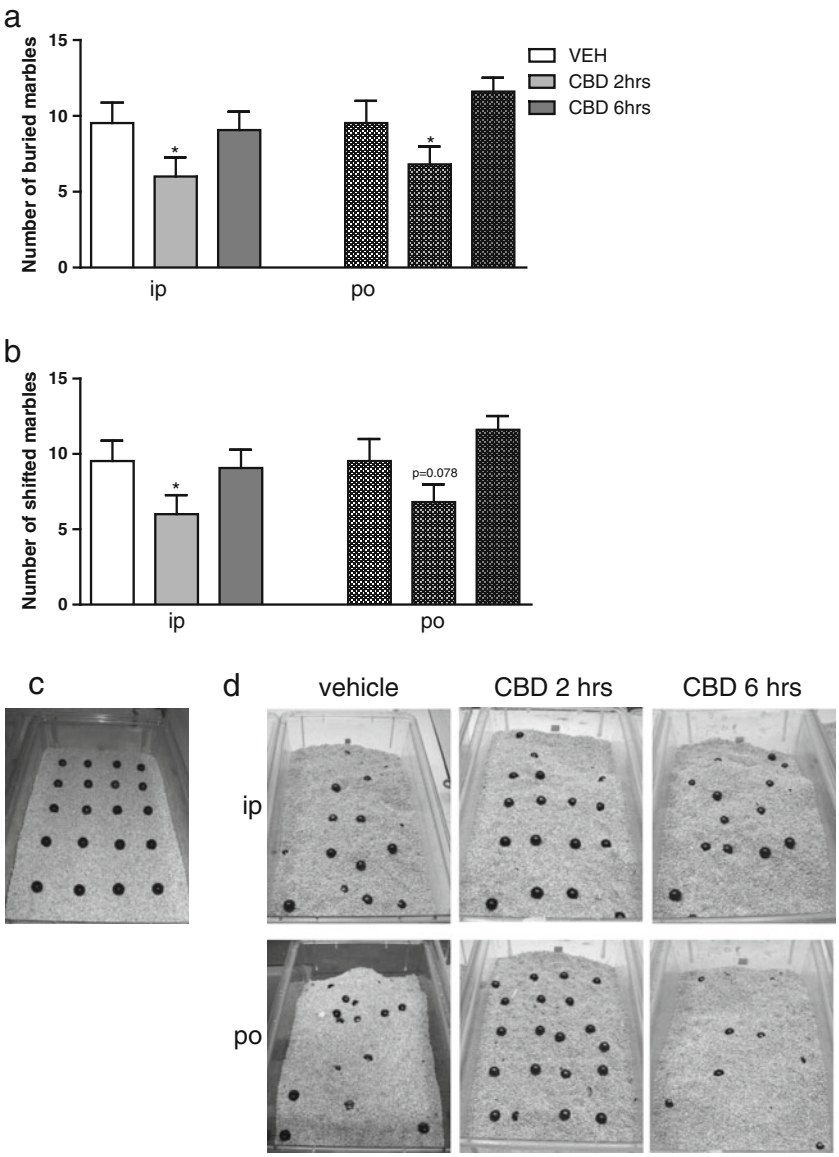

Fig. 6 Effect of oral and intraperitoneal CBD administration on marble burying in mice. CBD was administered 2 and 6 h pre-test; marble burying (**a**) and shifting (**b**) were scored. When administered 2 h prior to test, CBD reduced marble burying and shifting independent of administration route (ip or po). Mean+SEM. *Asterisks* represent $p<0.05$ versus vehicle-treated mice and versus 6 h post-treatment test. Representative pictures or marble burying show that marbles were equally distributed in the experimental cage at the start of test (**c**). Clear marble burying is shown following vehicle treatment (**d**), whereas CBD significantly reduced it at 2 h, but not 6 h post-injection

pounds. Towards this end, we reasoned that our compounds concurred with other cannabinoid agonists/antagonists such as WIN55,212-2 or rimonabant (SR141716A) (Valiveti et al. 2004; Barna et al. 2009) in that their absorption might be rapid and complete washout be achieved within 24 h. This assumption guided our time point selection for tissue harvesting. A second consideration pertained to the selection of vehicle for the highly lipophilic cannabinoids. Of recent interest in drug delivery methods are lipid nanocapsules (LNCs) containing surfactants such as polyethylene glycol (PEG) to prevent their recognition and capture by the immune system (Fang et al. 2009). Through additional physical methods, post-insertion incubation of liposomes with a micellar suspension of the added surfactant will result in its transfer inside the liposomal, lipidic bilayer. Solutol® HS 15 is a mixture of free PEG 660 and PEG 660 hydroxystearate that may be used for micelle formation (Perrier et al. 2010) to generate LNCs containing cannabinoids. By contrast, cremophor EL is a synthetic, nonionic surfactant that stabilizes emulsions of nonpolar materials in aqueous systems and has become a major component of self emulsifying drug delivery systems.

