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Acute effect of cannabidiol on the activity of various novel antiepileptic drugs in the maximal electroshock- and 6 Hz-induced seizures in mice: Pharmacodynamic and pharmacokinetic studies

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HIGHLIGHTS

- Cannabidiol potentiated the activity of some novel antiepileptic drugs.
- Cannabidiol decreased the activity of levetiracetam.
- Cannabidiol increased serum and/or brain concentrations of some antiepileptic drugs.
- Some antiepileptic drugs increased serum and/or brain concentrations of cannabidiol.
- This study indicates the need for monitoring of both antiepileptic drug and cannabidiol concentrations.

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ABSTRACT

Cannabidiol and cannabidiol-enriched products have recently attracted much attention as an add-on therapy for epilepsy, especially drug-resistant seizures. It should be, however, remembered that concomitant use of cannabidiol and antiepileptic drugs may pose a risk of interactions between them. For this reason, the aim of our study was to assess the effect of cannabidiol on the activity of selected new antiepileptic drugs in the electrically-induced seizure models in mice. We studied the effect of cannabidiol on the anticonvulsant action of topiramate, oxcarbazepine, lamotrigine, and pregabalin in the maximal electroshock-induced seizure test as well as on the activity of levetiracetam, tiagabine, lacosamide, and gabapentin in the 6 Hz seizure test in mice. We showed that cannabidiol increased the activity of topiramate, oxcarbazepine, pregabalin, tiagabine, and gabapentin. It did not affect the anticonvulsant effect of lamotrigine and lacosamide. Interestingly, cannabidiol attenuated the anticonvulsant activity of levetiracetam. Co-administration of antiepileptic drugs with cannabidiol did not cause adverse effects such as impairment of motor coordination, changes in neuromuscular strength or potentiation of the cannabidiol-induced hypolocomotion. Serum and brain levels of antiepileptic drugs and cannabidiol were determined by using HPLC in order to ascertain any pharmacokinetic contribution to the observed behavioral effects. Only interaction with levetiracetam was purely pharmacodynamic in nature because no changes in serum and brain concentration of either levetiracetam or cannabidiol were observed. Increased anticonvulsant activity of topiramate, oxcarbazepine, pregabalin, tiagabine, and gabapentin could be, at least in part, related to pharmacokinetic interactions with cannabidiol because there were changes in serum and/or brain concentrations of antiepileptic drugs and/or cannabidiol. Pharmacokinetic interactions cannot be also excluded between lacosamide and cannabidiol because cannabidiol increased brain concentration of lacosamide and lacosamide increased brain concentration of cannabidiol. Further pharmacokinetic studies are required to evaluate the type of interactions between cannabidiol and novel antiepileptic drugs.

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Abbreviations

CC_{50}	median convulsive current required to induce seizure response in 50% of mice
ED_{50}	median effective dose
ip	intraperitoneally
IS	internal standard
P-gp	glycoprotein P
MES	maximal electroshock seizure
MEST	maximal electroshock seizure threshold
SEM	standard error of the mean

1. Introduction

Cannabis sativa L. has been used as a medicinal plant to treat a vast array of different health problems for millennia. The plant contains over 500 compounds and new ones continue to be discovered (Jones et al., 2012; Gloss and Vickrey, 2014). Cannabidiol is a major non-psychoactive phytocannabinoid derived from *Cannabis*. A wide range of pharmacological effects of cannabidiol has been demonstrated, which makes it a promising candidate for the treatment of various conditions including neurodegenerative and inflammatory diseases, pain, cancer, anxiety, depression, and epilepsy (Pisanti et al., 2017; Scuderi et al., 2009).

A number of preclinical studies showed that cannabidiol exerts broad spectrum anticonvulsant activity. It was found to be active in a variety of acute seizure models in rodents including maximal electroshock (MES)- and 6 Hz-induced seizures, audiogenic seizures as well as seizures induced by isoniazid, cocaine and several GABA receptor antagonists, i.e., pentylenetetrazole, bicuculline, and picrotoxin (Devinsky et al., 2014; Consroe et al., 1982; Perucca, 2017). Moreover, cannabidiol showed anticonvulsant effects in the acute pilocarpine model of temporal lobe seizure (Jones et al., 2012; Patra et al., 2019) and the penicillin model of partial seizure in rats (Jones et al., 2012). It also reduced the number of rats that developed status epilepticus following intrahippocampal pilocarpine injection (Do Val-da Silva et al., 2017). In contrast, only few data exist on the effect of cannabidiol in animal models of chronic epilepsy and epileptogenesis. Its antiseizure activity was reported in electrically-evoked kindling (Turkanis et al., 1979), cobalt-induced epilepsy model in rats (Chiu et al., 1979), and most recently in the corneally kindled mice (Klein et al., 2017; Patra et al., 2019). Antiepileptogenic effect of cannabidiol was observed in the pentylenetetrazole-induced kindling model in rats (Mao et al., 2015).

Although anticonvulsant potential of cannabidiol has been reported since 1970s, in the last few years we have witnessed enormous interest in the use of medical marihuana or cannabidiol itself in the treatment of refractory epilepsy, especially intractable pediatric epilepsy (Brodie and Ben Menachem, 2018). This phenomenon results mainly from numerous anecdotal and case reports of remarkable responses following treatment with cannabidiol-enriched products in some patients. Very recently, several randomized, double-blinded, placebo-controlled clinical trials showed beneficial effects of cannabidiol in patients with Dravet and Lennox-Gastaut syndromes (Thiele et al., 2018; Devinsky et al., 2017, 2018a). There are, however, still many questions to answer before cannabidiol becomes a standard add-on therapy for refractory epilepsy. One of them is a question on possible interactions with antiepileptic drugs.

Data on the potential interactions between cannabidiol and currently used anticonvulsants are quite limited. In an animal study, cannabidiol potentiated the anticonvulsant activity of phenytoin and phenobarbital but it reduced the potency of chlordiazepoxide, clonazepam, trimethadione, and ethosuximide (Consroe and Wolkin, 1977). A pharmacokinetic drug-drug interaction between cannabidiol and clobazam was reported in a group of 13 pediatric patients (Geffrey et al., 2015). An open-label safety study revealed increased serum levels of N-desmethylclobazam (clobazam metabolite), topiramate, rufinamide, zonisamide, and eslicarbazepine in epileptic patients taking cannabidiol (Gaston et al., 2017). In another study, cannabidiol co-administration also resulted in elevated serum level of N-desmethylclobazam (but not topiramate, valproate, levetiracetam, and stiripentol) in children with Dravet syndrome (Devinsky et al., 2018b). Thus, further studies are required to provide a better insight into the interactions between cannabidiol and currently used antiepileptic drugs.

For this reason, the present study was undertaken to evaluate the effect of cannabidiol on the anticonvulsant action of several second and third generation antiepileptic drugs. We tested the influence of cannabidiol on the activity of lamotrigine, topiramate, oxcarbazepine, and pregabalin in the MES-induced seizure test. All of these drugs are highly effective against MES-induced seizure. The effects of cannabidiol on the activity of tiagabine, gabapentin, and levetiracetam were evaluated in the 6 Hz-induced seizure model in mice because these drugs are considered as virtually ineffective in the MES test (Löscher, 2011; Łuszczki and Czuczwar, 2006). We also investigated the effect of cannabidiol on the protective efficacy of lacosamide against 6 Hz-induced seizure to test at least one sodium channel blocker in this model. Brain and serum concentrations of cannabidiol and the tested antiepileptic drugs were determined to confirm or exclude any pharmacokinetic interactions between them. Finally, some acute adverse effects of cannabidiol, antiepileptic drugs alone and their combinations were evaluated.

2. Materials and methods

2.1. Animals

Inbred male albino Swiss mice (25–30 g), purchased from a licensed breeder (Laboratory Animals Breeding, Ilkowice, Poland), were used in the present study. Upon arrival, the animals were adapted to the laboratories for at least 7 days before being used in the experiments. All the animals were housed in groups of 8 in an environmentally controlled animal room (ambient temperature 21–24 °C; relative humidity 45–65%; artificial 12:12 light:dark cycle, lights on at 6:00 a.m.). A nutritionally-balanced rodent chow diet (Murigran, Agropol S.J., Motycz, Poland) and filtered water were freely available. All behavioral experiments were performed between 8:00 a.m. to 3:00 p.m., after a minimum 30-min adaptation period to the conditions kept in the experimental room.

