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The effect of Dapoxetine on Chronic Constriction Injury (CCI) induced neuralgia in rats

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ABSTRACT

Introduction. Recent studies have shown that SSRIs have anti-inflammatory effects, and their administration prevents their production by acting on inflammatory cytokines. Emphasizing that we did not find a study on Dapoxetine and its effect on neuropathic pain, in this study, we examined the effect of Dapoxetine on neuropathic pain in rats.

Material and methods. 42 rats were divided into six groups of 7, which included: sham, control, gabapentin, Duloxetine and Dapoxetine 1 and 3. Except for the sham group, other groups were induced with neuropathy, and no drug treatment was performed in the sham group. The groups were treated with normal saline, gabapentin (30 mg/kg/day), Duloxetine at (30 mg/kg/day) and Dapoxetine (1 and 3 mg/kg/day), respectively by intraperitoneal injection. Then, thermal hyperalgesia and mechanical and thermal allodynia experiments were performed on rats.

Results. The Dapoxetine-treated groups' mean response to thermal hyperalgesia and mechanical allodynia increased on days 7 and 14. The mean response of the 3 mg/kg Dapoxetine group on day 7 was significantly higher than the control group ($P < 0.05$). Mean response to mechanical and thermal allodynia-induced stimulation and thermal hyperactivity in the dapoxetine group 3 mg/kg at 14 days, compared with the Dapoxetine group 1 mg/kg showed significantly better results ($P < 0.05$).

Conclusion. Dapoxetine effectively reduces the behavioral response to painful and non-painful thermal and mechanical stimuli and painful thermal and mechanical stimuli. In addition to its short-term analgesic effects, it also has long-term effects.

Keywords: Chronic Constriction Injury (CCI), Dapoxetine, neuralgia

INTRODUCTION

Neuropathic pain is defined as pain associated with damage or disease of the nervous system. Damage to nerve cells and dysfunction of the central, peripheral, and autonomic nervous system can be caused by surgery and various conditions such as diabetes, herpes zoster infection, stroke, multiple sclerosis and spinal cord injury [1,2]. Overall, eighteen percent of the world's population suffers from neuralgia [3] and 6% of the world's population suffers from such pain each year [4]. According to global statistics, many people suffer from spinal cord injuries every year, which is 2.5 million people in the world, and 130,000 people are added to this number every year [5]. So sixty-four percent of these people suffer from chronic pain. In Tehran, 25.5% of people suffer from chronic pain [6].

Inflammatory and oxidative stress cytokines have been shown to increase stress in neuropathic pain. Examination of various mechanisms in the induction of neuropathic pain revealed that activation of N -methyl-D-aspartate (NMDA) receptors [7], stimulation of pain receptors by mediators such as histamine and chitin, increased inflammatory factors including TNF-α [8] and IL-6 [9], increased Malondialdehyde (MDA) [10] and decreased Superoxide dismutase (SOD) [11] can play a role in causing pain. First-line treatments include antidepressants such as Venlafaxine and Amitriptyline and anticonvulsants including Carbamazepine, Gabapentin, and Pregabalin [3]. Appropriate regular exercises such

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as swimming and hydrotherapy can be effective in relieving pain [3]. Hydroalcoholic extract of red onion relying on its antioxidant and anti-inflammatory effects can reduce neuropathic pain in mice [2]. The family of selective serotonin reuptake inhibitors (SSRIs) is common in treating depression and has been shown to have analgesic effects by increasing serotonin receptors in the spinal cord area [12]. In another study, the analgesic effects of Fluoxetine were evaluated in an experimental model of diabetic neuropathy, and the results of this study showed that SSRIs could prevent the progression of neuropathy and its pain [13]. Recent studies have shown that SSRIs have anti-inflammatory effects and their administration inhibits the production of inflammatory cytokines [14]. Dapoxetine is a SSRI that is not suitable for treating depression due to its rapid metabolism. It is a well-known drug to treat premature ejaculation [15]. Due to the frequency and severity of neuropathic pain and also the lack of definitive treatment following several studies in this field and emphasizing that we did not find a study on Dapoxetine and its effect on neuropathic pain, this study aimed to evaluate it to treat neuropathic pain in rats.

