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SYSTEMIC CANNABIDIOL DOES NOT REDUCE COMPOUND 48/80-INDUCED ITCHING BEHAVIOR IN MICE

Hatice Demirel[1], Elif Baksın[1], Ece Önay Özgür [1], Ruhan Deniz Topuz[2], Ahmet Ulugöl[2]

[1]Trakya University School of Medicine, Edirne, TURKEY
[2]Department of Medical Pharmacology, Trakya University School of Medicine, Edirne, TURKEY

ABSTRACT

Aims: Cannabinoids are chemical compounds including natural cannabinoids found in the Cannabis plant, their synthetic counterparts, and endocannabinoids. Cannabidiol, a phytocannabinoid derived from the Cannabis plant, exerts anticonvulsant, anxiolytic, anti-inflammatory, neuroprotective, analgesic effects. Although there are many similarities between the pathophysiological mechanisms of pain and itch, researches that investigate the effect of cannabinoids on itching are insufficient. Here, we aimed to examine the antipruritic effect of cannabidiol and the contribution of spinal cannabinoid receptors.

Methods: Male Balb/c mice, weighing 20-30 g, were used. Itching behavior was produced by intradermal injection of compound 48/80 (100 µg/50 µl); cannabidiol (1, 3, 10 mg/kg, ip) was administered 30 minutes before compound 48/80 injections. Then, scratching of the injected site by the hind paws was videotaped for 30 minutes. Locomotor performances were assessed using a rotarod apparatus.

Results: Cannabidiol had no effect on compound 48/80-induced itching behavior at any dose given; moreover, cannabidiol did not produce any impairment on motor function. AM-251, a cannabinoid receptor type 1 antagonist, and AM-630, a cannabinoid receptor type 2 antagonist were administered intrathecally to observe the contribution of spinal cannabinoid receptors to the antipruritic action of cannabidiol. We observed that cannabidol did not possess any effect on itching behaviour.

Conclusion: Our results indicate that systemic administration of cannabidiol does not attenuate compound 48/80 induced itching behavior in mice.

Keywords: Cannabidiol, compound 48-80, pruritus

INTRODUCTION

Pruritus can be described as an unpleasant and strange sensation of irritation, which may also involve tingling, biting or burning that initiates itching in the skin in related areas. Many similarities between the neuronal pathways and the pathophysiological mechanisms of pain and itching have been proposed; itching sensation is transmitted to spinal cord's dorsal horn by primary afferent C fibers, and then to the brain by spinothalamic pathways (1, 2). Glutamate is suggested as the principal excitatory neurotransmitter in the spinal cord not only for pain but also for itching; similarly, descending inhibition is involved in the development of both pain and itching sensations (3, 4). Similar symptoms to allodynia, hyperalgesia and abnormal pain also occur in pathological itching conditions (2, 5). Taken together, the spinal cord seems to be an attractive target for developing new drugs against pruritus (6-8).

Cannabinoids are chemical compounds including natural cannabinoids found in the Cannabis plant (phytocannabinoid), their synthetic counterparts, and substances that make up the endogenous cannabinoid system (endocannabinoid) synthesized in the body (9). Cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) receptors are the primary targets of all cannabinoids. Cannabis has been used for medical purposes, especially to treat pain, until the Marihuana Tax Act of 1937 which decreased its use rapidly. In recent years, there have been changes in policies which led to an increase in the use of medical cannabis in many countries (10, 11).

Address for Correspondence: Hatice DEMİREL, Trakya University School of Medicine, Edirne, TURKEY - e-mail: haticedemirel942@gmail.com
ORCID: orcid.org/0000-0002-2702-2619
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In addition to pain states, cannabinoids have been proposed as potential antipruritic drugs. Cannabinoid receptor agonists have been shown to reduce histamine-induced scratches, whereas cannabinoid receptor antagonists provoked pruritic responses via CB1 receptors (12, 13). Attenuation of itching replies by augmenting endocannabinoid tonus via the inhibition of the endocannabinoid degradative enzymes, such as fatty acid amide hydrolase and monoacylglycerol lipase which is a different promising method for treating pruritus (14-16). Our research group also indicated that the synthetic cannabinoid agonist WIN 55,212-2 exerts dose-dependent antipruritic effects and this effect is partially mediated by spinal cannabinoid receptors CB1 (17, 18).

Unlike most of the other cannabinoids, the non-psychoactive phytocannabinoid cannabidiol exhibits little or no orthosteric binding potential at cannabinoid CB1 and CB2 receptors (19). However, cannabidiol has extensive therapeutic properties, including anticonvulsant, anxiolytic, anti-inflammatory, neuroprotective, analgesic effects, etc (20). Thus, the purpose of this study is to investigate the antipruritic effect of cannabidiol in compound 48/80-induced itching behavior in mice and whether spinal cannabinoid receptors are involved in this action.