The study was carried out under experimental protocols approved by the Local Ethical Committee in Lublin. All procedures were in strict compliance with the European Union Directive of 22 September 2010 (2010/63/EU) and Polish legislation concerning animal experimentation. The total number of animals used in the present study was 1471. Each animal was exposed to an electrical stimulation only once. All efforts were made to minimize both the animal suffering and the number of animals used in the present study.

2.2. Drugs

Cannabidiol (THC Pharm GmbH, Frankfurt, Germany) and all the antiepileptic drugs (Toronto Research Chemicals Inc., Toronto, Canada) were suspended in a 1% solution of Tween 80 (POCH, Gliwice, Poland) and administered intraperitoneally (*ip*), as follows: tiagabine – 15 min, lacosamide and oxcarbazepine – 30 min, gabapentin, levetiracetam, lamotrigine, topiramate, cannabidiol – 60 min, and pregabalin – 120 min before the tests. The pretreatment times and route of administration of the drugs were based upon information about their biological activity from the literature and our previous experiments (Deiana et al., 2012; Florek-Łuszczki et al., 2015; Zolkowska et al., 2016; Socała et al., 2018). All drug suspensions were prepared freshly and administered at a volume of 0.1 ml per 10 g of body weight. Control animals received vehicle only.

2.3. Maximal electroshock seizure test

Seizures were induced by applying a sinusoidal alternating current (50 Hz; maximum output voltage 500 V, stimulus duration 0.2 s) via transcorneal electrodes with the usage of rodent shocker (type 221; Hugo Sachs Elektronik, Freiburg, Germany). Tonic hindlimb extension was taken as an endpoint. Two different experimental approaches were used: (1) the maximal electroshock seizure threshold (MEST) test was employed to evaluate the effects of cannabidiol on the threshold for tonic hindlimb extension and (2) the maximal electroshock seizure (MES) test was used to evaluate the effect of cannabidiol on the protective activity of antiepileptic drugs against tonic hindlimb extension. The seizure threshold from the MEST test was determined in groups of 20 mice and expressed as the median convulsive current (CC_{50} with confidence limits for 95% probability) predicted to produce tonic hindlimb extension in 50% of the animals tested. In the MES test, 3–5 groups of animals were treated with increasing doses of an antiepileptic drug (alone or in combination with cannabidiol) and they were subjected to the stimulation. Each group consisted of 8 animals (i.e., 8 animals/dose) with an exception for topiramate where 12 animals in one group were used. Each animal was stimulated only once. After constructing a dose-response curve, a log-probit method (Litchfield and Wilcoxon, 1949) was used to determine the median effective doses (ED_{50}) of antiepileptic drugs, i.e., doses (in mg/kg) that protect 50% of animals against MES-induced tonic hindlimb extension. The experimental procedures have been described in detail in our earlier studies (Socała et al., 2018; Wlaź et al., 2012).

2.4. Six hertz (6 Hz) psychomotor seizure test

Psychomotor seizures were induced by applying square-wave alternating current stimuli (pulse width 0.2 ms, duration 3 s, frequency 6 Hz) via transcorneal electrodes delivered by a Grass S48 stimulator coupled to a Grass model CCU1 constant current unit (Grass Technologies, Warwick, RI, USA). The 6 Hz-induced seizures were characterized by stunned posture, eye blinking, head nodding, rearing, forelimb clonus, twitching of the vibrissae, and elevated tail. Two methodologically different experimental approaches were used: (1) the 6 Hz seizure threshold test was employed to evaluate the effects of cannabidiol on the threshold for psychomotor seizure and (2) the 6 Hz seizure test at 32 mA (Giordano et al., 2015) was employed to evaluate the effect of cannabidiol on the protective activity of antiepileptic drugs against psychomotor seizures. The results from the 6 Hz seizure threshold test were expressed as the CC_{50} values (with confidence limits for 95% probability) determined in groups of 20 mice each. To determine the ED_{50} values of antiepileptic drugs, 3–4 groups of animals (8 mouse/group) were injected with increasing doses of antiepileptic drugs or their combinations with cannabidiol and subjected to the stimulation. Each animal was stimulated only once. After constructing a dose-response curve, a log-probit method (Litchfield and Wilcoxon, 1949) was used to calculate the ED_{50} of antiepileptic drugs, i.e., doses (in mg/kg) that protect 50% of animals against the 6 Hz-induced seizures. The experimental procedures were carried out as described earlier (Nieoczym et al., 2013; Wlaź et al., 2012).

2.5. Adverse effects tests

The acute adverse effects of cannabidiol (at the highest dose tested – 100 mg/kg), antiepileptic drugs (at doses corresponding to their ED_{50} values from the MES and 6 Hz test) and their combinations were quantified in the chimney test (assessment of motor coordination), the grip-strength test (assessment of neuromuscular strength) as well as in the spontaneous locomotor activity test. The tests were performed as described in detail elsewhere (Nieoczym et al., 2013; Socała et al., 2018).

2.6. Pharmacokinetic studies

For pharmacokinetic studies, mice were injected with antiepileptic drugs (at doses that corresponded to their ED_{50} values from the MES and 6 Hz tests) alone or in combination with cannabidiol at 100 mg/kg. The animals were decapitated at time points that were identical to those in the seizure tests. The trunk blood was collected into polypropylene tubes and allowed to clot at room temperature. Subsequently, it was centrifuged at 5,600 rpm for 10 min. Serum was collected into new polypropylene tubes. Immediately after the decapitation, brains were dissected from the skull and washed with cold 0.9% NaCl. Samples were kept at − 20 °C until analysis.

2.6.1. Determination of antiepileptic drugs concentrations

Murine brains containing studied antiepileptic drugs were homogenized in distilled water (1:4, w/v) with a tissue homogenizer TH220 (Omni International, Inc., Warrenton, VA, USA). To 50–100 µl of serum or 200 µl of brain homogenates appropriate internal standard (IS) solutions were added. The IS for levetiracetam quantification was caffeine, for topiramate and pregabalin – gabapentin, for gabapentin – pregabalin, and for tiagabine – pentoxifylline.

The samples containing levetiracetam and IS were extracted with dichloromethane on a shaker (IKA Vibrax VXR, Germany) for 20 min and centrifuged at 11,266 rpm (TDX fixed-speed centrifuge, Abbott Laboratories, Irving, TX, USA) for 4 min. The organic layers were transferred into conical glass tubes and evaporated to dryness at 37 °C under a gentle stream of nitrogen in a water bath. The residues were dissolved with 100 µl of methanol and aliquots of 5 µl were injected into the HPLC system. In the case of lamotrigine and lacosamide, to 50 or 100 µl of serum or 200 µl of brain homogenate containing any of these drugs and an appropriate IS 600 µl of ethanol (lamotrigine) or 500 µl methanol (lacosamide) was added. The samples underwent exactly the same procedure as the samples containing levetiracetam with the exception that after centrifugation supernatant (10 µl) was directly injected onto the column. The HPLC system consisted of an isocratic pump (model L-7100), an autosampler (model L-7200) both from Merck Hitachi (Darmstadt, Germany), and a UV variable-wavelength K-2600 detector (Knauer, Berlin, Germany). Data acquisition and processing were carried out using the D-7000 HSM software (Merck Hitachi). All analyses were performed on a 250 × 4 mm LiChrospher1100 RP-18 column with a particle size of 5 µm (Merck, Darmstadt, Germany) protected with a guard column (4 × 4 mm) with the same packing material. Chromatographic analyses were carried out at 21 °C and an analytical wavelength of 205 nm for levetiracetam and 210 nm for lamotrigine and lacosamide. For levetiracetam, the mobile phase consisting of acetonitrile and water was mixed at a ratio of 12:88 (v/v), for lamotrigine the mobile phase consisted of acetonitrile:0.05 M potassium dihydrogen phosphate buffer (pH = 3.0) (22:78, v/v) and for lacosamide it was composed of acetonitrile:methanol:water (0.9:31.6:67.5, v/v/v). The flow rate was set to 1 ml/min for all analytes.

