PHARMACOLOGICAL CHARACTERIZATION OF THE CANNABINOID RECEPTOR 2 AGONIST β-CARYOPHYLLENE ON RODENT MODELS OF SEIZURES

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ABSTRACT

Introduction: Accumulated experimental and clinical data support the suggestion that the endocannabinoid system represent a potential therapeutic tool for treatment of pathological conditions including epilepsy. The major goal of this study was to explore effect of β-caryophyllene (BCP), which posseses high affinity to cannabinoid receptor type 2 (CB_2), on seizure susceptibility in ICR mice.

Materials and methods: For studying anticonvulsant activity of this drug, maximal electroshock (MES) and subcutaneous pentylenetetrazol (scPTZ) tests were applied, after a single i.p. injection of this natural bicyclic sesquiterpene, at doses of 30, 100, and 300 mg/kg, respectively. In parallel, acute neurotoxicity was determined via the test for minimal motor impairment (rota-rod).

Results: No signs of acute toxicity were observed in all doses uses. BCP suppressed tonic-clonic seizures at dose of 30 $mg.kg^{-1}$, which effect was comparable to that of the referent drug phenytoin. In the scPTZ test, BCP was ineffective in the three doses used suggesting that the compound is unable to to raise the seizure threshold.

Conclusion: Taken together, the results provided evidence that BCF has potential anticonvulsant effect and deserves further exploration in models of epilepsy and psychiatric comorbities.

Key words: *β-caryophyllene, seizure susceptibility, status epilepticus, ICR mice*

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Introduction

Epilepsy is a neurological disorder that is characterized by spontaneous seizures and learning and memory impairment as a comorbid risk factor. Threatment by antiepileptic drugs (AEDs) prevent seizures but in most cases its effectiveness against cognitive disturbance in patients with epilepsy is neglected and uncertain (Agrawal and Govender, 2011). Therefore, current research in this field is focused on design and developing of alternative therapeutic approaches that prevent the epileptogenesis after status epilepticus (SE) and its deleterious consequences. During the last decade, drugs with potential memory-enhancing effects are explored as adjuvant to anticonvulsant therapy for treatment of deletirious comorbid behavioral changes. Kainic acid (KA), an analog of the excitatory amino acid glutamate, has been widely used as a tool for SE induction. Previous studies revealed that some natural substances such as curcumin, vineatrol, resveratrol and capsaicin demonstrate promising anticonvulsant, neuroprotective and antioxidant activity in acute seizure tests and SE model (Gupta et al., 2006).

Endocannabinoid system represents a potential therapeutic tool for treatment of pathological conditions including neuropathic pain, stroke, hypertension, psychosis as well as epilepsy (Sharma et al., 2015). Cannabidiol has agonistic activity on cannabinoid receptors (CB_1 and CB_2) and may possess

anticonvulsant activity (Cunha et al., 1980). The CB_2 receptors are distributed mainly in peripheral tissues (Sharma et al., 2015). Unlike CB_1 receptors, CB_2 receptors have been considered to be absent in brain. Recently, studies reported that agonists of CB_2 receptors could be exploited as novel pharmacological agents in the treatment of brain disorders (Galdino et al., 2012). Compounds which effects are mediated by CB_2 receptors deserve further exploration because of lack of side psychotropic activity of ligands of CB_1 receptors. In the recent years, numerous novel synthetic and natural compounds showing good affinity for CB_2 receptors are explored as good alternative of conventional drugs for disorders of CNS. Among them, a natural bicyclic sesquiterpene β-caryophyllene (BCP) is a common component of essential oils of spices (cinnamon, black pepper, oregano, clove, rosemary, thyme) and various plants, mainly Cannabis sativa and Copaifera spp. (Gertsch et al., 2008). BCP posseses high affinity to CB2 receptors and is considered a promising dietary phytocannabinoid deserving further exploration. This CB_2 receptor agonist has been found to be protective against alcohol addiction, inflammation, nociception, depression, cerebral ischemia and Alzheimer disease (Al Mansouri et al., 2010; Gertsch et al., 2008; Katsuyama et al., 2013). To date, only a few studies have reported its potential activity in animals under standardized experimental procedures.

The aim of the present work was to explore the anticonvulsant activity of BCP in seizure test in mice. Three different doses were applied intraperitoneally (i.p.) and these pharmacological treatments modified mouse behavior in a dose-dependent manner. The results provided evidence that BCP has potential anticonvulsant effect and deserves further exploration in models of epilepsy.

Materials and methods

*Animals***.** Male albino ICR mice weighing 25-30 g were used as experimental animals. The animals were maintained at an ambient temperature 22 ± 1°C, in groups of six per cage under standard laboratory conditions and allowed free access to food and water. All procedures were performed in agreement with the European Communities Council Directive 2010/63/EU. The experimental design was approved by the Institutional Ethics Committee at the Institute of Neurobiology.

