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Investigation of the Analgesic Properties L-759,633 and SER 601 in Experimental Neuropathic Pain Model in Rats and their Comparison with Pregabalin

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ABSTRACT

Despite the fact that narcotics and NSAIDs are the mainstays of nociceptive pain care, only a small proportion of neuropathic pain patients benefit from them. Cannabinoid agents could be a viable alternative to opioids in the management of chronic pain. The goal of our investigation was to assess the analgesic efficacy of SER 601 and L-759,633, cannabinoid receptor 2 (CB2) agonists, at various doses in a model of neuropathic pain generated in rat. The analgesic effect of CB2 agonists L-759,633 and SER 601 at various doses in a rat model of neuropathic pain created by partial sciatic nerve ligation was examined by the hot plate method. Furthermore, a comparison of analgesic effects of both drugs with pregabalin is also conducted. The two substances demonstrated a dose-dependent analgesic effect in this model. The analgesic response of SER601 and L-759,633 in the neuropathic pain model was higher compared to that of pregabalin. All in all, our data suggest that SER601 and L-759,633 may offer a beneficial treatment option for neuropathic pain in future.

Keywords: Neuropathic pain, L-759,633, SER 601, Hot plate, Pregabalin.

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Introduction

According to research, 13-50% of UK individuals suffer from chronic pain [1]. Neuropathic pain has been described as pain caused by a sensorimotor system damage or dysfunction, and it affects about one-fifth of people with chronic pain. [2, 3]. The sensory system is immediately affected by tissue destruction in neuropathic pain, resulting in ectopic discharges that circumvent transmission [4].

Two characteristics distinguish neuropathic pain from non-neuropathic pain. There is no transduction in neuropathic pain and the prognosis is bad. Nerve injury has a higher risk of causing persistent pain than injury to non-nervous tissues. Additionally, neuropathic pain is more resistant to standard painkillers than non-neuropathic pain (morphine derivatives and steroidal anti-inflammatory medicines) [5].

The absence of effective treatments is one explanation for the high incidence of neuropathic pain [5]. Despite the fact that morphine derivatives and non-steroidal anti-inflammatory medicines are the cornerstones of nociceptive pain medications, they have only a minor effect in the medication of neuropathic pain in a small percentage of patients. The primary cause for this is that the fundamental mechanisms are not fully targetable [6].

Tricyclic antidepressants (amitriptyline), serotonin-norepinephrine reuptake inhibitors (duloxetine), calcium channel α2- δ ligands (pregabalin) and lidocaine have exhibited effectiveness in neuropathic pain and they are used as first-line treatment. Opioid analgesics or tramadol, which are second-line drugs, can be used in patients that do not respond to the first-line treatment. In cases such as acute neuropathic pain, morphine derivatives such as tramadol are used as first-line drugs. Cannabinoids are used as a second-line drug in neuropathic pain caused by multiple sclerosis [7].

CB_2R expression has been demonstrated in the rodent in pain-related brain regions, including cerebral cortex, hippocampus, striatum, amygdala, thalamic nuclei, periaqueductal gray, cerebellum, and several brainstem nuclei. The upregulation of CB2R in the dorsal horn of the spinal cord during neuropathic or inflammatory pain was observed . Cannabinoids have a long history of medical application and are ~~growingly~~ increasingly approved for pain management. This advancement has been established by pre-clinical and experimental human research. Over the past ten years, six controlled trials have been reported evaluating the pain-relieving activities of cannabinoid-based medicines in experimental human settings. Cannabinoids have demonstrated efficacy in multiple chronic inflammatory and neuropathic pain models. There are tremendous evidences that specific cannabinoid receptor (CB_2R) agonists have anti-nociceptive effects and reduce the neuroinflammatory component of neuropathic pain [8].

It has been suggested that CB_2R selective agonists could be used to treat human neuropathic pain, a condition for which there are currently no consistently effective treatments. CB_2R selective agonists are not

estimated to have central nervous system side effects that limit the effectiveness of currently available drugs [9]. Up to now, the analgesic effect of L-759,633 and SER 601, cannabinoid receptor 2 (CB_2) agonists, in the rat model of neuropathic pain was not studied. In view of these findings, the analgesic effect of L-759,633 and SER 601, at various doses in the rat model of neuropathic pain was investigated in our experimentation using the hot plate method and the analgesic activity of these CB_2R agonists was compared to that of pregabalin.

Materials and Methods

Animals

In the experiments, 66 male adult Wistar albino rats ranging 210-235 g were employed. Four animals were located in each cage at 23 ± 0.5 ° C, with a 12-hour dark /12-hour light cycle and limitless connection with water and nutrient. There were six rats in each group. Cumhuriyet University's Animal Ethics Committee authorized the study protocols (Ethical Number: 65202830-050.04.04-280). Prior to testing, the animals were acclimatized to laboratory settings. Between 10 and 15 hours, all of the experiments were conducted blindly.