Topiramate, gabapentin, and pregabalin determinations were performed after precolumn derivatization with 4-chloro-7-nitrobenzofurazan

as a fluorescent labeling agent. To this end, 50–100 µl of serum or 200 µl of brain homogenate containing analyzed drugs and appropriate IS were extracted with 1 ml of dichloromethane (topiramate) or 300–600 µl of methanol (gabapentin and pregabalin) as described above. The obtained supernatants were transferred to new tubes and evaporated. To dry residue, 50 µl of 4-chloro-7-nitrobenzofurazan solution (6 mg/ml) in a mixture of methanol-acetonitrile (1:1, v/v), 50 µl of this solvent mixture, and 12.5 µl of 0.5 M borate buffer (pH = 10.5) were added. After vortexing for 30 s, the samples were incubated at 60 °C for 15 min in a dry heat sterilizer (model MOV-112S, Sanyo, Japan). The derivatized samples were centrifuged at 9,460 rpm for 5 min at 5 °C (EBA 12R, Hettich, Tuttlingen, Germany) and each supernatant was transferred to an autosampler vial and analyzed. The HPLC system (Merck-Hitachi LaChrom Elite) consisted of an L-2130 pump, an L-2200 autosampler, an L-2350 column oven set at 45 °C, and an L-2485 fluorescence detector set at an excitation wavelength of 470 nm and an emission wavelength of 530 nm. EZChrome Elite v. 3.2 (Merck-Hitachi) software was used for data acquisition. The mobile phase consisted of methanol:0.05 M potassium dihydrogen phosphate buffer (pH = 5.5) (47:53, v/v) and was pumped at a flow rate of 1.2 ml/min. The calibration curves constructed by plotting the peak area of the studied drugs or the ratio of the peak area of the studied drugs to the respective IS vs. drug concentrations were linear in the tested concentration ranges ($r > 0.998$). No interfering peaks were observed in the chromatograms, indicating the high selectivity of the developed methods. The intra- and inter-assay precision was below 10% for all studied compounds and tested concentrations, whereas the intra- and inter-assay accuracy ranged from 97.4 to 105% of the theoretical target concentrations. The mean extraction efficiencies of the analyzed drugs and IS ranged from 82.2 to 98.3%.

Serum and brain concentrations of tiagabine were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Serum samples (50 µl) were deproteinized at the ratio of 1:3 with 0.1% formic acid in acetonitrile containing IS, briefly vortexed and then centrifuged for 10 min at the speed of 8,000 rpm (Eppendorf miniSpin centrifuge, Hamburg, Germany). The supernatant was transferred into the autosampler vials. The brains were homogenized in distilled water (1:3, *w/v*) with a tissue homogenizer TH220 (Omni International, Inc., Warrenton, VA, USA). Then, the samples (500 µl) were mixed with 2.5 ml of heptane and shaken for 20 min (IKA Vibrax VXR, Germany). After centrifugation for 10 min at the speed of 3,500 rpm (Hettich Universal 32 centrifuge, Tuttlingen, Germany) the organic layer was discarded and the remaining solution was deproteinized, vortexed and centrifuged as with the preparation of serum samples. Next, the supernatant (1 ml) was evaporated to dryness at 37 °C under the gentle stream of nitrogen gas in the TurboVap evaporator (Zymark, Hopkinton, MA, USA). The dry residue was reconstituted with the 50 µl of 0.1% formic acid in acetonitrile and transferred into the autosampler vials. The HPLC system (Agilent 1100, Agilent Technologies, Waldbronn, Germany) consisted of a degasser, binary pump, column oven and an autosampler. Chromatographic separation was carried out on XBridge™ Amide analytical column (3 × 50 mm, 3.5 µm, Waters, Ireland) with the oven temperature set at 30 °C. The autosampler temperature was maintained at 10 °C and a sample volume of 30 µl was injected into LC-MS/MS system. The mobile phase containing 0.1% formic acid in acetonitrile and 0.1% formic acid in water was mixed at a ratio of 90:10 and run at 0.25 ml/min. Mass spectrometric detection was performed on an Applied Biosystems MDS Sciex (Concord, Ontario, Canada) API 2000 triple quadrupole mass spectrometer. Electrospray ionization in the positive ion mode was used for ion production. The tandem mass spectrometer was operated at unit resolution in the selected reaction monitoring mode, monitoring the transition of the protonated molecular ions *m/z* 376 to 247 and *m/z* 376 to 149 for tiagabine (first pair was used as an quantifier and the second for the identity verification – qualifier) and *m/z* 279 to 181 for pentoxifylline (IS). The mass spectrometric conditions were optimized for tiagabine by continuous infusion of the standard solution at the rate of 10 µl/min using a Harvard infusion pump. The ion source temperature was maintained at 450 °C. The ionspray voltage was set at 5500 V. The curtain gas was set at 30 and the collision gas at 5. The optimal collision energy was 40 V. The following parameters of ion path were used as the most favorable ones: declustering potential at 50 V, focusing potential at 200 V and entrance potential at 10 V. Data acquisition and processing were accomplished using the Applied Biosystems Analyst version 1.6 software. The calibration curves were constructed by plotting the ratio of the peak area of the studied drug to IS versus drug concentration and generated by weighted linear regression analysis. The validated quantitation ranges for this method were within the expected concentration ranges namely from 50 to 2000 ng/ml for serum and from 1.5 to 300 ng/g for brain tissue with accuracy from 89.18 to 115% and from 85.71 to 109.02% for serum and brain, respectively. No significant matrix effect was observed and there were no stability related problems during the routine analysis of the samples. The method for determination of oxcarbazepine concentrations was described elsewhere (Socała et al., 2018). Antiepileptic drug concentrations were expressed in µg/ml of serum or µg/g of brain tissue.

2.6.2. Determination of cannabidiol concentrations

Cannabidiol concentrations were measured using an HPLC method with UV detection. To isolate cannabidiol from biological material, 50 µl of serum or 100 µl of brain homogenate (1:4, w/v) were placed in Eppendorf tubes and 10 µl of IS (pterostilbene, 5 µg/ml) was added to each tube. The extraction procedure was similar to that described above for levetiracetam. Hexane was used as an extraction solvent. The analysis was performed at 21 °C on a 250 × 4 mm LiChrospher1100 RP-18 column with a particle size of 5 µm (Merck, Darmstadt, Germany) protected with a guard column (4 × 4 mm). The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer (pH = 4.5):acetonitrile (38:62, v/v) was pumped at a flow rate of 1 ml/min. The detector was programmed to change wavelengths during chromatographic run. The analytical wavelength was 321 nm for IS and 220 nm for cannabidiol.

2.7. Statistics

For statistical analysis of the data obtained in the MEST and the 6 Hz seizure threshold test, the CC_{50} values with 95% confidence limits were transformed into the mean value of logarithms (of convulsive current) with standard deviation. The ED_{50} values (with 95% confidence limits) of antiepileptic drugs were calculated by a log-probit method and the confidence limits were transformed into SEM. Subsequently, the one-way analysis of variance (one-way ANOVA) followed by the Dunnett's post hoc test were used to compare the changes in seizure thresholds and ED_{50} values. Effects of antiepileptic drugs and their combinations with cannabidiol on neuromuscular strength and spontaneous locomotor activity in mice were analyzed using one-way ANOVA followed by the Tukey's post hoc test. The effect of cannabidiol alone on the grip strength and locomotor activity was analyzed using unpaired Student's t-test. The Fisher's exact probability test was employed to compare the data from the chimney test, while serum and brain concentrations of either antiepileptic drugs or cannabidiol were statistically analyzed using unpaired Student's t-test. $p < 0.05$ was considered statistically significant. All calculations were performed using GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Effect of cannabidiol on seizure threshold in the MEST test

The effect of cannabidiol on the threshold for the tonic hindlimb extension in the MEST test is shown in Fig. 1A (one-way ANOVA: $F(3,34) = 30.04$, $p < 0.0001$). Cannabidiol injected at a dose of 25 mg/kg did not significantly affect the threshold for MES-induced seizures. However, when injected at higher doses of 50 and 100 mg/kg, it