MATERIALS AND METHODS

Forty two male Wistar rats weighing 250-300 g were used. Before starting the experiments, the environment was kept at a suitable temperature (25°C), with a humidity of 65-75% and a 12 hours dark-light cycle for a week to adapt the animals to the environment. The rats used water and food without any restrictions. Also, all ethical principles of working with laboratory animals were observed by following per the protocols approved by Shahid Sadoughi University of Medical Sciences, Yazd, Iran and based on the ethical guidelines for animal research developed by the Policy Council of the Ministry of Health and Medical Education and Medical Ethics and History Research Center of Yazd University of Medical Sciences.

Animal grouping

The animals were divided into six groups of 7 including sham, no surgery or injection was performed. Control neuropathic pain was induced by surgery, and they received normal saline orally; standard, surgical neuropathic rats were receiving gabapentin (Iran Drug Pharmaceutical Company, Iran) at a dose of 30 mg/kg/day by intraperitoneal injection [3];

Duloxetine, surgical neuropathic rats, receiving oral Duloxetine (Exir Pharmaceutical Company, Iran) at 30 mg/kg/day. Dapoxetin, neuropathic rats with surgery received dapoxetine (Exir Pharmaceutical Company, Iran) at doses of 1 or 3 mg/kg/day by intraperitoneal injection. The injections were given once and 30 minutes before the start of the behavioral tests.

Inducing pain

The Chronic Constriction Injury (CCI) model was developed in 1988 by Bennett and Xie. The CCI model induces neuropathic pain on the sciatic nerve in the left leg. Animals were deeply anesthetized by intraperitoneal injection of Ketamine hydrochloride (Alfasan, Netherlands, 50 mg/kg) and Xylazine (Alfasan, Netherlands, 10 mg/kg). Then, the left hind limb hair was shaved, and a 2 cm incision was made in the right thigh. After cutting the muscles, the joint of the three branches of the sciatic nerve was seen. Then the tissue surrounding the sciatic nerve was removed, and four not-so-tight knots were sutured at 4 to 1 mm intervals before the nerve was branched with 0-4 sutures (Supa, Iran). Then the muscles and skin were sutured with 0-6 sutures, and the surgical site was sterilized [16,17]. Then the rats were kept in a warm environment to recovery. Behavioral signs of spontaneous pain include licking of the hind paw, lameness of the rear foot, and avoidance of placing weight on the injured side, were observed in CCI rats.

Mechanical allodynia, thermal allodynia, and thermal hyperalgesia tests evaluated the pain behavior on days 7 and 14 after surgery. These tests were respectively done by Von Frey, acetone bubble, and hot air blower. In addition, the weight of the animals was measured before the beginning of the study, in the middle, and at the end of the study (Figure 1).

FIGURE 1. Chronic Constriction Injury (CCI)

Mechanical allodynia test

The animals were placed on a wire mesh inside a Plexiglas enclosure measuring 20x20 cm and 30 cm high. After the animal got used to the new environment, an electronic Von Frey (Elvamed, Iran) was used. The experiment was repeated three times in a row at intervals of 5 seconds and each time for 1 second on the sole of the animal's left foot.

Thermal allodynia test (Acetone test)

Acetone was sprayed on the sole to determine the sensitivity of the animals to thermal allodynia, which was used according to the method of Zabihi et al. In this procedure, the animal was placed on a wire mesh. A drop of acetone was sprayed into the sole of the animal's left foot through an insulin syringe with a narrow polypropylene tube instead of a needle. This test was performed five times, 3 minutes each time. Suppose the animal raised its leg when the acetone was sprayed that it was considered a positive response and otherwise a negative response. The response percentage was calculated by the number of positive responses of the animal to the total number of stimuli [18].

Thermal hyperalgesia test

In this test, using a hot air blower, hot air was applied to the soles of the operated feet of rats from a distance of 15 cm, and the time of pulling the rats' feet was recorded using a timer. All tests were performed two hours after medication on days 7 and 14 after surgery.

