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Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures

Nicholas A. Jones [a,b,*], Sarah E. Glyn [a,b], Satoshi Akiyama [b,c], Thomas D.M. Hill [a], Andrew J. Hill [a,b], Samantha E. Weston [a], Matthew D.A. Burnett [a], Yuki Yamasaki [b,c], Gary J. Stephens [a], Benjamin J. Whalley [a], Claire M. Williams [b]

[a] School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6AJ, UK
[b] School of Psychology, University of Reading, Whiteknights, Reading RG6 6AJ, UK
[c] Qs' Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-0192, Japan

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ABSTRACT

Cannabis sativa has been associated with contradictory effects upon seizure states despite its medicinal use by numerous people with epilepsy. We have recently shown that the phytocannabinoid cannabidiol (CBD) reduces seizure severity and lethality in the well-established *in vivo* model of pentylenetetrazole-induced generalised seizures, suggesting that earlier, small-scale clinical trials examining CBD effects in people with epilepsy warrant renewed attention. Here, we report the effects of pure CBD (1, 10 and 100 mg/kg) in two other established rodent seizure models, the acute pilocarpine model of temporal lobe seizure and the penicillin model of partial seizure. Seizure activity was video recorded and scored offline using model-specific seizure severity scales. In the pilocarpine model CBD (all doses) significantly reduced the percentage of animals experiencing the most severe seizures. In the penicillin model, CBD ($\geq$10 mg/kg) significantly decreased the percentage mortality as a result of seizures; CBD (all doses) also decreased the percentage of animals experiencing the most severe tonic–clonic seizures. These results extend the anti-convulsant profile of CBD; when combined with a reported absence of psychoactive effects, this evidence strongly supports CBD as a therapeutic candidate for a diverse range of human epilepsies.

1. Introduction

Dating back to 4000 BC, *Cannabis sativa* has a long history of medicinal use for the treatment of a variety of disorders such as rheumatism, chronic inflammation and pain management, in addition to control of convulsions.[1] More recently, *C. sativa* has been ascribed both pro-[2] and anti-convulsant effects[3] despite numerous people with epilepsy continuing to use *C. sativa* medicinally for seizure control.[4,5] Since Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of *C. sativa* was isolated,[6] more than 60 other phytocannabinoids (cannabis-derived components) have also been identified, isolated and shown to possess varied pharmacological activity.[7] One such phytocannabinoid is cannabidiol (CBD), typically the second most prevalent phytocannabinoid in *C. sativa*, whose structure was first described by Mechoulam and Shvo.[8] CBD currently represents the most promising phytocannabinoid candidate for clinical utilisation due to its non-psychotropic properties, low toxicity and high tolerability in humans and other animal species.[9–11]

Early preclinical work demonstrated that CBD possesses anti-convulsant properties.[12–14] In rats, CBD was an effective and relatively potent anti-convulsant in the maximal electroshock (MES) and audiogenic seizure models; findings that compared favourably with the clinically used AEDs employed in the same study.[15] In mice, CBD pretreatment prevented tonic convulsions caused by either MES seizures, γ-aminobutyric acid (GABA) antagonists or inhibitors of GABA synthesis, in addition to reliably protecting against 3-mercaptoproprionic acid-induced lethality.[10] Overall, these pre-clinical seizure studies confirmed CBD's anti-convulsant profile and are consistent with an assertion of therapeutic benefits in human epilepsies.

Interestingly and despite these promising pre-clinical results, only one clinical trial has thus far explored the potential anti-convulsant effects of CBD in humans.[11] Fifteen people experiencing secondary generalised epilepsy with temporal lobe focus that was

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; AED, anti-epileptic drug; CBD, cannabidiol; CCTVs, closed-circuit television cameras; CNS, central nervous system; GABA, γ-aminobutyric acid; mAChR, muscarinic acetylcholine receptor; MES, maximal electroshock; NMDA, *N*-methyl-D-aspartate; PTZ, pentylenetetrazole; TLE, temporal lobe epilepsy.