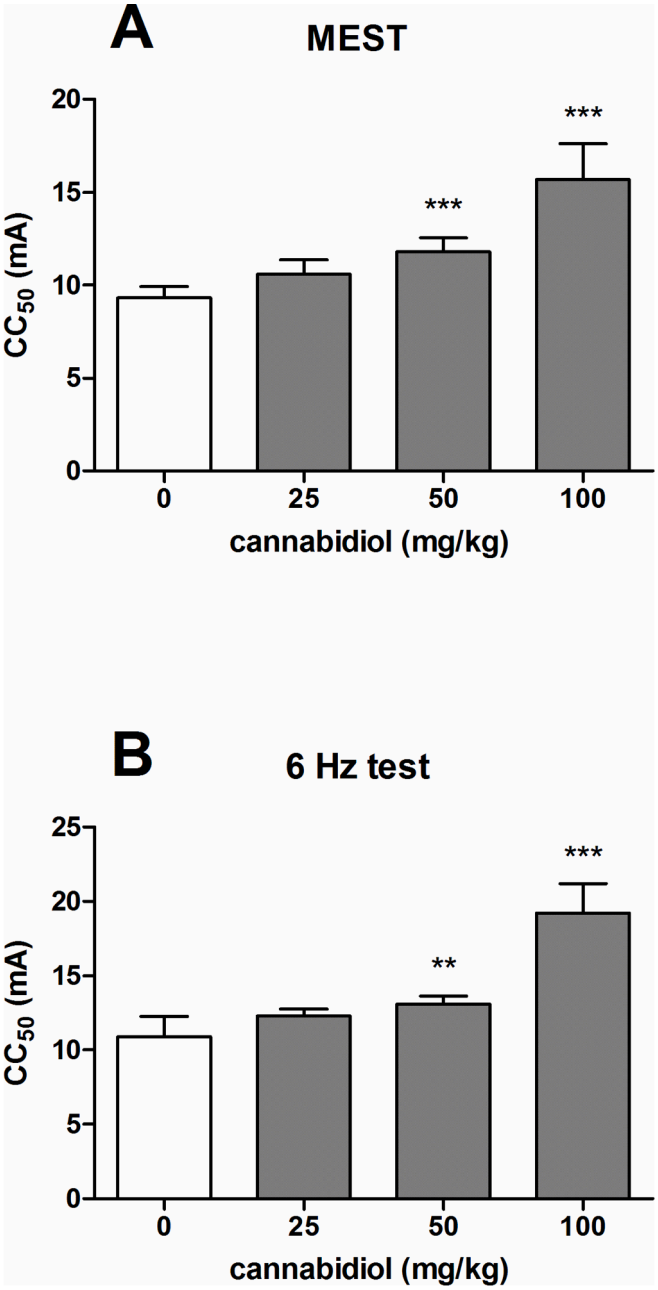

Fig. 1. Effect of cannabidiol on the seizure thresholds in the MEST (panel A) and the 6 Hz-induced seizure test (panel B) in mice. Cannabidiol was administered *ip* 60 min before the test. The doses are shown on the abscissa. Control animals received 1% Tween 80. Each experimental group consisted of 20 animals. Columns represent median convulsive currents (CC_{50} values with upper 95% confidence limits) required to produce seizure in 50% of the tested animals. **p < 0.01, ***p < 0.001 vs. the control group (one-way ANOVA followed by the Dunnett's post hoc test).

significantly raised the CC_{50} value from 9.33 (8.78–9.92) mA in control group to 11.80 (11.09–12.56) mA and 15.67 (13.94–17.61) mA, respectively (p < 0.001 for both experimental groups).

3.2. Effect of cannabidiol on seizure threshold in the 6 Hz seizure threshold test

The effect of cannabidiol on the threshold for psychomotor seizure in the 6 Hz seizure test is shown in Fig. 1B (one-way ANOVA: F(3,32) = 35.58, p < 0.0001). As in the MEST test, cannabidiol administered at a dose of 25 mg/kg was ineffective. The threshold for 6 Hz-induced psychomotor seizure was significantly elevated by cannabidiol at 50 and 100 mg/kg. Specifically, cannabidiol at a dose of 50 mg/kg increased the CC_{50} value from 10.88 (9.66–12.25) mA in control group to 13.08 (12.55–13.63) mA (p < 0.01), and at a dose of 100 mg/kg, it raised the threshold to 19.20 (17.39–21.21) mA (p < 0.001).

3.3. Effect of cannabidiol on the anticonvulsant activity of selected antiepileptic drugs in the MES test

The influence of cannabidiol on the anticonvulsant activity of lamotrigine, topiramate, oxcarbazepine, and pregabalin against MES-induced tonic hindlimb extension is shown in Fig. 2A–D (one-way ANOVA: F(3,76) = 0.38, p = 0.768 for panel A; F(3,72) = 2.86, p = 0.043 for panel B; F(3,92) = 10.74, p < 0.0001 for panel C, and F(3,84) = 5.74, p = 0.001 for panel D). All studied antiepileptic drugs administered alone exhibited a clear-cut anticonvulsant activity in the MES test in mice. The estimated ED_{50} values were as follows: 3.52 ± 0.34 mg/kg for lamotrigine, 34.28 ± 4.62 mg/kg for topiramate, 16.42 ± 2.38 mg/kg for oxcarbazepine, and 136.86 ± 27.89 mg/kg for pregabalin. Cannabidiol administered at doses of 25 mg/kg and 50 mg/kg had no significant impact on the anticonvulsant effects of all of the studied antiepileptic drugs in the MES test. At the highest dose tested, i.e., 100 mg/kg, cannabidiol had also no impact on the anticonvulsant action of lamotrigine. However, cannabidiol at 100 mg/kg significantly enhanced the activity of topiramate by reducing its ED_{50} value from 34.28 ± 4.62 mg/kg to 13.29 ± 1.52 mg/kg (p < 0.05). Likewise, co-administration of oxcarbazepine and pregabalin with cannabidiol at 100 mg/kg significantly reduced their ED_{50} from 16.42 ± 2.38 mg/kg to 1.98 ± 0.72 mg/kg (p < 0.001) and from 136.86 ± 27.89 mg/kg to 41.04 ± 9.49 mg/kg (p < 0.05), respectively.

3.4. Effect of cannabidiol on the anticonvulsant activity of selected antiepileptic drugs in the 6 Hz seizure test

The influence of cannabidiol on the anticonvulsant activity of tiagabine, gabapentin, lacosamide, and levetiracetam in the 6 Hz seizure test is shown in Fig. 3A–D (one-way ANOVA: F(3,92) = 5.81, p = 0.001 for panel A; F(3,68) = 8.59, p < 0.0001 for panel B; F(3,92) = 1.45, p = 0.235 for panel C, and F(3,100) = 3.15, p = 0.028 for panel D). All of the studied antiepileptic drugs exerted protective effect against 6 Hz-induced psychomotor seizures in mice. The estimated ED_{50} values were as follows: 0.92 ± 0.14 mg/kg for tiagabine, 119.97 ± 20.25 mg/kg for gabapentin, 4.97 ± 0.73 mg/kg for lacosamide, and 9.79 ± 4.22 mg/kg for levetiracetam. Co-administration of cannabidiol at 25 mg/kg did not produce any significant changes in the anticonvulsant activity of the studied drugs. Cannabidiol at doses of 50 and 100 mg/kg significantly enhanced the activity of tiagabine by decreasing its ED_{50} value from 0.92 ± 0.14 mg/kg in control group to 0.51 ± 0.08 mg/kg (p < 0.05) and 0.35 ± 0.07 mg/kg (p < 0.001), respectively. Likewise, the anticonvulsant activity of gabapentin was significantly increased by co-administration of cannabidiol at 50 and 100 mg/kg. The ED_{50} value of gabapentin was reduced from 119.97 ± 20.25 mg/kg (control group) to 52.50 ± 10.92 mg/kg (p < 0.01) and 27.62 ± 8.06 mg/kg (p < 0.001), respectively. By contrast, cannabidiol at 50 and 100 mg/kg had no significant impact on the anticonvulsant potency of lacosamide in the 6 Hz seizure test. Interestingly, cannabidiol at a dose of 100 mg/kg significantly decreased the activity of levetiracetam against 6 Hz-induced psychomotor seizure by increasing its ED_{50} value from 9.79 ± 4.22 mg/kg in control group to 31.56 ± 4.71 mg/kg (p < 0.05).