Average unoperated leg - Average operated leg = time difference of leg retraction.

If the difference between the mean delay of retraction of the two legs is negative, it indicates the phenomenon of thermal hyperalgesia (Figure 2).

FIGURE 2. Thermal hyperalgesia tests using a hot air blower

Data analysis

SPSS 21 was used for data analysis and descriptive statistics (mean tables and standard deviation), and the One-way analysis of variance (One-way ANOVA) with Tukey post hoc was used. The significancies were considered with a $P < 0.05$.

Ethical considerations of the plan

The dissertation was registered in the research system of Shahid Sadoughi University of Medical Sciences of Yazd with the number SSU-7699. By observing the ethical principles and laws of protection and care of laboratory animals, the code of ethics was obtained from the ethics committee of Shahid Sadoughi University of Medical Sciences of Yazd (IR.SSU MEDICINE.REC.1399.133).

RESULTS

There were no significant differences in rats' weight between the groups on days 7 and 14.

Mechanical allodynia

The mean response of the groups treated with Gabapentin, Duloxetine and Dapoxetine 1 and 3 mg/kg increased on days 7 and 14 and showed a significant difference compared to the control group ($P < 0.05$). On days 7 and 14, the mean response to stimulation due to mechanical allodynia in the dapoxetine group of 3 mg/kg increased significantly compared to 1 mg/kg ($P < 0.05$). (Figure 3)

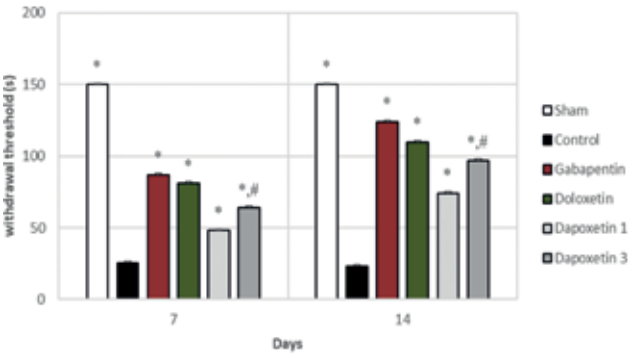

FIGURE 3. Mean response to stimulation due to mechanical allodynia in the Von-Frey test. Gabapentin, gabapentin at a dose of 30 mg/kg/day by intraperitoneal injection; Duloxetine, Duloxetine at an of 30 mg/kg/day orally; Dapoxetin 1: Dapoxetine at a dose of 1 mg/kg/day by intraperitoneal injection; Dapoxetin 3: Dapoxetine at an of 3 mg/kg/day by intraperitoneal injection; The results were analyzed using one-way ANOVA and Tukey post hoc. (*Significant difference compared to the control group with $P < 0.05$)

Thermal allodynia

On day 14, the mean response of thermal allodynia decreased in the groups treated with Gabapen-

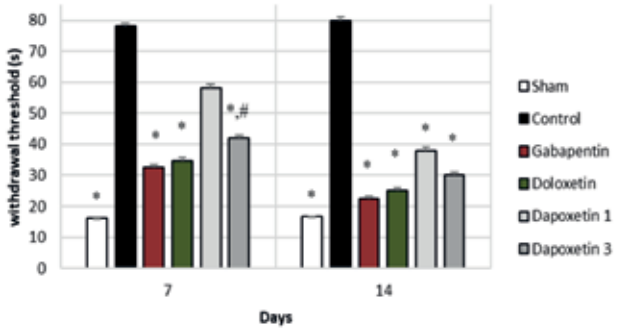

FIGURE 4. Mean response to the stimulation caused by thermal allodynia in the acetone bubble test. Gabapentin; gabapentin at a dose of 30 mg/kg/day by intraperitoneal injection; Duloxetine; duloxetine at a dose of 30 mg/kg/day orally; Dapoxetin 1, dapoxetine at a dose of 1 mg/kg/day by intraperitoneal injection; Dapoxetin 3, dapoxetine at a dose of 3 mg/kg/day by intraperitoneal injection. The results were analyzed using one-way ANOVA and Tukey post hoc. (* Significant difference compared to the control group with P <0.05)

