RESEARCH PAPER

Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB₁ receptor-independent mechanism

T D M Hill¹*, M-G Cascio²*, B Romano²,³, M Duncan⁴, R G Pertwee², C M Williams⁵, B J Whalley¹ and A J Hill¹,⁵

¹Reading School of Pharmacy, University of Reading, Reading, UK, ²School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK, ³Department of Pharmacy, University of Naples Federico II, Naples, Italy, ⁴GW Research Ltd, Salisbury, UK, and ⁵School of Psychology and Clinical Language Sciences, University of Reading, Reading, UK

BACKGROUND AND PURPOSE

Epilepsy is the most prevalent neurological disease and is characterized by recurrent seizures. Here, we investigate (i) the anticonvulsant profiles of cannabis-derived botanical drug substances (BDSs) rich in cannabidivarin (CBDV) and containing cannabidiol (CBD) in acute in vivo seizure models and (ii) the binding of CBDV BDSs and their components at cannabinoid CB₁ receptors.

EXPERIMENTAL APPROACH

The anticonvulsant profiles of two CBDV BDSs (50–422 mg·kg⁻¹) were evaluated in three animal models of acute seizure. Purified CBDV and CBD were also evaluated in an isobolographic study to evaluate potential pharmacological interactions. CBDV BDS effects on motor function were also investigated using static beam and grip strength assays. Binding of CBDV BDSs to cannabinoid CB₁ receptors was evaluated using displacement binding assays.

KEY RESULTS

CBDV BDSs exerted significant anticonvulsant effects in the pentylenetetrazole (≥100 mg·kg⁻¹) and audiogenic seizure models (≥87 mg·kg⁻¹), and suppressed pilocarpine-induced convulsions (≥100 mg·kg⁻¹). The isobolographic study revealed that the anticonvulsant effects of purified CBDV and CBD were linearly additive when co-administered. Some motor effects of CBDV BDSs were observed on static beam performance; no effects on grip strength were found. The Δ⁹-tetrahydrocannabinol and Δ⁹-tetrahydrocannabivarin content of CBDV BDS accounted for its greater affinity for CB₁ cannabinoid receptors than purified CBDV.

CONCLUSIONS AND IMPLICATIONS

CBDV BDSs exerted significant anticonvulsant effects in three models of seizure that were not mediated by the CB₁ cannabinoid receptor and were of comparable efficacy with purified CBDV. These findings strongly support the further clinical development of CBDV BDSs for the treatment of epilepsy.

Abbreviations

AED, antiepileptic drug; BDS, botanical drug substance; CBD, cannabidiol; CBDV, cannabidivarin; pCB, phytocannabinoid; PTZ, pentylenetetrazole; Δ⁹-THC, Δ⁹-tetrahydrocannabinol; Δ⁹-THCV, Δ⁹-tetrahydrocannabivarin
Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures, which affects approximately 50 million people worldwide (Leonardi and Ustun, 2002). Epilepsy’s co-morbidities include cognitive decline, depressive disorders and schizophrenia (Hermann et al., 2000; Kanner et al., 2012), which are worsened by poorly controlled seizures (Perucca et al., 2000). There are many treatments available (BNE, 2011); however, all have notable side effects (Ortinski and Meador, 2004; Schachter, 2007) and ~30% of the cases remain pharmacoresistant, resulting in poorly controlled seizures (Hittiris et al., 2007). This represents a major unmet clinical need for new well-tolerated antiepileptic drugs (AEDs) able to control previously pharmacoresistant epilepsies.

We and others have previously reported that cannabidiol (CBD), a non-psychoactive phytocannabinoid (pCB) of the cannabis plant, is anticonvulsant in several in vivo seizure models (Consroe et al., 1982; Wallace et al., 2001; Jones et al., 2010; 2012) and in humans (Cunha et al., 2010; 2012). As an example, amelioration of spasticity associated with multiple sclerosis by Δ9-tetrahydrocannabivarin (THCV; the propyl analogue of CBD) is anticonvulsant in vivo (Hill et al., 2012a). While the anticonvulsant mechanisms of CBD and CBDV are unidentified, their anticonvulsant and tolerability profiles do not suggest interaction with the CBD cannabinoid receptor (Wallace et al., 2001; Jones et al., 2010; Hill et al., 2012a).

An increasing body of evidence suggests that cannabis extracts enriched with a specific pCB can possess as much, or more, pharmacological efficacy/potency than the purified pCB (Wilkinson et al., 2003; Whalley et al., 2004; Ryan et al., 2006; De Petrocellis et al., 2011; Sagredo et al., 2011; Valdeolivias et al., 2012). As an example, amelioration of spasticity associated with multiple sclerosis by Δ9-tetrahydrocannabivarin (THCV) and CBD was greater in clinical trials when these two pCBs were combined as two plant extracts rather than as purified pCBs (Russo and Guy, 2006). The apparent benefits of extracts may arise from polypharmacological effects of the pCBs (McPartland and Russo, 2001) or the terpenoids present in cannabis extracts that possess their own pharmacology and can modulate pCB effects to enhance activity or reduce off-target effects (Formukong et al., 1988; McPartland and Russo, 2001), the ‘entourage effect’ (Russo, 2011).

Therefore, we extend our previous investigations of the anticonvulsant effects of CBDV and CBD (Jones et al., 2010; 2012; Hill et al., 2012a) to assess the anticonvulsant potential of extracts rich in CBDV and CBD ['botanical drug substances’ (BDDS)]. Here, we test two such extracts: one with pharmacologically significant (Järbe et al., 2002; Hill et al., 2010) Δ9-THC and Δ9-tetrahydrocannabivarin (THCV) content (‘unmodified CBDV BDS’) and another with Δ2-THC and Δ2-THCV removed (‘modified CBDV BDS’). This allows assessment of the effects of Δ9-THC and Δ9-THCV on the anticonvulsant and tolerability profiles of the compounds; both Δ9-THC and Δ9-THCV can be anticonvulsant in their own right (Corcoran et al., 1973; Fried and McIntyre, 1973; Wallace et al., 2001; 2003; Hill et al., 2010), although there are also some reports that Δ2-THC can be proconvulsant (Chesher and Jackson, 1974; Karler and Turkanis, 1980).