*Drugs and dosage***.** Control group (n=8) (treated with vehicle, 0.05% Tween 80 dissolved in 0.9% saline, negative control) and experimental groups (n=8) (treated with either referent drugs phenytoin sodium, diazepam (DZP) and BCP were injected intraperitoneally (i.p.) at different doses and were dissolved in polyoxyethylene sorbate (Tween 80) at a volume of 10 $mL.kg^{-1}$. BCP was administered at doses of 10, 25 and 50 mg/kg at interval of 30 minutes before each test.

*Maximal Electroshock Test (MES test)***.** A drop of a local anesthetic was applied to the eyes of each animal and corneal electrodes were applied followed by an electric stimulus of 50 mA, 60 Hz delivered for 0.2 s (Constant Current Shock Generator). Abolition of the hind limb extensor component following drug treatment was taken as an end point of the test. The tonic component was considered abolished, if the hind limb extension did not exceed a 90^o with the plane of the body. Abolition of tonic hindlimb extension (THE) in half or more of the animals was defined as protection.

Subcutaneous pentylenetetrazole (scPTZ) seizure test. A dose of 85 $mg.kg^{-1}$ utilized at the anticipated time of testing (0.5 h and 4.0 h) during the scPTZ test, produced clonic seizures lasting for a period of at least five seconds in 97 percent (CD97) of animals tested. The mice were further observed during a period of 20 min. The protection against clonic seizures in half or more of the animals was defined as an anticonvulsant activity.

Rota-rod test. The minimal motor impairment in mice was measured using the rota-rod test. The mice were trained to stay on an accelerating rota-rod of diameter 3.2 cm rotating at 10 rpm. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at

least one minute in each of the three trials. The dose at which 50 % of the animals were unable to balance themselves and fell off the rotating rod was determined as toxic.

*Statistical analysis.*The results were expressed as mean ± standard error of mean (SEM). Chi square test was used to analyze percentage of animals with seizures and mortality rate. Differences were considered statistically significant when $p < 0.05$.

Results

Screening evaluation was executed at two different time intervals, namely 0.5 h and 4 h. The results are shown in Table 1. No signs of acute toxicity suggestive of neurotoxicity were observed in all doses uses. In MES, BCP suppressed tonic-clonic seizures at dose of 30 $mg.kg^{-1}$ after 4.0 h which effect was comparable to that of the referent drug phenytoin. In the scPTZ test, BCP was ineffective in the three doses used suggesting that the compound is unable to to raise the seizure threshold.

Table 1. Anticonvulsant activity and neurotoxicity of beta-caryophyllene in ICR mice

Drug	Maximal electroshock test		Subcutaneous pentylenetetrazole test		Test for neurotoxicity (rota-rod)	
	0.5 h	**4.0 h**	**0.5 h**	**4.0 h**	**0.5 h**	**4.0 h**
Beta-caryophyllene	–	30	–	–	–	–
Phenytoin	30	30	–	–	100	100
Diazepam	–	–	30	30	30	30

Data in the table indicate the minimum dose, whereby bioactivity or neurotoxicity was demonstrated in at least 50% of treated animals. A dash indicates the absence of activity or neurotoxicity at the maximum dose administrated (300 mg/kg).

Discussion

In the present study, we have shown that BCF is able to block tonic-clonic seizures at the lowest dose of 30 $mg.kg^{-1}$ used, which effect was comparable to that of the referent drug phenytoin. The MES test is the best-validated to predict potential drugs against generalized tonic-clonic seizures with activity to suppress propagation of seizure attacks (Swinyard & Kupferberg, 1985). However, the anticonvulsant activity of BCP was demonstrated at 4rd hour after injection suggesting long-term potency. In the scPTZ test, BCF was ineffective in the three doses used revealing that the compound is unable to to raise the seizure threshold. In contrast to our finding, de Oliveira et al. (2016) reported that BCP posses anticonvulsant effect against PTZ-induced myoclonic seizures. However, the authors revealed that BCP suppress only the latency to myoclonic jerks induced by a 60 $mg.kg^{-1}$ PTZ, which model minimal clonic seizures but not tonic-clonic seizures as in our work. The mechanism involving GABAergic system activation is associated with sedation and loss of muscle tone. In our study, diazepam at a dose of 30 $mg.kg^{-1}$ demonstrated a profound ataxia in a rotarod test. The lack of activity in the PTZ seizure test and sedative effect in the rotarod test of BCP suggest that it does not interact with GABAergic inhibitory system.

Conclusion

Although the results of this study showed that the highest dose of 300 mg/kg BCP failed to prevent the incidence of clonic seizures in PTZ seizure test its effectiveness was comparable to the standard drug phenytoin as concern the incidence of THE in the MES test. Therefore, BCP is as potent as phenytoin suggesting that this drug is worthy of further experimental study.

Acknowledgements: This work was supported by the Sofia University "St.Kliment Ohridski", contract No.80-10-104/2017.

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