Drugs

SER601 and L-759,633 (Cayman Chemical Company, USA) were dissolved in the solution containing 10% dimethyl sulfoxide (DMSO) and 90% normal saline. Pregabalin (Cumhuriyet University Hospital, Sivas, Turkey) was dissolved in 0.9% NaCl solution. On the days of the experiments, fresh solutions were prepared. L-759,633, SER601 (3, 6, 12 mg/kg) and pregabalin (30 mg/kg) were injected intraperitoneally. A pilot study was carried out to determine the doses of L-759,633, SER601. However, the dose of pregabalin was determined according to the study conducted by Meymandi [10].

Analgesia Tests

The thermal pain was measured using a hot plate method (May AHP 0603 Analgesic HP, Commat) [11]. The animals were located on a hot plate. The temperature was determined as 53 ± 0.6 ° C. The time taken for the first action (licking or jumping) to avoid heat was calculated and taken as a measure of pain threshold.. The cut of time is 30 seconds to avoid damaging the claw. This test's hyperalgesic responses demonstrate pain processes in the central nervous systems [11].

Surgical Intervention

A neuropathic pain model was created by partially ligating the sciatic nerve. Surgical procedures were carried out in the Experimental Animals Laboratory of Sivas Cumhuriyet University. Intramuscular ketamine at dose of 90 mg/kg and xylazine at dose of 3 mg/kg were used for anesthesia. An about 1 cm incision was made in the biceps femoris under aseptic circumstances. After that the sciatic nerve was reached in the right leg's middle thigh level. The sciatic nerve was then carefully separated from the supporting tissues and firmly bound with 4.0 chromic catgut. 4.0 silk was used to close the incision. In sham group, the rat's nerve was separated but not tied [12,13].

Protocol

Rats were randomly divided into 11 groups. In order to create a neuropathic pain model, the sciatic nerve binding method was applied to rats and it was predicted that neuropathic pain would occur during two weeks. At the end of this period, basal latencies were obtained before applying the drugs and compared with basal latencies obtained before surgery. Then the agonists were applied in 3 various doses. The antihyperalgesic effects of L-759,633, SER601 and pregabalin in the rats were examined at 0, 15, 30, 60, 90, and 120 minutes by hot-plate method. In the sham group, rats received DMSO. Table 1 below explains the animal groups (11 groups) in detail.

Table 1. Some topological parameters of BCPs for gas phase calculations.

	Experimental and Control Groups (Each dose was administered once intraperitoneally)	Number of Rats
1	Sham (DMSO)	6
2	Sham (saline)	6
3	Neuropathic Pain (saline)	6
4	Neuropathic Pain (DMSO)	6
5	L-759,633 3 mg/kg	6
6	L-759,633 6 mg/kg	6
7	L-759,633 12 mg/kg	6
8	SER 601 3 mg/kg	6
9	SER 601 6 mg/kg	6
10	SER 601 12 mg/kg	6
11	Pregabalin 30 mg/kg	6

Data Analysis and Statistical Analysis

To determine percentage of the maximal anti-nociceptive effects (% MPE), lick/escape latencies were translated to percentage of anti-nociceptive effects using this formula:

% MPE = [(test latency – baseline) / (cutoff (30) – baseline)] * 100 [14]

The results were evaluated using paired student t-test and one-way analysis of variance (ANOVA) and repeated measures ANOVA followed by a Tukey post hoc test (SPSS 20.0 for Windows) for multiple comparisons between groups. All results are displayed as a mean ± SEM. The level of significance was set at $p < 0.05$.

Results and Discussion

Detection of Neuropathic Pain Generation by Sciatic Nerve Ligation

The hot plate basal latencies obtained after surgery were significantly lower than those obtained before it ($p<0.05$) (Fig. 1). In this context, the formation of neuropathic pain in rats was established.,

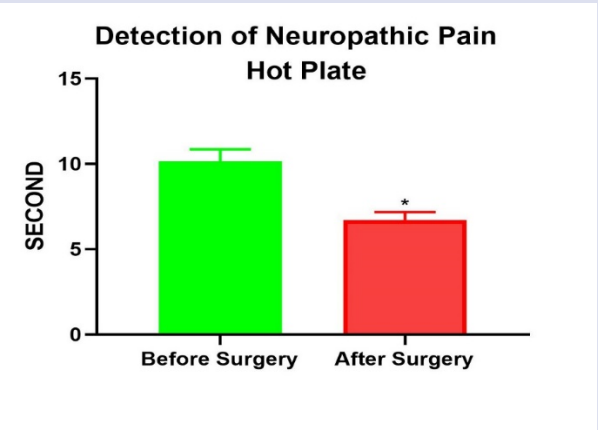

Figure 1. The basal latencies of rats before and after operation (paired student t-test, *p<0.05)