MATERIAL AND METHODS

The investigations were approved by the institutional ethics committee of Trakya University. This experiment was carried out in young male Balb/c mice (obtained from Center of the Laboratory Animals, Trakya University), weighing 20-30 g (n=8 for each group). Animals were maintained under a 12-12 h light/dark cycle at a constant temperature of 21±2 °C with food and water ad libitum. Mice were housed in a group of 8 per cage and the experiments were conducted in a quiet room between 10:00 and 17:00. Animals were allowed to acclimate to laboratory conditions for one week before the experiments were performed; each mouse was tested only once. All procedures involving mice were carried out in strict accordance with "Guide for the Care and Use of Laboratory Animals" published by National Academy of Sciences (21).

Itching behavior was produced by intradermal injection of compound 48/80 (100 µg/50 µl); compound 48/80 is a well-known histamine releasing agent which produce scratches subsequent to mast cell degranulation. Scratching injected site by the hind paws was accepted as the itching behavior; mice scratched several times after compound 48/80 injection, and this reaction is counted as one bout of scratching. Scratches were video recorded in a quiet room, and then counted for 30 min. Testing was accomplished according to previously described procedures (22-24).

Locomotor performances were assessed using a rotarod apparatus (Commat, Ankara, Turkey). The animals were acclimatized to the apparatus before the assessments. Then, mice were placed on the drum rotating at 16 rpm and the performance time until the mice fell from the drum. 180 seconds cut-off frequency was adjusted before the experiments.

Groups of eight mice each received increasing doses of cannabidiol (1, 3, 10 mg/kg, ip). Cannabidiol was administered 30 min before compound 48/80 injections. Then, the cannabinoid CB1 receptor antagonist AM-251 (1g/mouse) and the cannabinoid CB2 receptor antagonist AM-630 (4g/mouse) were given intrathecally 10 min prior to cannabidiol administration in order to determine whether spinal cannabinoid receptors are involved in the effect of cannabidiol on itching behavior.

Cannabidiol was purchased from Tocris (UK), while compound 48/80, AM-251 and AM-630 from Sigma-Aldrich (St Louis, MO, USA. Compound 48/80 was dissolved in physiological saline, whereas cannabidiol, AM-251, and AM-630 were given in 20% dimethyl sulfoxide (DMSO), 1% Tween 80, 1% ethanol, and 78% saline. Cannabidiol was administered intraperitoneally in a volume of 0.05 ml/10 g body weight of mice, AM-251 and AM-630 were injected intrathecally (5l/mouse), and compound 48/80 was given intradermally (100 µg/50 µl). Previous studies guided doses and treatment (23-25).

Differences in the number of scratches in durations on the rotating rod were evaluated using analysis of variance and were followed by Bonferroni's multiple comparison tests. All data are expressed as mean SEM; $p<0.05$ was considered to be significant for all experiments.

RESULTS

Treatment with cannabidiol (1, 3, 10 mg/kg, ip) had no effect on compound 48/80-induced itching behavior at any dose given (Figure 1). 3mg/kg dose of cannabidiol seemed to reduce the number of scratches, but this reduction was not statistically significant (p=0.4499).

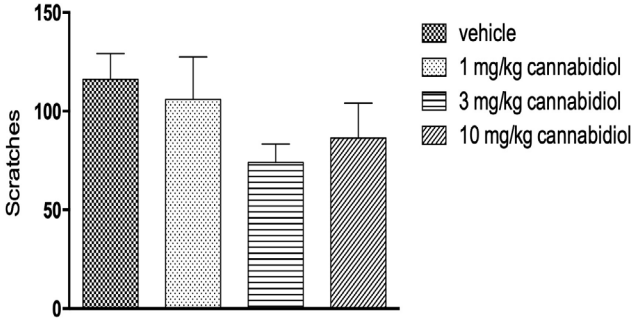

Figure 1: The effect of cannabidiol (1, 3, 10 mg/kg, ip) on the number of scratches.

The effect of cannabidiol (1, 3, 10 mg/kg, ip) on locomotion was evaluated in the rotarod test, where no significant change on motor function was observed (Figure 2).

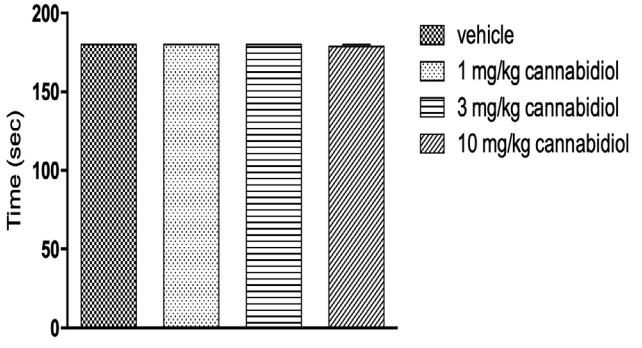

Figure 2: The effect of cannabidiol (1, 3, 10 mg/kg, ip) on locomotion.