Here, for the first time, we demonstrate significant anticonvulsant actions of CBDV BDSs in rat and mouse in vivo seizure models. Investigation of the interactions between the principal constituent pCBs indicated that CBD and CBDV act additively to suppress seizures. In motor assays, while unmodified CBDV BDS adversely affected balance and coordination at all doses, limited effects were only seen at the highest dose of modified CBDV BDS; neither CBDV BDS caused deficits in forelimb grip strength. Radioligand binding indicated that the Δ9-THC/Δ9-THCV content of the unmodified CBDV BDS was responsible for the majority of CB2 cannabinoid receptor binding; parallel behavioural experiments demonstrated that they were not required for anticonvulsant effects.

Methods

Animals

All animals were housed on a 12 h light–dark cycle, with food and water available ad libitum. Studies using male Wistar Kyoto rats (70–110 g; Harlan, Bicester, UK) were undertaken at the University of Reading. Mouse whole brain membranes for binding studies (University of Aberdeen) were obtained from adult (25–40 g) male MF1 mice (Harlan, Blackthorn, UK). In both cases, work was conducted in accordance with the Animals (Scientific Procedures) Act of 1986. Audiogenic seizure experiments used DBA/2 mice (10–14 g, 3–4 weeks old; Elevage Janvier, Le Genest-Saint-Ise, France), were designed and analysed by the authors, and performed by Poroslt Research Laboratory (Le Genest-Saint-Ise, France) in accordance with the French legislation under licence from the French Ministry for Agriculture and Fisheries. All animal work was carried out in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny et al., 2010; McGrath et al., 2010); 595 rats and 290 mice were used for in vivo studies in total.

Drug administration

Animals received either cannabis-derived CBDV BDSs or purified pCBs. pCBs (10–200 mg·kg−1) and cannabis-derived CBDV BDSs (50–422 mg·kg−1; GW Pharmaceuticals Ltd, Salisbury, UK); were suspended in ethanol, Cremophor EL and saline [0.9% (w/v) NaCl, 2:1:17, respectively; all from Sigma-Aldrich, Poole, UK]; and administered via i.p. injection 1 h prior to experimental procedures to achieve brain T\text{max} (Delana et al., 2012). Each experiment contained a control group that received vehicle, to which other groups were compared. In seizure experiments, group sizes were n = 10 for mice and n = 15 for rats. Unmodified CBDV BDS contained 47.4% CBDV, 13.9% CBD, 1% Δ9-THC and 2.5% Δ9-THCV; modified CBDV BDS lacked Δ9-THC/Δ9-THCV and contained 57.8% CBDV and 13.7% CBD; remaining content comprised plant matter. A CBDV BDS with most pCB content removed (termed BDS-pCB) contained no Δ9-THCV/Δ9-THC and 4.3% CBDV and 0.1% CBD. For clarity, in vivo experiments are numbered and detailed in Table 1 where the treatments used in each experiment and the doses of CBDV, CBD, Δ9-THC and Δ9-THCV received are outlined. The standardization and reproducibility of all CBDV BDSs employed in this study complied with the US Food and Drug Administration (FDA) guidelines for botanical drug products (FDA, 2004).
Rat seizure experiments

Pentylenetetrazole (PTZ; 85 mg·kg⁻¹; Experiments 1.1–1.4 and 2.1) or pilocarpine (380 mg·kg⁻¹; Experiments 4.1 and 4.2) was administered i.p. 1 h after pCB/CBDV BDS/vehicle treatment in 0.9% (w/v) NaCl. Methylscopolamine [1 mg·kg⁻¹; i.n. 0.9% (w/v) NaCl] was administered i.p. 45 min before pilocarpine to minimize pilocarpine’s peripheral effects. Seizure activity was recorded (Farrimond et al., 2009) for 30 min (PTZ) or 1 h (pilocarpine); video records were blinded and subsequently coded offline using the Observer XT software (Noldus, Wageningen, the Netherlands) and modified Racine scales (PTZ: 0, normal behaviour; 0.5, abnormal behaviour; 1, isolated myoclonic jerk; 2, atypical clonic seizure; 3, bilateral forelimb clonus; 3.5, bilateral forelimb clonus with body

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**Table 1**

Experimental design and pCB content of *in vivo* Experiments 1.1–4.2

<table>
<thead>
<tr>
<th>Experiment/Figures</th>
<th>Compound</th>
<th>Doses (mg·kg⁻¹)</th>
<th>CBDV/CBD content (mg·kg⁻¹)</th>
<th>THC/THCV content (mg·kg⁻¹)</th>
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<td>159/38</td>
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<td>346</td>
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<td>Unmodified CBDV BDS</td>
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<td>2/5</td>
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<td></td>
<td>CBDV + CBD</td>
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twist; 4, tonic–clonic seizure with suppressed tonic phase; 5, fully developed tonic–clonic seizure. Pilocarpine: 0, normal behaviour; 1, mouth clonus; 2, unilateral forelimb clonus; 3, bilateral forelimb clonus; 4, bilateral forelimb clonus with rearing and falling; 4.5, tonic–clonic convulsions; 5, tonic–clonic convulsions with righting reflex lost). In pilocarpine experiments, purified CBDV was added to unmodified CBDV BDS to match the CBDV content of modified CBDV BDS.