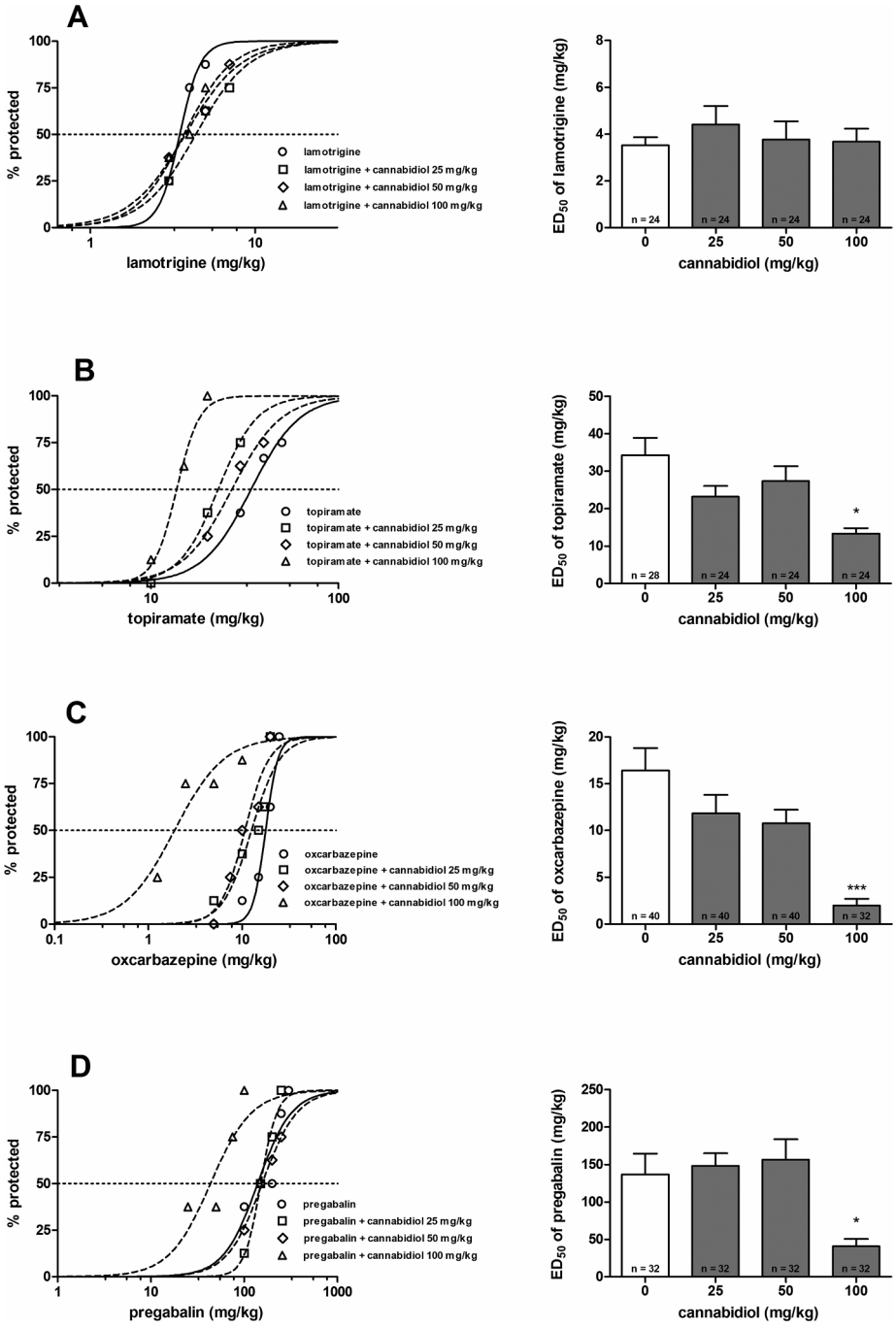

Fig. 2. Effects of cannabidiol on the anticonvulsant activity of lamotrigine (panel A), topiramate (panel B), oxcarbazepine (panel C), and pregabalin (panel D) in the MES test in mice. The graphs on the left illustrate dose-response functions (sigmoidal curves) for the anticonvulsant activity of the studied drugs. Each data point represents the percentage of mice protected from seizures (n = 8–12 mice/data point). Sigmoidal curves are the result of a least squares fit of dose–response function for each antiepileptic drug alone or in combination with cannabidiol. Points of intersections with the dashed line at 50% represent the approximate ED_{50} values of antiepileptic drugs alone or in combination with cannabidiol. The graphs in the right represent the median effective doses (ED_{50} in mg/kg + SEM) of antiepileptic drugs, protecting 50% of animals tested against MES-induced tonic hindlimb extension; n represents the total number of animals used to determine the ED_{50} value. Oxcarbazepine was administered 30 min, lamotrigine, topiramate and cannabidiol 60 min, while pregabalin 120 min prior to the test. All drugs were injected *ip*. Control animals received an antiepileptic drug and 1% Tween 80 instead of cannabidiol. Results are presented as *p < 0.05, ***p < 0.001 vs. the control group (one-way ANOVA followed by the Dunnett's post hoc test).

3.5. Effect of cannabidiol, antiepileptic drugs and their combinations on neuromuscular strength, motor coordination and locomotor activity

As shown in Table 1, cannabidiol (at a dose of 100 mg/kg), antiepileptic drugs administered alone (at doses corresponding to their ED_{50} values from the MES and the 6 Hz seizure tests) or combinations of cannabidiol with antiepileptic drugs did not significantly affect neuromuscular strength and motor coordination, as assessed in the grip-strength test and the chimney test, respectively.

However, cannabidiol injected alone significantly decreased locomotor activity in mice ($p = 0.002$). None of the studied antiepileptic drugs altered locomotor activity. Joint administration of cannabidiol with antiepileptic drugs did not produce further decrease in the locomotor activity as compared to the cannabidiol-treated group. Although pregabalin injected alone did not affect locomotor activity, its combination with cannabidiol caused statistically significant reduction in activity counts as compared to both control group and pregabalin-treated group ($p < 0.05$).

3.6. Pharmacokinetic studies

Effects of cannabidiol on serum and brain concentrations of the studied antiepileptic drugs are shown in Table 2. Cannabidiol significantly raised serum concentrations of topiramate ($t = 2.41$, $df = 18$, $p = 0.027$) and oxcarbazepine ($t = 3.21$, $df = 18$, $p = 0.005$) without affecting their concentrations in the brain. Co-administration of cannabidiol with gabapentin produced a significant increase of gabapentin concentration in both serum ($t = 3.18$, $df = 16$, $p = 0.006$) and brain ($t = 2.83$, $df = 16$, $p = 0.012$). Moreover, cannabidiol raised the concentrations of tiagabine ($t = 2.82$, $df = 18$, $p = 0.011$) and lacosamide