The Effect of Various Doses of SER601 on Neuropathic Pain

The effect of SER601 at doses of 3, 6 and 12 mg/kg on neuropathic pain was assessed using a hot plate test to determine the anti-hyperalgesic responses for the different doses of this agent from 15 to120 minutes using hot plate test. All doses of SER601 were demonstrated to be efficient on neuropathic pain in comparison with the sham group at all minutes (Fig. 2). In addition, statistically significant differences were found between %MPE values produced by these three doses. As a result, it was revealed that the drug's effect on neuropathic pain is dose-dependent (Fig. 2).The maximum %MPE was recorded after 60 minutes of administration of these three doses.

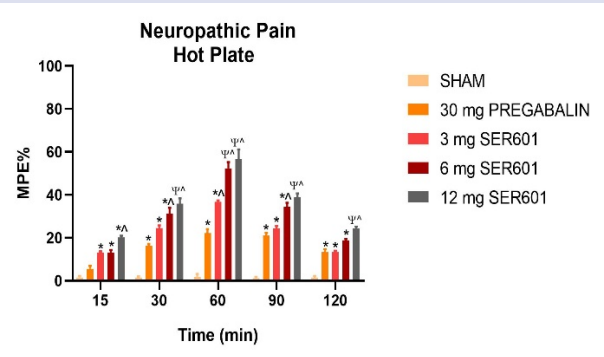

Figure 2. The effect of SER601 on the neuropathic pain model represented as a percentage of the maximum potential effect (MPE). Pregabalin was used as positive control. The agents were administered intraperitoneally. The results are presented as mean ± SEM for 6 rats. One-way ANOVA was applied.*p < 0.05, MPE% is significantly higher when compared to the correspondence time of sham group. Ψp< 0.05, MPE% is significantly higher when compared to the correspondence time of sham and 3 mg/kg SER601 groups. ^p < 0.05, MPE% is significantly higher when compared to the correspondence time of pregabalin group.

The Effect of Various Doses of L-759,633 on Neuropathic Pain

The effect of L-759,633 at doses of 3, 6 and 12 mg/kg on neuropathic pain was assessed using a hot plate test to determine anti-hyperalgesic responses for the different doses of this agent from 15 to120 minutes using hot plate test. 6 and 12 mg/kg L-759,633 were demonstrated to be efficient on neuropathic pain in comparison with the sham group at all minute points. 3 mg/kg L-759,633 from 30 to 120 minutes was demonstrated to be efficient on neuropathic pain in comparison with the sham group (Fig. 3). In addition, statistically significant differences were found between %MPE values produced by these three doses. As a result, it was revealed that the drug's effect on neuropathic pain is dose-dependent (Fig. 3). The maximum %MPE was recorded after 60 minutes of administration of these three doses.

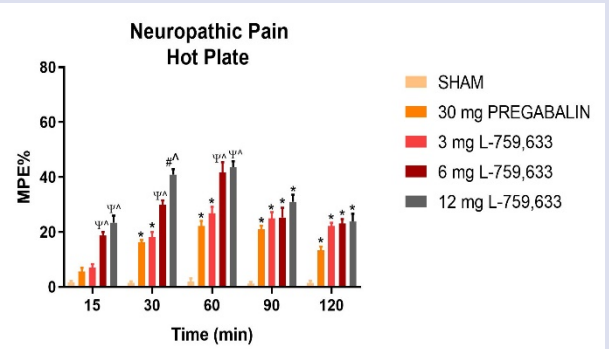

Figure 3. The effect of L-759,633 on the neuropathic pain model represented as a percentage of the maximum potential effect (MPE). Pregabalin was used as positive control. The agents were administered intraperitoneally. The results are presented as mean ± SEM for 6 rats. One-way ANOVA was applied. *p < 0.05, MPE% is significantly higher when compared to the correspondence time of sham group. Ψp< 0.05, MPE% is significantly higher when compared to the correspondence time of sham and 3 mg/kg L-759,633 groups .#p< 0.05 MPE% is significantly higher when compared to the correspondence time of sham, 3 mg/kg L-759,633 and 6 mg/kg L-759,633 groups. ^p< 0.05, MPE% is significantly higher when compared to the correspondence time of pregabalin group.