Audiogenic seizures in mice (Experiments 3.1–3.4)

DBA/2 mice were individually placed in a Plexiglas jar (40 cm wide, 35 cm high) containing an electric bell that sounded for up to 60 s (110–120 dB) to induce seizure activity (wild running, clonic convulsions and tonic convulsions), which was recorded by experienced technicians during experiments (Jensen et al., 1983).

Isobolographic experimental design and analysis determines whether two compounds that exert similar (e.g. anticonvulsant) pharmacological effects behave synergistically, additively or antagonistically when co-administered (Tallarida, 2006). Here, this approach was applied using the audiogenic seizure model to investigate any pharmacological interactions between CBD and CBDV. Initially, purified CBDV and CBD (each 10–200 mg·kg⁻¹, i.p.) were administered separately and their dose–response relationships (DRRs) calculated for protection against development of clonic convulsions (Experiment 3.3). CBDV and CBD were then co-administered in 1:1 effect size ratios (10 equally separated effect sizes, ED₁₀–ED₁₀₀) calculated from the previously calculated DRRs (Experiment 3.4). Thereafter, the individual DRRs of each pCB were used to create isoboles describing theoretical lines of additivity at multiple effect sizes (e.g. ED₁₀₀, ED₅₀) for the two compounds in combination. Finally, experimental effect sizes obtained from the co-administration study were statistically compared with the theoretical line of additivity on isoboles in accordance with the isobolographic method for full agonists described by Tallarida (2006).

Motor assays (Experiment 2.2)

Static beam and grip strength tasks were used as described in Jones et al. (2012) to assess motor function after administration of unmodified CBDV BDS (150, 275 or 422 mg·kg⁻¹, i.p.), modified CBDV BDS (150, 275 or 346 mg·kg⁻¹, i.p.) or CBDV BDS vehicle (control). Treatment order was randomized, and each animal (n = 10) received all treatments during the study with a minimum of 48 h between each treatment.

Radioligand binding

Materials. CP55940 was supplied by Tocris (Bristol, UK) and [³H]CP55940 (160 Ci-mmol⁻¹) by PerkinElmer Life Sciences, Inc. (Boston, MA, USA).

CHO cells. CHO cells stably transfected with complementary DNA encoding human CB₁ cannabinoid receptors (PerkinElmer Life Sciences, Inc.) were maintained in DMEM nutrient mixture F-12 HAM, supplemented with 1 mM L-glutamine, 10% FBS, 0.6% penicillin-streptomycin and Genetin G418 (600 μg·mL⁻¹). Cells were maintained at 37°C with 5% CO₂ and were passaged twice a week using non-enzymatic cell dissociation solution (EDTA, 1 mM).

Membrane preparation. Binding assays with [³H]CP55940 were performed with membranes obtained from MF1 mouse whole brain or hCB₁-CHO cells (Ross et al., 1999; Thomas et al., 2004) and frozen as a pellet at −20°C until required. Before use cells were defrosted, diluted in Tris buffer (50 mM Tris–HCl and 50 mM Tris–base) and homogenized with a 1 mL handheld homogenizer. Protein assays were performed using a Bio-Rad DC Kit (Hercules, CA, USA).

Radioligand displacement assay

Assays were carried out in Tris-binding buffer (50 mM Tris–HCl, 50 mM Tris–base, 0.1% BSA, pH 7.4), total assay volume 500 μL, using the filtration procedure described by Ross et al. (1999). Binding was initiated by addition of mouse brain membranes (33 μg protein per well) or hCB₁-CHO cell membranes (50 μg protein per well). All assays were performed at 37°C for 60 min before termination by addition of ice cold Tris-binding buffer and vacuum filtration using a 24 well sampling manifold (Brandel Cell Harvester; Brandel Inc., Gaithersburg, MD, USA) and Brandel GF/F filters that had been soaked in wash buffer at 4°C for at least 24 h. Each reaction well was washed six times with Tris-binding buffer (1.2 mL). The filters were oven-dried for 60 min and placed in 5 mL of scintillation fluid (Ultima Gold XR, PerkinElmer, Seer Green, Buckinghamshire, UK). Radioactivity was quantified by liquid scintillation spectrometry. Specific binding was defined by the presence and absence of 1 μM unlabelled CP55940. The concentration of [³H]CP55940 used in our displacement assays was 0.7 nM. All CBDV BDSs and CBDV were stored as stock solutions (10 mM) in dimethyl sulfoxide (DMSO); the vehicle concentration in all assay wells was 0.1% DMSO. [³H]CP55940 binding parameters were 2.33 fmol·mg⁻¹ (Bmax) and 2.31 nM (Kd) in mouse brain membranes (Thomas et al., 2004), and 57.0 pmol·mg⁻¹ (Bmax) and 1.1 nM (Kd) for human CB₁ CHO cells.

Statistical analyses

Statistical procedures were performed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA, USA). Effects on seizure severity were analysed by Kruskal–Wallis with post hoc Mann–Whitney U-tests. Parameters reported as percentages were analysed by chi-squared tests with post hoc Fisher exact tests. Body temperatures were analysed using a Student’s t-test. Grip strength, distance travelled and foot slips/metre parameters in motor assays were analysed using one-way ANOVA with post hoc Tukey tests, performed on the daily averages of each animal. Isoboles were analysed by comparing the Cartesian coordinates for the experimental result with those of the two points on the theoretical line of additivity. The x coordinate was calculated by setting the y coordinate of the experimental value and solving for x on the line of additivity, and vice versa for the y coordinate. The three coordinates were compared using a chi-squared test. Binding assay values were expressed as means and variability as SEM or as 95% confidence limits. The concentrations of test compounds that produced a 50% displacement of radioligand from specific binding sites (IC₅₀ values) were determined, and the
corresponding $K_i$ values were calculated using the equation of Cheng and Prusoff (1973). All receptor and ion channel nomenclature conforms to Alexander et al. (2011).