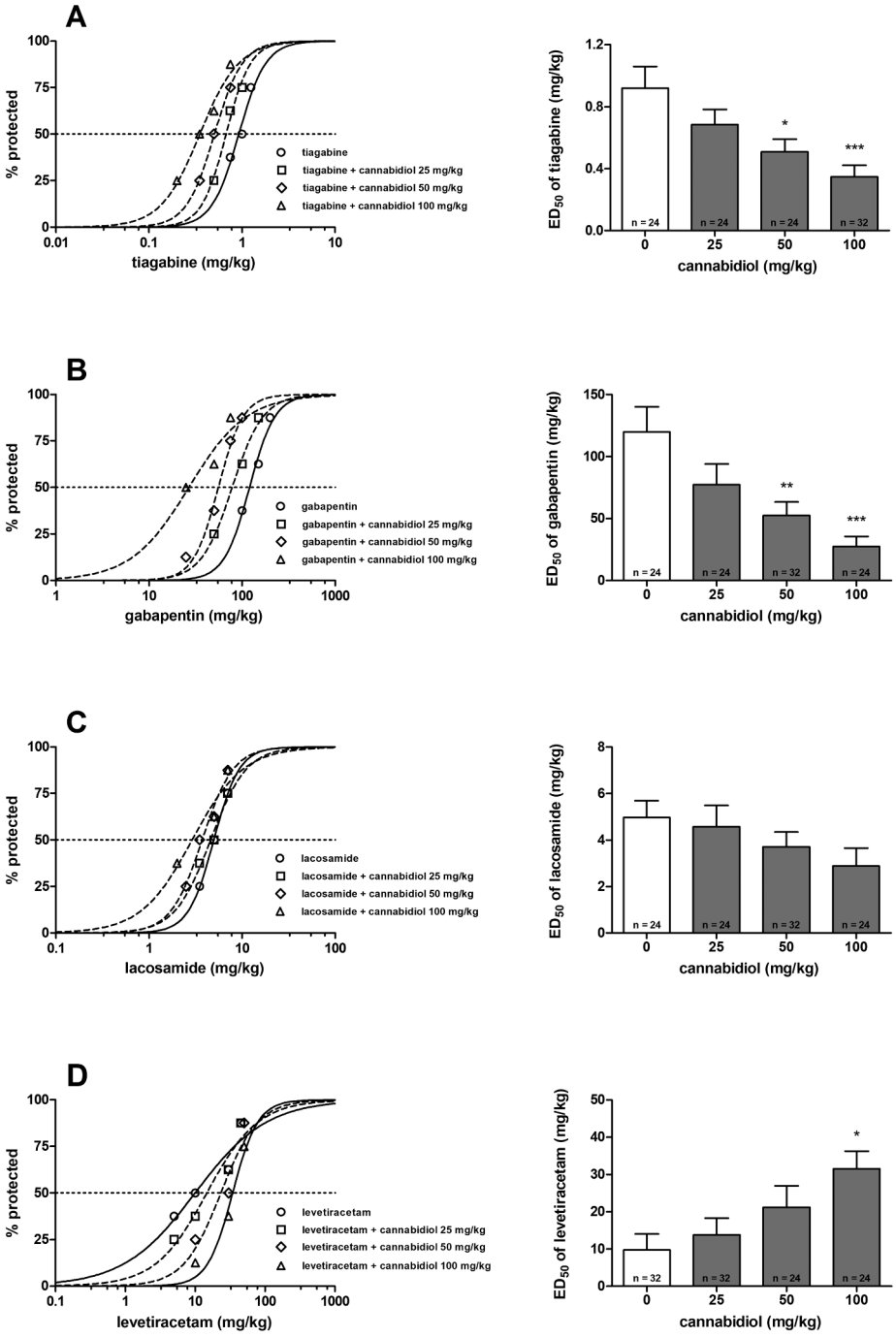

Fig. 3. Effects of cannabidiol on the anticonvulsant activity of tiagabine (panel A), gabapentin (panel B), lacosamide (panel C), and levetiracetam (panel D) in the 6 Hz seizure test in mice. The graphs on the left illustrate dose-response functions (sigmoidal curves) for the anticonvulsant activity of the studied drugs. Each data point represents the percentage of mice protected from seizures (n = 8 mice/data point). Sigmoidal curves are the result of a least squares fit of dose–response function for each antiepileptic drug alone or in combination with cannabidiol. Points of intersections with the dashed line at 50% represent the approximate ED_{50} values of antiepileptic drugs alone or in combination with cannabidiol. The graphs in the right represent the median effective doses (ED_{50} in mg/kg + SEM) of antiepileptic drugs, protecting 50% of animals tested against 6 Hz-induced psychomotor seizure; n represents the total number of animals used to determine the ED_{50} value. Tiagabine was administered 15 min, lacosamide 30 min, while gabapentin, levetiracetam and cannabidiol 60 min prior to the test. All drugs were injected *ip*. Control animals received an antiepileptic drug and 1% Tween 80 instead of cannabidiol. *p < 0.05, **p < 0.01, ***p < 0.001 vs. the control group (one-way ANOVA followed by the Dunnett's post hoc test).

(t = 3.46, df = 18, p = 0.003) in brain without changing their concentrations in serum. No significant changes in serum and brain concentrations of lamotrigine, pregabalin, and levetiracetam were observed.

Effects of antiepileptic drugs on serum and brain concentrations of cannabidiol are shown in Table 3. Lamotrigine, tiagabine, gabapentin, and levetiracetam did not produce any significant changes in concentrations of cannabidiol either in serum or in brain. Co-administration of cannabidiol with topiramate caused a significant increase in both serum (t = 2.37, df = 18, p = 0.029) and brain concentration (t = 3.03, df = 18, p = 0.007) of cannabidiol. Elevated brain concentrations of cannabidiol were also observed after its co-administration with oxcarbazepine (t = 4.29, df = 15, p < 0.001), pregabalin (t = 3.23, df = 17, p = 0.005), and lacosamide (t = 2.57, df = 17, p = 0.02).

4. Discussion

Several recent clinical studies have proved the efficacy of cannabidiol as an add-on therapy in drug-resistant epilepsy (Devinsky et al., 2018a, 2018b, 2019; Thiele et al., 2018, 2019). There are, however, also evidences showing that cannabidiol can significantly affect serum concentration of some antiepileptic drugs (Devinsky et al., 2018b; Gaston et al., 2017; Geffrey et al., 2015).

In the present study, we aimed to provide more data on the influence of cannabidiol on the anticonvulsant activity of various novel antiepileptic drugs in two acute seizure tests in mice. The MES test was employed as a model of generalized tonic–clonic seizures (Löscher and Schmidt, 2011), whereas the 6 Hz-induced seizure test was used as an experimental model of psychomotor (limbic) seizures occurring in human partial epilepsy (Barton et al., 2001; Löscher, 2017). In the first

Table 1
Effect of cannabidiol, antiepileptic drugs, and their combinations on neuromuscular strength, motor coordination, and spontaneous locomotor activity in mice.

Treatment (mg/kg)	Neuromuscular strength (mN/g)	Impairment of motor coordination (%)	Activity counts/10 min
control	28.78 ± 0.82	0	2760 ± 377
cannabidiol (100)	29.49 ± 1.82	0	1267 ± 116**
	t = 0.36, df = 18, p = 0.724		*t = 3.62, df = 17, p = 0.002*
lamotrigine (3.68)	29.64 ± 0.84	0	2349 ± 183
lamotrigine (3.68) + cannabidiol (100)	31.50 ± 0.35	0	1465 ± 181##
	F(3,35) = 1.03, p = 0.393		*F(3,35) = 8.70, p = 0.0002*
topiramate (13.29)	31.44 ± 1.74	0	2124 ± 229
topiramate (13.29) + cannabidiol (100)	29.64 ± 0.50	0	2081 ± 330
	F(3,35) = 0.69, p = 0.563		*F(3,35) = 4.38, p = 0.010*
oxcarbazepine (1.98)	29.77 ± 1.26	0	2439 ± 395
oxcarbazepine (1.98) + cannabidiol (100)	28.75 ± 1.58	0	1977 ± 441
	F(3,36) = 0.13, p = 0.941		*F(3,35) = 3.06, p = 0.041*
pregabalin (41.04)	27.76 ± 1.25	10 *(p = 1.000 vs. control)*	2670 ± 420
pregabalin (41.04) + cannabidiol (100)	28.01 ± 0.89	30 *(p = 0.211 vs control,*	1237 ± 321# ^
	F(3,36) = 0.39, p = 0.763	*p = 0.582 vs. pregabalin)*	*F(3,35) = 6.28, p = 0.002*
tiagabine (0.35)	28.99 ± 1.51	0	2277 ± 277
tiagabine (0.35) + cannabidiol (100)	29.13 ± 1.04	0	2213 ± 276
	F(3,36) = 0.05, p = 0.985		*F(3,35) = 4.64, p = 0.008*
gabapentin (27.62)	27.04 ± 1.95	10 *(p = 1.000 vs. control)*	2058 ± 215
gabapentin (27.62) + cannabidiol (100)	27.01 ± 0.74	0	1651 ± 384
	F(3,36) = 0.75, p = 0.530		*F(3,35) = 4.41, p = 0.010*
lacosamide (2.88)	29.44 ± 0.96	0	2854 ± 507
lacosamide (2.88) + cannabidiol (100)	25.45 ± 0.54	10 *(p = 1.000 vs. control,*	1838 ± 192
	F(3,35) = 2.59, p = 0.069	*p = 1.000 vs. lacosamide)*	*F(3,34) = 4.71, p = 0.008*
levetiracetam (31.56)	30.09 ± 1.21	0	1950 ± 74.90
levetiracetam (31.56) + cannabidiol (100)	31.78 ± 1.29	0	1775 ± 306#
	F(3,36) = 0.92, p = 0.439		*F(3,35) = 5.71, p = 0.003*

The antiepileptic drugs were injected at doses corresponding to their ED_{50} values from the MES- and 6 Hz-induced seizure tests. All drugs were administered *ip*, as follows: tiagabine – 15 min, lacosamide and oxcarbazepine – 30 min, gabapentin, levetiracetam, lamotrigine, topiramate, cannabidiol – 60 min, and pregabalin – 120 min before the tests. Data are expressed as means ± SEM grip strengths in millinewtons per gram of mouse body weight (mN/g) from the grip-strength test, as a percentage of animals showing motor coordination impairment in the chimney test; and means of activity counts/10 min ± SEM from the locomotor activity test. Experimental groups consisted of 9–10 animals.
*$p < 0.05$ vs. the control group (Student's t-test), #$p < 0.05$, ##$p < 0.01$ vs. the control group, ^$p < 0.05$ vs. the pregabalin-treated group (one way ANOVA followed by the Tukey's post hoc test).