In our pilot study, we compared these two solvents for CBD and obtained greatly varying exposure of CBD (see Fig. 2 and Suppl. Fig. 2). Although solutol drastically enhanced CBD absorption following oral administration, it had no effect on the pharmacokinetic profile following ip injections. Unexpectedly, behavioural side effects such as piloerection and drowsiness occurred therefore precluding solutol-based drug administration when animals were assessed in cognitive and behavioural tasks. We take it that this was due to the relatively high concentration of solutol (30%) used here to achieve the correct suspension of CBD. Lower concentrations of solutol as described in the

literature (~7–10%) may be devoid of such side effects. Drug pharmacokinetics are subject to vehicle-induced changes in absorption and exposure (Cornaire et al. 2004), and our data are in line with the notion that solutol can increase plasma C_{max} and overall exposure of drugs compared to other solvents (Bittner et al. 2003). Whether the enhanced concentration of CBD in plasma and brain administered in solutol was due to heightened solubility in gastrointestinal fluids or increased drug permeability remains uncertain. Clearly, the presence of co-solvents (like ethanol in cremophor) may indeed modulate gastric and intestinal drug absorption (and thus their pharmacokinetics) depending on concentration and chemistry of the drug (Claassen. 1994). Gastric absorption may indeed be low under solutol so that liver metabolism and clearance are reduced relative to other solvents. However, we have no data to determine extraction of CBD depending on solvents. Additional factors that may also have contributed to increased oral exposure are possible vehicle-induced inhibition of CBD metabolism and/or transport processes as well as direct enhancement of tissue permeability for micellous surfactants. Consequently, it appears that the drug half-life can be correlated with the emulsifier half-life (Woodburn et al. 1995).

Given their lipophilicity, all tested phytocannabinoids crossed the BBB showing consistent brain penetration. Nevertheless, subtle differences in drug absorption to brain mainly concerned the route of administration (Ho et al. 1971; Abel et al. 1974). A direct comparison between the different purified cannabinoid extracts is difficult given that administered doses were not identical so that values may not be fully compatible. However, some general comments about plasma and brain levels and compartmentalisation can be made. It is noticeable that in mice, ip administration of drugs always resulted in higher C_{max} in both plasma and brain indicating that drug absorption in the gastrointestinal system or liver metabolism provided a major difference for drug distribution. This was less so in rats for which only CBD and THCV yielded higher plasma concentrations after ip administration. For CBG, both treatment routes lead to similar levels in tissues and CBDV was higher in plasma and brain after oral gavaging. However, when tissue partitioning was based on $AUC_{0–6\ h}$, it appeared that oral administration in both species lead to higher brain/plasma ratios for CBD, CBDV and CBG, while the distribution was similar for THCV, confirming that the overall more efficient route was oral in terms of brain penetration but, with few exceptions, ip for absolute concentrations and it should be obvious that drug efficacy in vivo may be determined by C_{max} or exposure rather than ratios. While results are novel here for CBDV, Δ^9-THCV and CBG, it has been observed when various administration routes were compared for CBD across different species including rats (Alozie et al. 1980), dogs (Samara et al. 1988), monkeys (Jones et al. 1981) and humans (Agurell et al. 1986), to which we now add the mouse as a novel species. However, only minor qualitative differences between absorption were obtained compared with rats (Fig. 2). Plasma and brain levels of CBD increased within 2 h post-administration, then constantly declined to zero within 24 h in mice. While we take this as clear evidence for different absorption profiles of CBD from different body compartments, it should be noted that oral gavaging was conducted in overnight fasted animals. Consequently, gastrointestinal absorption may have been artificially enhanced and bio-distribution of drugs accelerated in fasted subjects. This is in agreement with clinical trials on rimonabant which suggest that absorption and metabolism is strongly dependent on weight and food intake (Bifulco et al. 2007).