**Results**

**Effects of modified CBDV BDS and constituents on PTZ-induced seizures in rats**

Initially, we investigated the effects of modified CBDV BDS (containing no $\Delta^9$-THCV or $\Delta^9$-THC; Table 1) on PTZ-induced seizures in Experiments 1.1–1.4. The highest modified CBDV BDS dose was selected to contain the most effective anticonvulsant dose of purified CBDV tested to date (200 mg·kg$^{-1}$; Hill et al., 2012a). In Experiment 1.1, modified CBDV BDS had a significant anticonvulsant effect on seizure severity ($U = 13.84, P \leq 0.05$; Figure 1A); 200 and 275 mg·kg$^{-1}$ significantly suppressed the observed severity ($P \leq 0.05$). Seizure-associated mortality was also affected by modified CBDV BDS administration [$\chi^2(5) = 29.97, P \leq 0.01$; Figure 1B]; ≥200 mg·kg$^{-1}$ significantly reduced mortality ($P \leq 0.05$). We then compared the anticonvulsant effects of modified CBDV BDS with those of purified CBDV in Experiment 1.2. Administration of purified CBDV or modified CBDV BDS, matched by absolute weight, significantly affected the observed seizure severity ($U = 12.72, P \leq 0.05$; Figure 1C); both ≥50 mg·kg$^{-1}$ of

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**Figure 1**

Effects of CBDV BDS components in the PTZ model of acute convulsion. (A, B) Experiment 1.1: dose response of modified BDS. (C, D) Experiment 1.2: purified CBDV compared with modified BDS. (E, F) Experiment 1.3: comparison of modified CBDV BDS against matching levels of CBDV and CBD. (G, H) Experiment 1.4: BDS-pCB. (A, C, E, G) Maximum observed convulsion severity (median severity in grey, box represents interquartile range, whiskers represent maxima and minima (Kruskal–Wallis test, with a post hoc Mann–Whitney U-tests). (B, D, F, H) Mortality (chi-squared test, with post hoc Fisher exact test); $n = 15$ for each dose; $^aP \leq 0.1, ^*P \leq 0.05, ^{**}P \leq 0.01$. In all panels ‘V’ represents vehicle treatment.
purified CBDV ($P \leq 0.05$) and 100 mg·kg$^{-1}$ ($P \leq 0.01$) of modified CBDV BDS significantly suppressed seizure severity; additionally, 200 mg·kg$^{-1}$ of modified CBDV BDS produced a strong trend ($P \leq 0.1$) towards seizure severity suppression. Mortality was also significantly reduced by purified CBDV and modified CBDV BDS ($\chi^2(6) = 21.05$, $P \leq 0.01$; Figure 1D), with significant reductions caused by administration of $\geq$100 mg·kg$^{-1}$ ($P \leq 0.01$) of either substance.

As 100 mg·kg$^{-1}$ modified CBDV BDS and purified CBDV each suppressed seizure activity to a similar extent despite modified CBDV BDS containing less CBDV, we proceeded to compare the anticonvulsant properties of the modified CBDV BDS with combinations of purified CBD and CBDV to assess potential benefits of BDSs in Experiment 1.3. Administration of both modified CBDV BDS and matched levels of purified CBDV plus CBD caused a trend in the observed seizure severity ($U = 12.28$, $P \leq 0.1$; Figure 1E), where the highest dose of the purified pCBs ($P \leq 0.05$) and middle dose of the modified CBDV BDS ($P \leq 0.01$) significantly suppressed seizure severity. Mortality was significantly affected by administration of modified CBDV BDS or matched doses of pCBs ($\chi^2(6) = 26.81$, $P \leq 0.001$; Figure 1F), where middle or greater purified pCBs ($P \leq 0.05$) and all modified CBDV BDS ($P \leq 0.05$) doses significantly reduced mortality. In a further experiment (Experiment 1.4), we examined the effects of the BDS-pCB in the PTZ model of seizure; only a near-significant trend in seizure severity ($U = 6.555$, $P \leq 0.1$; Figure 1G) was seen due to 100 mg·kg$^{-1}$, suggesting an increase in seizure severity ($P \leq 0.1$) but with no effect on mortality (Figure 1H).

Thus, when these data are considered as a whole, modified CBDV BDS had a strong anticonvulsant effect in the PTZ model of seizures, producing comparable anticonvulsant effects as purified constituent pCBs (Figure 1E–G), but with lower pCB levels.

**Effects of modified and unmodified CBDV BDS on PTZ-induced convulsions and motor function**

Next, we compared modified and unmodified CBDV BDSs to determine whether the presence of $\Delta^2$-THC and $\Delta^2$-THCV affects the anticonvulsant profile of CBDV BDS (Experiment 2.1; Table 1). Administration of both modified and unmodified CBDV BDS significantly affected the observed seizure severity ($U = 21.57$, $P \leq 0.01$; Figure 2A); seizure severity was significantly suppressed by $\geq$150 mg·kg$^{-1}$ of both unmodified ($P \geq 0.05$) and modified ($P \leq 0.01$) CBDV BDS. Mortality was also significantly affected by CBDV BDS administration ($\chi^2(6) = 26.81$, $P \leq 0.001$; Figure 2B); 150–275 mg·kg$^{-1}$ ($P \leq 0.05$) of unmodified and $\geq$150 mg·kg$^{-1}$ ($P \leq 0.05$) of modified CBDV BDS significantly reduced mortality.