tin, Duloxetine, and Dapoxetine 1 and 3 mg/kg compared with the control group (P <0.05). On day 7, the mean response to thermal allodynia stimulation in the 3 mg/kg Dapoxetine group was significantly reduced compared to the Dapoxetine group of 1 mg/kg (P <0.05). However, on day 14, the mean response between the two groups was reduced. Dapoxetine 1 and 3 mg/kg did not show a significant difference (P> 0.05). (Figure 4)

Thermal hyperalgesia

The mean response time of Dapoxetine-treated groups increased on days 7 and 14. The mean response of the Dapoxetine-treated group (3 mg/kg)

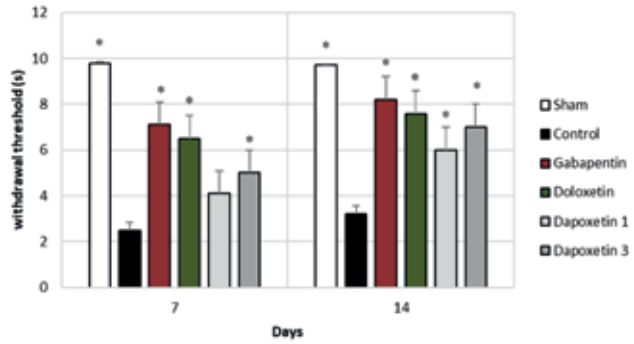

FIGURE 5. The mean response time to the stimulation was caused by thermal hyperalgesia after receiving the drug. Gabapentin at a dose of 30 mg/kg per day was injected intraperitoneally, and Duloxetine at a dose of 30 mg/kg per day was used orally; Dapoxetine 1 contained 1 mg/kg Dapoxetine, and Dapoxetine 3 contained 3 mg/kg Dapoxetine, both of which were administered by intraperitoneal injection. The results were analyzed using one-way ANOVA and Tukey post hoc. (* Significant difference compared to the control group with P <0.05).

On day 7 was significantly higher than the control group (P <0.05). On day 14, the groups treated with Dapoxetine 1 and 3 mg/kg were significantly higher than the control group (Figure 5).

DISCUSSION

Neuropathic pain is a non-tolerable and complex disease resulting from nerve damage and dysfunction[19]. Pain naturally occurs when a severely painful or traumatic stimulus activates the primary sensory neurons of pain with a high activity threshold. Still, in neuropathic pain, the threshold of the pain-conducting neurons decreases, and it often manifests itself in two ways. One is spontaneous pain (independent of the stimulus), and the other is aroused pain (dependent on the stimulus), which is caused by the presence of a stimulus after the damage. Evoked pain has two characteristics: allodynia (a painful response to non-painful stimulus) and hyperalgesia (increased intensity of response to a painful stimulus) [20]. The pathophysiology of pain is relatively complex, involving peripheral and central mechanisms that can include changes in the expression of ion channels, the release of neuronal neurotransmitters changes in the path of inhibitory pain, and so on [21,22]. Neuropathic pain is accompanied by an unpleasant burning sensation, tingling, increased sensitivity to painful stimuli and a feeling of pain in the face of non-painful stimuli [23]. An inflammatory reaction occurs during tissue destruction and wound healing, leading to activation of the pain receptor [24]. Both hyperalgesia and allodynia are present in inflammatory and neuropathic pain [25,26].

The role of inflammatory [27] and oxidative [28] markers in various diseases have been well studied. It has also been shown that inflammatory cytokines [29] and oxidative stress [7] increase neuropathic pain.

Given the adverse effects of pain, the importance of its treatment is not hidden from anyone. Short-term drug use is an effective way to treat neuropathic pain, but its use is limited due to concerns about its complications. Some pharmacotherapists like the prescription of Tricyclic Antidepressants (TCAs) and anticonvulsants (like Gabapentin and Pregabalin) as a basis for the treatment of chronic neuropathic pain and improve quality of life (QOL) [30].