* Corresponding author at: School of Pharmacy, University of Reading, Whiteknights, Hopkins Building, Reading RG6 6UB, UK. Tel.: +44 0118 378 8464; fax: +44 0118 3787 8703.
E-mail addresses: n.a.jones@reading.ac.uk, najones84@googlemail.com (N.A. Jones).

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unresponsive to prescribed AED treatments were recruited. Four of eight of those receiving CBD in conjunction with their existing AEDs remained virtually seizure-free during the supplementation period and the remainder of this patient group exhibited a marked improvement in seizure control.[11] Surprisingly however, no further clinical trials employing CBD have been published.

The therapeutic potential of the phytocannabinoids attracted renewed interest following the discovery and characterisation of the endocannabinoid signalling system that comprises the G protein-coupled cannabinoid CB_1 and CB_2 receptors, a family of endogenous cannabinoid receptor ligands and several enzymes involved in their metabolism and degradation.[16] Whilst a number of phytocannabinoid actions are mediated *via* CB_1 and/or CB_2 receptors,[7,17] including the now well-known CB1 receptor-mediated modulation of epileptiform and seizure activity,[18,19] CBD exhibits negligible affinity for either CB_1 and/or CB_2 receptors.[7,20,21] Consequently, it is likely that the anti-convulsant effects of CBD described above arise *via* cannabinoid receptor-independent mechanisms.[17,22–24]

Recently, we have shown that CBD inhibits epileptiform activity *in vitro* and reduces seizure severity and lethality in the pentylenetetrazole (PTZ) model of generalised seizures *in vivo*, strongly supporting reconsideration of the use of CBD in the treatment of human epilepsies.[22] However, in order to strengthen earlier findings and inform appropriate human study design, assessment of the anti-convulsant potential of CBD against untested seizure phenotypes *in vivo* is required.

In this present study, we have investigated whether CBD exerts anti-convulsant effects in the acute pilocarpine-induced model of temporal lobe seizure and the penicillin-induced model of partial seizure in rat. Furthermore, four behavioural tests were undertaken to assess the effects of CBD on rodent motor function, providing complementary evidence of CBD's lack of toxicity.

2. Materials and methods

2.1. Animals

Adult male Wistar Kyoto rats (Harlan, Bicester, UK) were used in both seizure models and the rotarod test described below (acute pilocarpine model of temporal lobe seizure: >P21, 70–110 g; penicillin model of partial seizure: >P40, 250–300 g; motor function tests: >P28, starting weight 110–140 g). Animals were housed at room temperature on a 12:12-h day/night cycle (lights on at 0800) and given *ad libitum* access to food and water. On days prior to seizure induction, animals were habituated to handling, experimental procedures and the test environment. All experiments were carried out in accordance with UK Home Office regulations (Animals (Scientific Procedures) Act, 1986).

2.2. CBD administration

CBD penetrates the blood–brain barrier such that 120 mg/kg delivered intraperitoneally in Wistar Kyoto rats provides C_{max} = 6.8 μg/g at T_{max} = 120 min and, at the same dosage, no major toxicity, genotoxicity, or mutagenicity has been observed (personal communication with GW Pharmaceuticals Ltd.; Study Report UNA-REP-02). Prior to seizure or motor function protocols, animals received (i.p.) 1, 10 or 100 mg/kg CBD in all seizure experiments or 50, 100 or 200 mg/kg CBD in motor function tests (GW Pharmaceuticals, Porton Down, Wiltshire, UK). The vehicle employed was a 1:1:18 solution of ethanol, Cremophor (Sigma–Aldrich, Poole, UK) and 0.9% (w/v) NaCl. In each experiment, a group of animals that received volume-matched doses of vehicle alone served as a negative control.