We next evaluated the effects of modified and unmodified CBDV BDSs on motor function, using static beam and grip strength assays (Experiment 2.2; Table 1). CBDV BDS significantly affected the number of animals that failed the static beam ($\chi^2(6) = 15.77$, $P \leq 0.05$; Figure 2C); $\geq$150 mg·kg$^{-1}$ of unmodified ($P \leq 0.05$) and 346 mg·kg$^{-1}$ of modified ($P \leq 0.01$) CBDV BDS significantly increased the failure rate. Notably, the greater failure rate of unmodified CBDV BDS-treated animals arose from exceeding the time limit. The foot slips/ metre and distance travelled on the static beam were unaffected by either modified or unmodified CBDV BDS, as was forelimb grip strength. The data presented in Figure 2 taken as a whole demonstrate that CBDV BDS anticonvulsant activity is unaffected by the presence or absence of $\Delta^2$-THC and $\Delta^2$-THCV. However, poor static beam performance following a lower dose of unmodified CBDV BDS than modified CBDV BDS indicates that, despite their opposing pharmacological actions at CB$_2$ cannabinoid receptors (see the Discussion section), $\Delta^2$-THC and/or $\Delta^2$-THCV content negatively affects motor.

**Modified and unmodified CBDV BDSs and isobolographic analysis of CBDV and CBD in the audiogenic seizure model**

Having demonstrated significant anticonvulsant effects of all CBDV BDSs in the PTZ model, we next evaluated their anti-convulsant activity in the audiogenic model of generalized seizure in mouse (Experiments 3.1 and 3.2).

In Experiment 3.1 (Table 1), the unmodified CBDV BDS significantly affected the proportion of animals that developed: wild running [$\chi^2(3) = 19.55$, $P \leq 0.001$; Figure 3A], where $\geq$100 mg·kg$^{-1}$ significantly reduced incidence ($P \leq 0.01$); clonic convulsions [$\chi^2(3) = 17.94$, $P \leq 0.001$; Figure 3A], where $\geq$100 mg·kg$^{-1}$ significantly reduced incidence ($P \leq 0.01$); and tonic convulsions [$\chi^2(3) = 17.14$, $P \leq 0.001$; Figure 3A], where $\geq$50 mg·kg$^{-1}$ ($P \leq 0.05$) significantly reduced their incidence. Body temperature was significantly lower in animals administered 100 mg·kg$^{-1}$ [36.5°C ± 0.2; $t(18) = 3.28$, $P \leq 0.01$] and 200 mg·kg$^{-1}$ [34.5°C ± 0.2; $t(18) = 4.78$, $P \leq 0.001$] of unmodified CBDV BDS than vehicle (37.3°C ± 0.1). Thereafter, we tested the modified CBDV BDS in Experiment 3.2 (Table 1), where administration significantly affected: wild running [$\chi^2(3) = 26.81$, $P \leq 0.001$; Figure 3B], where $\geq$50 mg·kg$^{-1}$ significantly reduced incidence ($P \leq 0.01$); and clonic convulsions [$\chi^2(3) = 21.18$, $P \leq 0.001$; Figure 3B], where $\geq$50 mg·kg$^{-1}$ ($P \leq 0.05$) significantly reduced incidence. Body temperature was significantly lower in animals administered 200 mg·kg$^{-1}$ unmodified CBDV BDS [33.7°C ± 0.6; $t(18) = 5.73$, $P \leq 0.001$] when compared with vehicle-treated animals (37.2°C ± 0.2).

As both modified and unmodified CBDV BDS reduced seizure activity at lower doses than purified cannabinooids in the PTZ model of seizure, we also used the tractable nature of the audiogenic seizure model to investigate any therapeutic interaction between CBDV and CBD using an isobolographic approach (Experiments 3.3 and 3.4). Firstly, in Experiment 3.3 we demonstrated that purified CBDV and CBD both reduced clonic convulsion incidence [$\chi^2(8) = 34.21$, $P \leq 0.001$; Figure 3C], where $\geq$100 mg·kg$^{-1}$ of both CBDV ($P \leq 0.05$) and CBD ($P \leq 0.05$) significantly reduced incidence. Furthermore, calculation of respective ED$_{50}$ values revealed that CBDV (64 mg·kg$^{-1}$) was more potent than CBD (80 mg·kg$^{-1}$). The clonic convulsion parameter was selected to evaluate seizure suppression due to the linear DRRs, and each dose producing a unique effect (Tallarida, 2006). As DRRs were significantly non-parallel [$F_{(3,6)} = 121.63$, $P \leq 0.01$; Figure 3C], in Experiment 3.4 we co-administered CBDV and CBD in a 1:1 dose effect ratio using 10 theoretical effect sizes (ED$_{1\alpha}$−ED$_{10\alpha}$) derived from the DRRs of the purified CBDV and CBD in Figure 3C. The ED$_{50}$ of co-administered CBDV and CBD did not differ significantly from the theoretical line of additivity [$\chi^2(2) = 3.44$, $P \geq 0.1$; Figure 3D; see the Methods section]
CBDV-rich extracts are anticonvulsant