Table 2
Effect of cannabidiol on the concentrations of antiepileptic drugs.

Treatment (mg/kg)	Drug concentration	
	Serum (μg/ml)	Brain (μg/g)
lamotrigine (3.68)	3.11 ± 0.10	2.81 ± 0.12
lamotrigine (3.68) + cannabidiol (100)	3.27 ± 0.08	2.82 ± 0.09
topiramate (13.29)	1.86 ± 0.19	3.88 ± 0.39
topiramate (13.29) + cannabidiol (100)	2.40 ± 0.12*	4.52 ± 0.56
oxcarbazepine (1.98)	0.54 ± 0.03	0.33 ± 0.04
oxcarbazepine (1.98) + cannabidiol (100)	0.66 ± 0.03**	0.43 ± 0.05
pregabalin (41.04)	8.84 ± 0.95	3.63 ± 0.59
pregabalin (41.04) + cannabidiol (100)	9.54 ± 1.08	3.77 ± 0.30
tiagabine (0.35)	0.29 ± 0.02	0.07 ± 0.01
tiagabine (0.35) + cannabidiol (100)	0.34 ± 0.04	0.10 ± 0.01*
gabapentin (27.62)	10.97 ± 0.76	5.81 ± 0.41
gabapentin (27.62) + cannabidiol (100)	14.53 ± 0.83**	8.10 ± 0.75*
lacosamide (2.88)	2.01 ± 0.24	1.09 ± 0.08
lacosamide (2.88) + cannabidiol (100)	2.29 ± 0.14	1.47 ± 0.07**
levetiracetam (31.56)	23.21 ± 1.31	13.48 ± 0.50
levetiracetam (31.56) + cannabidiol (100)	26.80 ± 1.47	12.43 ± 0.35

The antiepileptic drugs were injected at doses corresponding to their ED_{50} values from the MES- and 6 Hz-induced seizure tests. All drugs were administered *ip*, as follows: tiagabine – 15 min, lacosamide and oxcarbazepine – 30 min, gabapentin, levetiracetam, lamotrigine, topiramate, cannabidiol – 60 min, and pregabalin – 120 min before the tests. Data are expressed as means ± SEM. Experimental groups consisted of 8–10 animals. *$p < 0.05$, **$p < 0.01$ vs. the respective control group (Student's t-test).

Table 3
Effect of antiepileptic drugs on the concentrations of cannabidiol.

Treatment (mg/kg)	Cannabidiol concentration	
	Serum (μg/ml)	Brain (μg/g)
cannabidiol (100)	3.75 ± 0.50	1.82 ± 0.32
lamotrigine (3.68) + cannabidiol (100)	4.42 ± 0.67	3.05 ± 0.53
topiramate (13.29) + cannabidiol (100)	6.63 ± 1.11*	3.63 ± 0.51**
oxcarbazepine (1.98) + cannabidiol (100)	4.63 ± 0.51	3.97 ± 0.39***
pregabalin (41.04) + cannabidiol (100)	4.89 ± 0.35	3.31 ± 0.33**
tiagabine (0.35) + cannabidiol (100)	3.62 ± 0.30	1.78 ± 0.21
gabapentin (27.62) + cannabidiol (100)	4.12 ± 0.86	2.08 ± 0.39
lacosamide (2.88) + cannabidiol (100)	4.63 ± 0.41	3.10 ± 0.39*
levetiracetam (31.56) + cannabidiol (100)	3.27 ± 0.50	1.86 ± 0.38

The antiepileptic drugs were injected at doses corresponding to their ED_{50} values from the MES- and 6 Hz-induced seizure tests. All drugs were administered *ip*, as follows: tiagabine – 15 min, lacosamide and oxcarbazepine – 30 min, gabapentin, levetiracetam, lamotrigine, topiramate, cannabidiol – 60 min, and pregabalin – 120 min before the tests. Data are expressed as means ± SEM. Experimental groups consisted of 9–10 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. cannabidiol-treated group (Student's t-test).

experiment, we evaluated the effect of cannabidiol alone on seizure thresholds to select the dosage regimen for further studies. The obtained results showed that cannabidiol at 25 mg/kg was ineffective, while higher doses (50 and 100 mg/kg) produced significant increase of the CC_{50} values. Based on this observation, a sub-effective dose of 25 mg/kg was selected as an initial dose for studies with antiepileptic drugs. Since the dose did not affect significantly the activity of any of the studied antiepileptic drug, it was increased up to 50 and 100 mg/kg. Results from the MES test showed that cannabidiol (only at the highest dose tested, i.e., 100 mg/kg) significantly potentiated the anticonvulsant potency of topiramate, oxcarbazepine, and pregabalin but not lamotrigine. In the 6 Hz seizure test, cannabidiol (at 50 and 100 mg/kg) enhanced the activity of tiagabine and gabapentin without affecting the activity of lacosamide. Interestingly, a significant

reduction of the anticonvulsant activity of levetiracetam against 6 Hz-induced seizures following cannabidiol co-administration was observed.

The changes in the anticonvulsant action of the studied drugs caused by concomitant treatment with cannabidiol could result from pharmacodynamic and/or pharmacokinetic interactions between them. Pharmacodynamic interaction occurs when one drug alters the effect of another drug without changing its concentration. The drugs may act at the same or different molecular targets, which results in potentiation or reduction of the observed effects (Hoffelt and Gross, 2016). However, to classify the observed interactions as supra-additive (synergistic), additive, sub-additive (antagonistic) or neutral (indifferent) further isobolographic studies are needed. Pharmacology of cannabidiol is not completely understood and this makes it difficult to determine by which specific mechanism(s) the pharmacodynamic interactions between cannabidiol and the studied antiepileptic drugs could occur. Cannabidiol acts on multiple targets and its effects are not directly mediated by the endocannabinoid system because of the low affinity for the cannabinoid CB_1 and CB_2 receptors (Gaston and Friedman, 2017). It may, however, indirectly enhance the endocannabinoid signaling by inhibiting the re-uptake and hydrolysis of anandamide. Moreover, cannabidiol antagonizes the GPR55 receptor – a putative novel cannabinoid receptor (dos Santos et al., 2015), and acts as an agonist of the transient receptor potential of vanilloid type 1 channel (TRPV1) that is also activated by anandamide. Cannabidiol was recently shown to produce inhibitory effects on the voltage-gated sodium channels, which may significantly contribute to its anticonvulsant potential (Patel et al., 2016; Ghovanloo et al., 2018). Some other mechanisms by which cannabidiol may, at least in part, produce antiepileptic effects include an inhibition of T-type calcium channels (Ross et al., 2008), a modulation of the adenosine system (Devinsky et al., 2014), a direct action at various $GABA_A$ receptor subtypes (Bakas et al., 2017), or an activation of mTOR pathway and a decrease in glutamate release (Gobira et al., 2015). It is rather unlikely that the anticonvulsant action of cannabidiol is related solely (or predominantly) to only one of the above-mentioned mechanisms. Thus, a variety of molecular mechanisms could underlie the pharmacodynamic interactions between cannabidiol and antiepileptic drugs.

The changes in the anticonvulsant activity of the studied drugs could also result from pharmacokinetic interactions with cannabidiol. In brief, pharmacokinetic drug–drug interaction occurs when one drug affects absorption, distribution, metabolism or elimination of another drug, thereby changing its concentration. Changes in drug metabolism may be a result of induction or inhibition of hepatic microsomal drug-metabolizing enzymes activity and/or expression. In humans, cannabidiol is metabolized in liver, predominantly by CYP3A4 and CYP2C19 isozymes. Other human CYP enzymes involved in cannabidiol metabolism include CYP1A1, CYP1A2, CYP2C9, CYP2D6, and CYP3A5 (Jiang et al., 2011). In *in vitro* studies, cannabidiol was reported to be a potent inhibitor of human CYP1A, CYP2C19, CYP3A4, CYP3A5, and CYP2D6 isozymes (Ujvary and Hanus, 2016; Zendulka et al., 2016). Moreover, a single injection of cannabidiol (120 mg/kg, *ip*) inhibited mouse liver microsomal CYP2C and CYP3A subfamilies of CYP enzymes (Bornheim et al., 1993). Since many antiepileptic drugs are substrates for CYP3A4 and CYP2C19 isozymes, interactions of cannabidiol with these drugs are possible. Cannabidiol may also affect absorption, disposition, and excretion of co-administered drugs because it was shown to inhibit *P*-glycoprotein (*P*-gp)-mediated drug transport (Zhu et al., 2006). Noteworthy, cannabidiol itself is not a substrate for *P*-gp (Brzozowska et al., 2016). Since both cannabidiol and antiepileptic drugs may influence cytochrome P450 activity, they may also affect neurosteroidogenesis which is involved in status epilepticus (Meletti et al., 2017, 2018) and epileptogenesis (Biagini et al., 2010).