AM-251, a CB1 antagonist; AM-630, a CB2 antagonist, were going to be administered intrathecally to observe the contribution of spinal cannabinoid receptors to the antipruritic action of cannabidiol, but they were not given when we observed cannabidiol did not exert any effect on itching behavior.

DISCUSSION

The non-psychoactive phytocannabinoid cannabidiol is a compound that does not produce typical subjective effects of marijuana. Different from the classical cannabinoids, including those found in the Cannabis plant, cannabidiol exhibits very low affinity with CB1 and CB2 (19). On the other hand, possible therapeutic uses of cannabidiol include analgesia, epilepsy, anxiety, schizophrenia, depression, and many more. Other than activity on cannabinoid receptors, there are multiple potential mechanisms underlying this wide spectrum of potential beneficial effects of cannabidiol. Firstly, recent findings also indicate that cannabidiol is a negative allosteric modulator of CB1 (26). Moreover, cannabidiol has been shown to be a transient receptor potential cation channel subfamily V member 1 (TRPV1) agonist which desensitize TRPV1 even at lower concentrations (27). Additionally, its pharmacological effects have been assigned to peroxisome proliferator-activated receptor (PPAR) agonism, intracellular calcium release and serotonin 1A receptor (5-HT1A) agonism (28, 29). Cannabidiol also appears to act via fatty acid amide hydrolase (FAAH) inhibition and augment endocannabinoid levels (28).

Considering similarities between pain and itching sensations and the potent analgesic effect of cannabidiol in different types of pain states, one would expect cannabidiol to show antipruritic action in mice (1-5). Its ineffectiveness in compound 48/80-induced itching behavior may be attributed to above-mentioned mechanisms unrelated to classical cannabinoid actions (26, 27, 29). Moreover, variations in physiological state, age, strain, and sex of the mouse, dose range and volume, route of administration and method of restraint are among the factors influencing this kind of behavioral research. Differences in assessment methods and existing animal models also seem to be important; for example, applying the pruritogens intradermally into the rostral part of the neck has been indicated not to discriminate pain and itching sensations but may give false positive results with analgesic drugs (23). Furthermore, the characteristics of the environment and the history of the subjects, such as exposure to stress, are suggested to interfere with the activity of cannabinoids in behavioral studies (30). Since cannabinoids have been proposed to excite circadian clock neurons and the activity of the endocannabi-

noid system is profoundly modulated by circadian rhythmicity, the ineffectiveness of cannabidiol in reducing scratches may have also resulted from the timing of drug administration (32, 33).

As we mentioned before, spinal cord appears promising for developing novel antipruritic drugs (6-8). In addition to the well-known involvement of spinal opioid receptor, gastrin-releasing peptide receptor and N-methyl-D-aspartate glutamate receptor in pruritus, serotonin, histamine, substance P and bradykinin receptors are among potential itching treatment targets (6, 7). It has also been demonstrated that blockade of spinal cannabinoid CB1 receptors partially reverse the antipruritic effect of synthetic cannabinoid WIN 55,212-2 (17). Here, we weren't able to investigate the contribution of spinal cannabinoid receptors since systemic cannabidiol had no effect on itching behavior.

Cannabidiol is used in some countries against pruritus; however, our findings suggest that systemic administration of cannabidiol does not diminish compound 48/80-induced itching behavior in mice. Recently, reduced pruritus and improved pain scores have been reported in patients with epidermolysis bullosa after combined treatment with tetrahydrocannabinol and cannabidiol (33). The effectiveness of cannabidiol in alleviating itching behavior should be investigated with further studies by using different itching models and experimental protocols.

Ethics Committee Approval: This study was approved by the Scientific Researches Committee of Trakya University School of Medicine.
Informed Consent: N/A
Conflict of Interest: The authors declared no conflict of interest.
Author contributions: Concept: RDT, AU. Design: HD, EB, EÖÖ, RDT, AU. Supervision: RDT, AU. Resources: RDT, AU. Materials: HD, EB, EÖÖ, RDT, AU. Data collection and/or processing: HD, EB, EÖÖ, RDT, AU. Analysis and/or Interpretation: HD, EB, EÖÖ, RDT, AU. Literature Search: HD, EB, EÖÖ, RDT, AU. Writing Manuscript: RDT, AU. Critical Review: HD, EB, EÖÖ, RDT, AU.
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