Figure 2
Anticonvulsant and motor effects of modified and unmodified BDSs. (A, B) Experiment 2.1: effect of BDSs on the severity (A) and associated mortality (B) of PTZ-induced convulsion. In (A) median severity is shown in grey, box represents interquartile range and whiskers represent maxima and minima (Kruskal–Wallis test, with a post hoc Mann–Whitney U-tests); in (B), mortality is given as a percentage (chi-squared test, with post hoc Fisher exact test). (C–F) Experiment 2.2: side effect profile of BDSs in motor assays. (C–E) Performance on the static beam assay showing the failure rate (C; chi squared, Fisher exact post hoc test), the mean number of foot slips per metre (D) and the mean distance covered (E) after treatment with BDS or vehicle. (F) Mean forelimb grip strength (kgf). Data in (D–F) presented as mean ± SEM and analysed by ANOVA with Tukey post hoc test. (A, B) n = 15, (C–F) n = 10; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001.
Figure 3
Effects of CBDV BDS modified and unmodified, and isobolographic study of CBDV and CBD. (A, B) Experiments 3.1–3.2: percentage of animals exhibiting each convulsion parameter (WR, wild running; clonic, clonic convulsions; tonic, tonic convulsions; chi-squared test, with post hoc Fisher exact test) for unmodified BDS (A) and modified BDS (B). (C–E) Experiments 3.3–3.4: isobolographic determination of CBDV and CBD interactions. (C) Dose–response relationships of CBD and CBDV. (D) ED$_{50}$ isobole of CBDV and CBD with theoretical and actual ED$_{50}$s marked (chi-squared test). (E) Results from co-administration study and predicted results based on individual pCB studies (two-way ANOVA); $n = 10$; *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. 
plotted between the ED_{50}s of each drug when administered in isolation, thereby indicating an additive anticonvulsant action when the two compounds are combined at this effect size. Moreover, this additive interaction was present across the entire dose range, with no significant difference between the theoretical additive DRR \( F_1,9 = 0.49, P \geq 0.1; \) Figure 3E, derived from the pCBs in isolation, and the experimental DRR follows co-administration.

As a whole, these results demonstrate that modified and unmodified CBDV BDSs each have strong anticonvulsant activity in the mouse audiogenic seizure and complement the data produced in the PTZ seizure model. In addition, we demonstrate that anticonvulsant properties of CBDV and CBD are additive in the mouse audiogenic seizure model.

**Effects of CBDV BDSs on pilocarpine-induced convulsions in rats**

Both modified and unmodified CBDV BDSs were investigated in the pilocarpine model of acute, temporal lobe convulsion (Experiments 4.1 and 4.2; Table 1). In Experiment 4.1, unmodified CBDV BDS significantly affected convulsion severity \( (U = 13.15, P \leq 0.01; \) Figure 4A); \( \geq 100 \text{ mg} \cdot \text{kg}^{-1} \) significantly reduced severity \( (P \geq 0.05). \) No effect on mortality was observed (data not shown). In Experiment 4.2, using the dose at which unmodified CBDV BDS exerted its optimal anticonvulsant effect (\( 200 \text{ mg} \cdot \text{kg}^{-1} \); see Figure 4A), the effects of modified and unmodified CBDV BDSs and co-administered purified pCBs with matching doses of CBDV and CBD were compared. Drug treatment significantly affected convulsion severity \( (U = 10.64, P \leq 0.05; \) Figure 4A); unmodified CBDV BDS \( (P \leq 0.05) \) and purified pCBs \( (P \leq 0.001) \) reduced severity, and modified CBDV BDS produced a trend towards severity reduction \( (P \leq 0.1). \) Drug administration produced a trend towards a reduction in mortality \( [\chi^2(3) = 6.67, P \leq 0.1], \) where unmodified CBDV BDS reduced mortality \( (P \leq 0.1); \) data not shown.

**Radioligand binding assays**

First, displacement binding assays to compare the pharmacological profiles of unmodified CBDV BDS with purified CBDV were performed. Unmodified CBDV BDS showed greater affinity for CB1 cannabinoid receptors than purified CBDV in both MF1 mouse brain and hCB1-CHO cell membranes (Figure 5A,B and Table 2); purified CBDV only displaced \([\text{H}]\text{CP55940}\) at the highest concentration tested \( (10 \mu\text{M}). \) Next, to investigate the greater affinity of unmodified CBDV BDS over purified CBDV for CB1 cannabinoid receptors, displacement binding assays using MF1 whole brain membranes were performed with unmodified CBDV BDS in parallel with the modified CBDV BDS. Interestingly, removal of \( \Delta^9\text{-THC} \) and \( \Delta^9\text{-THCV} \) did not significantly affect the potency with which modified CBDV BDS displaced \([\text{H}]\text{CP55940}\) (Figure 5C and Table 2). However, the mean displacement, at the highest concentrations tested \( (1 \text{ and } 10 \mu\text{M}), \) was significantly reduced by this removal \( (P < 0.05) \) (Figure 5C and Table 2). To further assess the contribution made by \( \Delta^9\text{-THC} \) plus \( \Delta^9\text{-THCV} \) to the potency with which unmodified CBDV BDS displaced \([\text{H}]\text{CP55940}, \) we tested unmodified CBDV BDS in parallel with BDS-pCB to which a pCB \( (\text{CBDV, } \Delta^9\text{-THCV or } \Delta^9\text{-THC}) \) had been added. The resulting percentage concentration of the added pCB was the same as the percentage concentration of this pCB in unmodified CBDV BDS. Interestingly, we found...
that BDS-pCB + purified CBDV displaced [3H]CP55940 with significantly less potency than unmodified CBDV BDS (Figure 5D and Table 3). The addition of Δ9-THCV to BDS-pCB resulted in this BDS displacing [3H]CP55940 with slightly, but not significantly, less potency than unmodified CBDV BDS (Figure 5E and Table 3). Addition of Δ9-THC had no statistically significant effect on the potency or maximum percentage of displacement with which unmodified CBDV BDS displaced [3H]CP55940 from mouse brain membranes (Figure 5F and Table 3). Taken together, these results suggest that both Δ9-THC and Δ9-THCV together in the unmodified CBDV BDS accounts for its ability to displace [3H]CP55940 with greater potency than purified CBDV from specific binding sites in membranes from both MF1 whole brain and human CB1-CHO cells.