Previous reports have explained the effect of SSRIs on neuropathy [31]. Dapoxetine hydrochloride is an SSRI that commonly use for depression treatment [32]. This is also the first and only drug in men aged 18 to 64. Due to its anti-inflammatory and immune-modulating effects, Dapoxetine hydrochloride is a promising drug for treating inflammatory

diseases, including rheumatoid arthritis [33]. However, no study has been conducted to investigate the effect of this drug on neuropathic pain. One of the causes of the analgesic effects of this drug can be related to the anti-inflammatory properties of this drug and the other SSRIs. Inflammation is probably one of the most acceptable humoral links between chronic pain and depression. Suppose SSRIs have inherent anti-inflammatory and antioxidant properties and can modulate inflammatory processes. In that case, this may explain their therapeutic effect in managing chronic pain, Although the exact mechanism of the action remains unknown [34].

Studies have shown that in the standard CCI model, an increase in TNF-α levels can cause pain and neurological disorders [35]. On the other hand, interleukin 6 (IL_6) levels that increased significantly in the nerve area and ganglia have been shown to induce neuronal damage [36]. SSRIs may be suitable candidates for the treatment of inflammatory diseases because they reduce pro-inflammatory cytokines such as IL-6 and tumor necrosis factor (TNF-α) and suppress the expression of cyclooxygenase-2 (COX-2) protein [37].

It has also been shown that SSRIs have analgesic effects by increasing serotonin receptors in the spinal cord area [12]. Tembhurne S. also showed that SSRIs could prevent the progression of neuropathy and its pain in diabetic neuropathy [13]. Heba F. Salem showed that Dapoxetine locally relieves rats' paw edema, which indicates the anti-inflammatory properties of this drug even in topical form [38]. Another study in 2020 examined the effect of Dapoxetine on reducing the severity of rheumatoid arthritis in mice, and the results showed that this drug significantly reduces the serum levels of IL-6 and the severity of arthritis [39].

In 2017, Abbaszadeh et al. studied the anti-inflammatory and antioxidant effects of Fluoxetine in an experimental model of neuropathic pain in mice. The results of this study showed that Fluoxetine reduces neuropathic pain symptoms. Fluoxetine also decreased TNF-α, IL-6, and MDA levels and increased SOD levels. Finally, the results of this study showed that this drug has effective analgesic effects in the experimental model of neuropathic pain, which is probably due to its anti-inflammatory and antioxidant effects [40]. Ohgi Y et al. stated that SSRIs have considerable anti-inflammatory effects by increasing IL-10 by peripheral blood leukocytes and decreasing the production of inflammatory cytokines [41]. Also, in the study by Bannister et al. in 2016, it was concluded that the analgesic effects of SSRIs are induced by HT7-5 receptors [42]. On the other hand, SSRIs have been reported to reduce pain transmission from the environment to the spinal cord by increasing HT7-5 levels in the spinal cord.

In the same way, HT7-5 receptors can produce the analgesic effects of morphine in the spinal cord. The results of the studies are consistent with ours. This study aimed to evaluate the effect of Dapoxetine on neuropathic pain induced by chronic constriction injury in rats. The results showed that intraperitoneal administration of Dapoxetine significantly reduced their sensitivity to pain tests. Also, these effects were not dose-dependent. Performing pain tests before prescribing this drug showed that this drug also has long-term analgesic effects, so that, on day 14, the maximum long-term effect of the drug was seen.

CONCLUSION

The animals in the control group showed a considerable behavioral response to non-painful thermal and mechanical stimuli and painful thermal and mechanical stimuli compared to those in the dapoxetine group. In addition to short-term analgesic effects, this drug also has long-term consequences.

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Authors contribution

Mohsen Zabihi conceived and designed research. Mohsen Zabihi and Akram Zare Bidaki conducted experiments and analyzed data. All authors read and approved the manuscript, and all data were generated in-house, and no paper mill was used.

Conflict of interest: none declared
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