In our study, only interactions of cannabidiol with levetiracetam were purely pharmacodynamic in nature because neither cannabidiol changed serum and brain concentrations of levetiracetam nor did levetiracetam affect serum and brain concentrations of cannabidiol. Pharmacokinetic interactions occurred between cannabidiol and topiramate. Cannabidiol increased serum concentration of topiramate (by ~30%), while topiramate increased both serum and brain concentrations of cannabidiol (by ~70 and ~100%, respectively). Topiramate is not extensively metabolized in liver and about 70% of the administered dose is excreted unchanged in urine (Sommer and Fenn, 2010; Benedetti, 2000). It has been shown that topiramate is a substrate of *P*-gp (Luna-Tortos et al., 2009). Thus, the increased serum topiramate concentrations could be due to its decreased renal and/or bile excretion as a result of *P*-gp inhibition by cannabidiol. Although no alterations in brain topiramate concentration were noted, we cannot exclude the possibility that the increased anticonvulsant activity of topiramate was, at least in part, caused by the fact that cannabidiol affects pharmacokinetics of this drug. However, it seems that the decreased ED_{50} value of topiramate in mice co-injected with cannabidiol was mainly related to a high increase in serum and brain concentrations of cannabidiol. Topiramate inhibits human CYP2C19 and thereby, it may affect metabolism of CYP2C19 substrates, including cannabidiol.

A similar bidirectional pharmacokinetic interaction occurred between oxcarbazepine and cannabidiol. Cannabidiol increased serum concentration of oxcarbazepine (by ~20%) and oxcarbazepine caused a marked increase (by ~120%) of cannabidiol brain concentration. In humans, oxcarbazepine is rapidly and almost completely metabolized by a cytosolic aldo-keto reductase into a biologically active 10-monohydroxymetabolite (MHD) which is subsequently conjugated to the MHD glucuronide by a microsomal UDP-glucuronyl transferase (UGT). The protein binding of oxcarbazepine is about 67%, whereas the protein binding of its metabolite is only about 38% (Tomson, 2002). In rats, oxcarbazepine is reduced into MHD to a lesser extent and plasma oxcarbazepine concentrations are higher than those of MHD (Łuszczki et al., 2003). For this reason, we decided to determine the level of oxcarbazepine only. Cannabidiol inhibits some UGT isoforms (Stout and Cimino, 2014) and it could affect oxcarbazepine metabolism by inhibiting its glucuronidation. Since oxcarbazepine is a substrate for *P*-gp, cannabidiol might also increase oxcarbazepine concentrations by affecting its excretion. Moreover, oxcarbazepine markedly increased brain content of cannabidiol without affecting its serum concentration. This suggests that oxcarbazepine significantly enhanced the brain uptake of cannabidiol. Likewise, increased brain (by ~80%), but not serum concentration of cannabidiol was seen after its co-injection with pregabalin, which could contribute to the increased anticonvulsant activity of pregabalin.

Although no statistically significant changes in the activity of lacosamide were observed, it seems that pharmacokinetic interactions between these two compounds are also possible. Cannabidiol caused a 35% increase in the brain concentration of lacosamide and lacosamide increased the brain level of cannabidiol by ~70%, which suggests that these two drugs increased each other's penetration across the blood-brain barrier. In humans, lacosamide is metabolized by CYP2C19, CYP2C9, and CYP3A4 isozymes (Dean, 2012-2018). At very high concentrations, it inhibits CYP2C19. Therefore, despite the lack of changes in serum concentrations of both lacosamide and cannabidiol at the time point studied, we cannot exclude the possibility that both drugs inhibit each other's CYP2C-mediated metabolism in mice.

Since cannabidiol increased brain concentrations of tiagabine (by ~50%), a pharmacokinetic interaction between these two drugs is also possible. In humans, about 96% of tiagabine is bound to plasma proteins. The drug is extensively metabolized by the hepatic CYP3A isozymes (Benedetti, 2000; Johannessen and Landmark, 2010). Being a CYP3A inhibitor (Bornheim et al., 1993; Zendulka et al., 2016), cannabidiol could have decreased tiagabine metabolism and increased its brain concentration. Moreover, since tiagabine was proved to be a *P*-gp substrate (Nakanishi et al., 2013), cannabidiol could contribute to the increased brain uptake of tiagabine.

The increase in both serum and brain concentrations of gabapentin

(by ~30 and 40%, respectively) indicate a pharmacokinetic interaction between gabapentin and cannabidiol. Gabapentin does not bind to plasma proteins, it is not metabolized by microsomal CYP450 enzymes and is eliminated by renal excretion as an unchanged drug (Taylor et al., 1998; Radulovic et al., 1995). Thus, it seems that cannabidiol may affect gabapentin penetration into the brain and/or its elimination via kidneys.

Lack of changes in concentrations of pregabalin, lamotrigine, and levetiracetam as well as increased serum concentration of topiramate are in line with observation made by Gaston et al. (2017) who monitored antiepileptic drugs levels in patients treated simultaneously with cannabidiol. However, their study showed no changes in serum levels of oxcarbazepine and lacosamide, which is in contrast with our observations (Gaston et al., 2017).

Concomitant treatment with two drugs frequently leads to a potentiation of their adverse effects and may pose a risk of some new side effects. Therefore, we also investigated the influence of cannabidiol and the studied antiepileptic drugs alone as well as their combinations on motor coordination, neuromuscular strength and locomotor activity. The obtained results suggest that the studied combinations of cannabidiol and antiepileptic drugs should have promising safety profile with regard to the three studied parameters.

Although animal models are commonly used in the preclinical studies, several issues should be taken into consideration when extrapolating the present results to humans. Firstly, animal drug-metabolizing systems differ from those in humans. For example, mouse CYP isoforms are not identical with respect to expression and catalytic activity with human cytochrome P450 isozymes (Martignoni et al., 2006), which means that the biotransformation of cannabidiol and/or the studied antiepileptic drugs may be a bit different in mice and humans. Secondly, only concentrations of unchanged antiepileptic drugs and cannabidiol were determined in this study. Of note, metabolites may also contribute to drug-drug interactions. They can be inhibitors of CYP isozymes or transporters and may alter drug elimination or displace other drugs from plasma or tissue binding sites (Ujvary and Hanus, 2016; Yeung et al., 2011). Thirdly, cannabidiol was administered at much higher doses than those used in clinics, which led to much higher blood concentrations of this compound. In our study, serum cannabidiol concentrations were 100–1000 times higher than those reported in human studies (Ujvary and Hanus, 2016; Iffland and Grotenhermen, 2017). At such supratherapeutic concentrations, some additional effects of cannabidiol could have occurred, which will not necessarily occur at the therapeutic blood level. Finally, all of the drugs were injected acutely, which is a limitation of the study because antiepileptic drugs are usually given chronically. Some different effects, such as changes in the expression of metabolizing enzymes, may occur after chronic treatment (Ujvary and Hanus, 2016).

5. Conclusion

Our study illustrates that cannabidiol may enhance the activity of some antiepileptic drugs and some of the occurring interactions may be, at least in part, pharmacokinetic in nature. We also showed that some antiepileptics may have the ability to increase cannabidiol concentration, which indicates the need for monitoring of not only antiepileptic drug but also cannabidiol concentrations. Moreover, a decreased anticonvulsant activity of levetiracetam in the presence of cannabidiol gives cause for concerns and it deserves further investigation. More detailed preclinical and clinical studies are required to fully evaluate the interactions between cannabidiol and the currently used antiepileptic drugs.

Conflicts of interest

The authors declare no conflict of interest.

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