**Discussion**

Here, for the first time, we demonstrate that cannabis extracts rich in CBDV and CBD can exert significant anticonvulsant effects. Importantly, these effects were observed in three separate models of seizure across two species. Both the modified and the unmodified CBDV BDSs had broadly similar efficacies...
to purified CBDV as well as CBDV and CBD in combination. Thereafter, we determined that CBDV and CBD act together in an additive manner against audiogenic seizures. While the presence of Δ⁹-THC and Δ⁹-THCV in the extracts did not affect anticonvulsant profile, their presence adversely affected motor function and was responsible for the displacement of CB₁ cannabinoid receptor ligands in radioligand binding assays. Additionally, this is the first study to examine CBDV binding at the CB₁ cannabinoid receptor where affinity was found to be very limited.

Anticonvulsant effects of CBDV BDSs

The modified CBDV BDS was investigated first as Δ⁹-THC at sufficiently high doses can induce psychoactive effects via the CB₁ cannabinoid receptor, an undesirable clinical side effect. Although Δ⁹-THCV, a neutral CB₁ cannabinoid receptor antagonist, can exert limited anticonvulsant effects, the clinical profile of such compounds remains unclear but is likely to be distinct from that of inverse agonists, for example, rimonabant (Pertwee, 2005).

Modified CBDV BDS dose-dependently reduced seizure severity and mortality, clearly demonstrating that the modified CBDV BDS was anticonvulsant. When purified CBDV and the modified CBDV BDS were compared, both reduced seizure severity and mortality; CBDV affected severity at a lower dose than modified CBDV BDS, but doses suppressing mortality were comparable. Subsequently, instead of comparing by absolute weight to the principal pCB, we compared by CBDV and CBD content to see if co-administered CBDV and CBD further reduced seizure severity, a broadly similar anticonvulsant effect resulted. In addition, modified CBDV BDS significantly reduced mortality at a lower dose than purified pCBs, a possible benefit of the BDS over purified pCBs. However, in Experiments 1.1–1.3 and 2.1, the highest doses of both the modified and the unmodified CBDV BDSs did not appear as efficacious as the preceding dose. While a definitive cause for this remains to be determined, this effect was not due to any significant pro-convulsant activity of the non-cannabinoid fraction (see Experiment 1.4). However, the complex nature (~400 discrete non-cannabinoid constituents) of standardized cannabis extracts (Elsohly and Slade, 2005) means that concentrations of some constituents that could affect cannabinoid pharmacokinetics might only appear at higher doses to produce the differences seen.

Overall, these results indicate that CBDV BDS could be efficacious in the treatment of generalized seizures (Löscher, 2011), and its anticonvulsant activity against PTZ-induced

### Table 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compound</th>
<th>$K_i$ (nM) (95% CL)</th>
<th>Maximum % displacement (95% CL)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*</td>
<td>Pure CBDV</td>
<td>No plateau</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td>A*</td>
<td>Unmodified CBDV BDS</td>
<td>127.6 (52.5 and 310.2)</td>
<td>113.6 (98.2 and 129.0)</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>Unmodified CBDV BDS</td>
<td>722.6 (403.7 and 1293)</td>
<td>98.6 (83.8 and 113.4)</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>Modified CBDV BDS</td>
<td>1010 (223.5 and 4564)</td>
<td>55.5 (33.4 and 77.6)</td>
<td>4</td>
</tr>
</tbody>
</table>

*Mean $K_i$ and maximum % displacement values for pure CBDV and unmodified CBDV BDS in hCB₁-CHO cell membranes were not significantly different from those obtained in MF1 whole mouse brain membranes (see Results and Figure 5A,B). CL, confidence limit.

### Table 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compound</th>
<th>$K_i$ (nM) (95% CL)</th>
<th>Maximum % displacement (95% CL)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Unmodified CBDV BDS</td>
<td>300.8 (159.2 and 568.5)</td>
<td>89.2 (78.7 and 99.7)</td>
<td>4</td>
</tr>
<tr>
<td>A</td>
<td>BDS-pCB + pure CBDV</td>
<td>2151 (643.9 and 7184)</td>
<td>80.2 (51.8 and 108.6)</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>Unmodified CBDV BDS</td>
<td>363.9 (141.4 and 936.5)</td>
<td>85.8 (70.4 and 101.3)</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>BDS-pCB + pure THCV</td>
<td>960.0 (203.8 and 4523)</td>
<td>73.0 (44.6 and 101.3)</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>Unmodified CBDV BDS</td>
<td>604.5 (252.5 and 1447)</td>
<td>99.5 (79.7 and 119.3)</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>BDS-pCB + pure THC</td>
<td>861.7 (195.1 and 3805)</td>
<td>86.5 (57.4 and 115.6)</td>
<td>4</td>
</tr>
</tbody>
</table>

CL, confidence limit.
seizures justifies further investigation of its utility against absence seizures using absence epilepsy models such as the GAERS and WAG/Rij rats (Coenen et al., 1992; Marescaux and Vergnes, 1995; Hosford and Wang, 1997).

Both unmodified and modified CBDV BDS reduced seizure severity when compared directly, with little appreciable difference in efficacy in the PTZ model. However, when the static beam task was employed to evaluate any motor side effects of the modified and unmodified CBDV BDSs, a drug-induced increase in the number of animals failing the task was seen. This effect was observed for all doses of unmodified CBDV BDS, but only the highest dose of modified CBDV BDS, suggesting that Δ9-THC and/or Δ9-THCV were responsible for a significant proportion of the observed motor deficits. The majority of unmodified CBDV BDS-treated animals failed due to the task’s 5 min time limit being exceeded as animals remained stationary. This is consistent with previous reports on the effects of Δ9-THC on motor function (Järbe et al., 2002) and further suggests that the Δ9-THCV present in the unmodified CBDV BDS may have been insufficient to overcome Δ9-THC-mediated CB1 cannabinoid receptor partial agonism. In contrast to the effects on static beam performance, neither CBDV BDS elicited grip strength deficits, a test for muscle relaxation (Nevins et al., 1993) and, putatively, functional neurotoxicity (Sed et al., 2008). Many currently available AEDs produce notable side effects in people with epilepsy, including motor function deficits (Schachter, 2007), which reduce quality of life. These deficits are often also seen in non-clinical species (Löschler, 2011), potentially reducing the observed seizure severity in models of seizure (Hill et al., 2012a).