2.3. Acute pilocarpine in vivo seizure model

Pilocarpine is a muscarinic acetylcholine receptor agonist that, following systemic administration, causes localised seizure foci in the limbic system consistent with temporal lobe seizures[25] ($n \geq 14$ for each group). 15 min after CBD or vehicle administration, animals were injected with the muscarinic receptor antagonist methylscopolamine (Sigma–Aldrich, Poole, UK; 1 mg/kg; i.p.) to minimise peripheral pilocarpine-induced side-effects. 45 min later, pilocarpine (Sigma–Aldrich, Poole, UK; 380 mg/kg; i.p.) was administered to induce seizures and animal behaviour was monitored for a further 60 min. On completion of the experimental procedure animals were euthanised by CO_2 inhalation.

2.4. Penicillin in vivo seizure model

Penicillin selectively antagonises $GABA_A$-receptor mediated inhibitory postsynaptic potentials in the central nervous system (CNS).[26,27] Surgical implantations of cannulae were required to enable the focal administration of penicillin G potassium salt (penicillin; Sigma–Aldrich) directly into the cerebral ventricles to induce partial seizures.[28] Prior to surgery, animals were placed in an isoflurane anaesthetic induction chamber (Vet Tech Solutions Ltd., Cheshire, UK) which was attached to an isoflurane machine (Vet Tech Solutions Ltd.) with an isoflurane vaporiser, oxygen tank and active scavenging unit. The isoflurane (National Veterinary Services, Stoke on Trent, UK) concentration for induction was set to 5% and the oxygen flow rate was set to 2 L/min. The anaesthetised animals were then placed on a stereotaxic frame (David Kopf, Bilaney Consultants Ltd., Kent, UK) with an anaesthesia mask (David Kopf Model 906, Bilaney) attached to the patient breathing circuit of the isoflurane machine. Isoflurane concentration was initially 4% before reduction to 3.5% for maintenance of anaesthesia during the surgery. The oxygen flow rate was set to 1.5 L/min throughout the surgery. Fucithalmic Vet (Dechra Veterinary Products A/S, Uldum, Denmark) eye ointment was applied to the eyes during the surgery. After cranial midline incision, a 26-gauge guide cannula (Bilaney) was implanted using flat-skull stereotaxic technique into the right lateral cerebral ventricle. In all surgeries, bregma was used as a reference point and implantation co-ordinates were taken from the atlas of Paxinos and Watson[29] (lateromedial: +2.0 mm; anteroposterior: −0.6 mm; dorsoventral: −4.2 mm). After fixation to the skull with two stainless steel screws (1 mm diameter; Bilaney) and dental cement (Advanced Healthcare Limited, Kent, UK) each cannula was sealed with a stylet (Bilaney) to maintain patency. Post-operatively, buprenorphine hydrochloride (Reckitt Benckiser Healthcare (UK) Ltd., Hull, UK; 1 mg/kg; s.c.) and 0.9% (w/v) NaCl (1 ml; s.c.) were administered as required. Animals were housed individually and allowed at least one week to recover from surgery.

One hour after CBD administration, 525 IU penicillin was infused into the right lateral ventricle in 1.5 μl 0.9% (w/v) NaCl to induce partial seizures (n = 17–18 for each group). Intracerebroventricular infusions were made by attaching the implanted cannula to a 10 μl Hamilton syringe (Fisher Scientific, Loughborough, UK; infusion rate 1.5 μl/min) *via* a polyethylene tube (Bilaney). Animal behaviour was then monitored for 120 min after penicillin administration. On completion of the experimental procedure, animals were euthanised by CO_2 inhalation before being decapitated. Removed heads were placed in 4% (w/v) paraformaldehyde (Sigma), left to fix for one week at room temperature, then dissected and cannula placement confirmed as right lateral ventricle (conducted blind with respect to seizure scoring results). Results from any animals which exhibited an incorrect cannula position were omitted from the study.