Feeding can indeed play an important role on a drug PK profile (De Leo et al. 2010). One advantage of reduced food uptake is the concomitant reduction in liver blood flow and the corollary of lower drug clearance through the liver. This may, overall, reduce clearance/enhance drug absorption after oral administration, but appears to be less of a concern in case of ip dosing (Kast and Nishikawa. 1981; Aghazadeh-Habashi et al. 2006). Hence, we selectively fasted animals for oral dosing and fed subjects for ip routes. This would still enable direct comparison of results.

For all drugs, mice showed faster drug absorption, with T_{max} values ranging between 30 and 120 min for plasma and 30 to 360 min for brain, possibly owing to their higher metabolic activity compared to rats. While this would not necessarily explain higher absorption of drug, the data suggest that active transport or passive diffusion in mice is higher than in rats. It seems to coincide, however, with differential levels of free drug in plasma and brain between mouse and rat and in some cases even better retained cannabinoids in sub-compartments of the brain. Overall, the higher metabolic rate in mice may support a faster equilibrium on both sides of the blood–brain barrier and maintain a high free drug concentration capable of passive diffusion into brain (Smith et al. 2010). Interesting was also the observation that some phytocannabinoids were readily absorbed into brain tissue and maximal concentrations exceeded those in plasma. This was not observed for Δ^9-THCV, which is structurally similar to Δ^9-THC but contains a shorter side chain on its phenyl-ring. This may facilitate BBB penetration, but at the same time hamper first pass and thus favour ip dosing. Yet, we cannot exclude any higher affinity in protein binding for Δ^9-THCV. Since CBDV also contains a short side chain consisting of three methyl groups, but otherwise differs in chemical structure, and yet is readily detectable in brain, this argues against our hypothesis of first pass effects due to side chain chemistry. Brain/plasma ratios were somewhat better in rats indicating differences in the ability to transport drugs

across the BBB between these two rodents or other mammalian species (Plotkin et al. 2000; Syvanen et al. 2009). In this respect at least, T_{max} after oral gavage of CBD in rats appears to be consistent even across laboratories (Siemens et al. 1980).

Species-specific differences affected mainly the elimination half-life, which was considerably shorter in mice. Such short elimination was independent of administration route, suggesting that higher metabolic rate in this species could be responsible. It is difficult to draw any conclusions on absolute concentrations in plasma and brain, primarily because no dose–response relationships were assessed, but also because drug solubility was vastly different so that maximal doses administered remained non-comparable. Their adequate absorption and brain penetration within the first 2 h post-injection therefore does provide important guidance for the selection of time points when analysing phytocannabinoid effects on behaviour.

We here performed a behavioural investigation of CBD effects on obsessive–compulsive behaviour as a pharmacodynamic parallel to our PK data. In line with previous reports (Casarotto et al. 2010), we confirmed inhibition of marble burying (Thomas et al. 2009) by CBD. The drug action correlated with plasma concentrations that peaked at 2 h and continuously declined thereafter. Important to the result is the finding that plasma concentrations differ between oral and ip administration in mice (Fig. 2a); yet, there was a similar impairment in marble burying after both treatment routes which we take as evidence for already saturated drug efficacy for negative effects, to which further increases in drug levels did not add on a behavioural level. Whether CBD acts through CB_1 receptors, as indicated by Casarotto et al. (2010), has not been tested here, but saturated receptor occupancy in brain may explain impairments despite differences in plasma concentrations. The progressive drug elimination in plasma at 6 h attained concentrations at which normal marble burying behaviour was observed, further confirming that plasma/brain levels of CBD above a certain threshold determine behavioural outcome. A further decline of CBD in plasma at 24 h is indicated from Fig. 2a making it unlikely to obtain a behavioural difference. Consequently, this time point was not examined.

In summary, we describe the first pharmacokinetic profile of CBD, CBDV, CBG and Δ^9-THCV in both rats and mice that also discriminates between the most commonly used pre-clinical administration routes po and ip. Relative rapid absorption and distribution to brain was observed for all drugs with somewhat higher concentrations in tissue after ip treatment. As a pharmacodynamic correlate for CBD, marble burying was impaired at the time point of C_{max}, but not when lower concentrations were observed in plasma/brain.

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