Cannabis-based BDSs have been reported to possess different pharmacological activity to their principal pCB constituent, where the presence of the non-principal pCBs and pCB-free fraction can enhance or decrease activity in some in vitro assays (De Petrocellis et al., 2011). Given this a priori knowledge and because the results obtained from our comparisons of CBDV BDS and purified pCB effects in seizure models suggested possible advantages of CBDV BDSs, we also investigated the actions and interactions of the pCB and pCB-free components of CBDV BDSs. Importantly, we showed that the BDS-pCB exerted no significant effect upon seizure severity in the PTZ model of seizure. In order to investigate interactions between CBDV and CBD, we employed the mouse audiogenic seizure model. Prior to the isobolographic study, we confirmed a dose-dependent anticonvulsant effect of the modified and unmodified CBDV BDSs in the audiogenic model comparable to that in PTZ. A core temperature below 34.5°C in DBA/2 mice can reduce seizure incidence in the audiogenic model (Essman and Sudak, 1964); however, significant reductions in seizure severity were seen in animals with body temperatures higher than this threshold, indicating that anticonvulsant effects were not primarily due to hypothermia. Using the isobolographic study design, we demonstrated an additive anticonvulsant effect of purified CBDV and CBD co-administration; there were no deleterious effects from co-administering these pCBs.

We have previously reported that administration of ≤200 mg·kg⁻¹ CBDV did not produce anticonvulsant effects in the pilocarpine model (Hill et al., 2012a) except in highly powered experiments (n = 60 CBDV-treated animals), and that CBD did not affect convulsion severity in this model despite reduced tonic-clonic seizure incidence (Jones et al., 2012). In contrast, here we have demonstrated that ≥116 mg·kg⁻¹ CBDV and ≥27 mg·kg⁻¹ CBD co-administered as unmodified CBDV BDS or a combination of purified pCBs significantly reduced convulsion severity, demonstrating a clear advantage to combinatorial use of CBDV and CBD to treat temporal lobe convulsions in this model.

CB1 cannabinoid receptor binding of CBDV BDSs

In MF1 mouse whole brain membranes, we found that unmodified CBDV BDS showed greater affinity for CB1 cannabinoid receptors than purified CBDV, with mean Kᵢ values for the micromolar range; Pertwee, 2008, both Δ9-THC and Δ9-THCV are potent CB1 cannabinoid receptor ligands (mean Kᵢ values in the nanomolar range; Pertwee, 2008). Interestingly we found that (i) although the modified CBDV BDS’s affinity was not significantly affected by the removal of Δ9-THC and Δ9-THCV, its maximum percentage displacement was significantly lower than that of unmodified CBDV BDS; (ii) the addition of Δ9-THC or Δ9-THCV to the BDS-pCB had no statistically significant effect on the ability of this BDS to displace [3H]CP55940 from mouse brain membranes compared with unmodified CBDV-BDS. In contrast (Figure 5D), the BDS-pCB plus CBDV mixture displaced [3H]CP55940 with significantly less potency than unmodified CBDV-BDS, and is consistent with our finding that purified CBDV binds to CB1 cannabinoid receptors with only very weak affinity (limited effect and no plateau was reached at the highest concentration tested). Together, these in vitro results suggest that the presence of both Δ9-THC and Δ9-THCV accounts for the ability of unmodified CBDV BDS to displace [3H]CP55940 with higher affinity than purified CBDV. Furthermore, as modified and unmodified CBDV BDS display a similar anticonvulsant action in vivo, the binding data suggest that the anticonvulsant mechanism of action of the CBDV BDSs is not CB1 cannabinoid receptor mediated. Despite CBD inhibiting ion channel function (e.g. T-type Ca²⁺ channels; Ross et al., 2008) and increasing inhibitory activity at some GPCRs (e.g. 5-HT₁A; Russo et al., 2005), the specific molecular target(s) underlying CBD’s anticonvulsant effects remain unconfirmed (Hill et al., 2012b). While limited structural changes can profoundly affect cannabinoid pharmacology (e.g. Δ9-THC and Δ9-THCV at CB1 cannabinoid receptors), it is not unreasonable to suggest that CBDV may share some
pharmacological properties with CBD. CBD and CBDV have agonist effects at TRPA1, TRPV1 and TRPV2 receptors, and antagonistic properties at TRPM8 (De Petrocellis et al., 2011). Although TRPV1 expression has been reported in the brain (Toth et al., 2005), as with other TRP receptors to which CBDV can bind, its functional role in health and disease is unclear. CBDV has also been reported to inhibit diacylglycerol lipase-α (De Petrocellis et al., 2011), an enzyme responsible for the synthesis of the endocannabinoid, 2-arachidonoylglycerol, and to stimulate fibroblast formation via a mechanism that can be functionally blocked by concurrent CB2 cannabinoid receptor antagonism (Scutt and Williamson, 2007). However, no evidence yet exists to link any of these mechanisms to CBDV’s anticonvulsant activity.

Conclusions

In conclusion, these results demonstrate a strong CB1 cannabinoid receptor-independent anticonvulsant action of both modified and unmodified CBDV BDSs in three models of seizure, across two species. While unmodified CBDV BDS negatively affected performance in one motor function task, this was in stark contrast to the modified CBDV BDS that was well tolerated at anticonvulsant doses. This indicates that both modified and unmodified CBDV BDSs have potential to treat generalized or temporal lobe seizures with the modified CBDV BDS being best tolerated.

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Conflict of Interest

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