For comparison, the effect of 30 mg/kg dose of pregabalin on neuropathic pain was assessed at 0, 15, 30, 60, 90, and 120 minutes. Compared to the sham group, it was determined that pregabalin was effective against neuropathic pain at all minutes (except the 15-minute). The effect of the drug reached its peak at the 60-min then started to decrease. The antihyperalgesic effect of 12 mg/kg SER601 from 15 to 120 minutes, 6 mg/kg SER601 from 30 to 90 minutes and 3 mg/kg SER601 at 60-min on neuropathic pain were higher than the effect of

pregabalin group (Fig. 2). The antihyperalgesic effect of 12 and 6 mg/kg L-759,633 from 15 to 60 minutes on neuropathic pain were greater than the effect of pregabalin group (Fig. 3).

There is an inadequately treated pain epidemic, and it has been considered as a main public health issue. Sever and chronic pain management is the burden of clinicians [15]. Nonsteroidal anti-inflammatory medications opiates, antidepressants, anti-convulsants, ketamine, and other drugs have been used to manage a variety of pathological pain conditions. However, the side effects lead to a limitation of the amount of doses that can be used in the treatment and thus a decrease in the therapeutic efficacy. Though there are advances in the comprehension of pathophysiological processes that generate chronic pain status and in the determination of different analgesic pathways, there is still an excessive need for treatment approaches for chronic pain that are effective and don't cause undesirable central side effects [16]. CB2 receptor expression has also been discovered to be altered in different regions of the pain pathways during inflammatory and neuropathic pain conditions. An elevation of CB2 receptor mRNA level in the medulla spinalis was discovered in neuropathic pain situations caused by ligation of sciatic or spinal nerve. In animal models of neuropathic pain generated by nerve injury or chemotherapeutic drugs, systemic or local treatment of a variety of selective CB2 receptor agonists was found to be useful in alleviating neuropathic pain.[17]. Cannabinoid-based therapies may be a feasible alternative to opioids for the chronic pain management. Cannabinoids have been demonstrated in clinical studies to considerably relieve chronic pain in multiple sclerosis, fibromyalgia, diabetic or other neuropathy, and rheumatoid arthritis patients. [18,19]. Due to undesirable properties of opioids, drug discovery and development efforts have become more focused on finding effective new drugs that do not have these unwanted properties [20,21]. Undesirable effects of cannabinoids are reported to be relatively mild and well tolerated, and some studies have also shown that these drugs may cause improvements in sleep [18,19]. Sheng et al. showed that CB2 receptor agonists JWH015, Gp1a, and JWH133 were efficacious in lowering mechanical allodynia caused by prolonged retroviral infection in mice when evaluated 2 hours after injections [22]. In a similar study, It has been demonstrated that CB2 agonist GW405833 eliminated the mechanical allodynia generated in inflammatory and neuropathic pain models in wild type WT mice and the effect was dose-dependent [23]. Pasquiniet al., showed that SER-601, a selective and potent CB2 agonist, has antinociceptive effect in a formalin-induced pain model at 3 mg/kg and because of its poor affinity for the CB1 receptor, it has no cannabis-like behavioral activities [24,25].

No research has been conducted to determine the effect of the selective CB2 agonists L-759,633 and SER-601 on neuropathic pain. In our research, the effects of these agents on neuropathic pain were evaluated. SER601 and L-759,633 were administered at doses of 3, 6, and 12 mg/kg. These two drugs showed dose-dependent analgesic activity in neuropathic pain model induced by sciatic nerve ligation. Our results are consistent with earlier data as CB2R agonists have analgesic activities on neuropathic pain. The analgesic effects of SER601 and L-759,633 achieved their peak at 60- minute and then began to diminish. The antinociceptive effects of these agents were evaluated as %MPE (maximal possible effect). The analgesic response of SER601 and L-759,633 in the neuropathic pain model was higher compared to that of pregabalin. When comparing the analgesic response to SER601 and L-759,633, no statistically significant differences were found. The effect of 10% diluted DMSO on pain response was detected and compared with saline. 10% Diluted DMSO did not produce any alteration in the pain response. In addition, the control solution was 10% diluted DMSO, which prevents errors in outcome assessment.

We did not examine the mechanisms contributing to the analgesic effects of SER601 and L-759,633. However, other studies have reported that activation of cannabinoid type 2 receptors produce antinociceptive effects by inhibiting glutamatergic transmission [26], calcium influx [27] and stimulating β-endorphin release [28].

In summary, L-759,633 and SER601 showed an analgesic effect in neuropathic pain and could be candidate drugs for neuropathic pain treatment. The study of analgesic effects of CB2 agonists deserves further effort, as these agents do not cause central side effects and exert strong analgesic activity. In addition, the mechanisms of analgesic effect of SER601 and L-759,633 were not investigated in our study so further studies should be done to reveal the mechanisms involved in this effect.

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Conflicts of interest

There are no conflicts of interest in this work.

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