RESEARCH ARTICLE

Evaluation of the Effect of Vortioxetine on Pain Threshold By Hot-Plate Test in Mice

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ABSTRACT

Introduction: Vortioxetine is an antidepressant that has a multimadal action mechanism and has recently come into use. The present study was planned to determine whether vortioxetine affects pain threshold in mice.

Method: The experimental animals were divided into four groups with 10 mice in each group. The distilled water was given to the control group, 5 mg/kg of vortioxetine was intraperitoneally administered to the first group, 10 mg/kg of vortioxetine was intraperitoneally administered to the second group and 20 mg/kg of vortioxetine was intraperitoneally administered to the third group. Mice were placed on a hot-plate at 30 and 90 minutes. Hind paw licking and jumping times of the mice on the hot plate surface (55°C) were recorded..

Results: With increasing dose (0 mg $p > 0.05$, 5 mg $p < 0.001$, 10 mg $p < 0.001$, 20 mg $p < 0.001$) and increasing time (30th minute $p < 0.01$, 90th minute $p < 0.01$), it was observed that the reaction time per minute, which was a reflection of pain treshold was decreased.

Conclusion: The results of this study shows that vortioxetine may have a decreasing effect on pain threshold in mice. Further studies are needed to determine the mechanism by which vortioxetine exerts its hyperalgesic effect.

Keywords: Pain, animal experiments, pain threshold, nociception, vortioxetine

INTRODUCTION

Depression and chronic pain often occur together (1). Psychological problems play an important role in chronic pain. Prolonged pain causes anxiety with a progressive depressive state and increases the perception of pain. The co-occurrence of chronic pain and depression is associated with functional decline (1), poorer response to treatment (2), and higher health care costs (3). Although antidepressants were not designed as analgesics, they have been shown to have analgesic effects in chronic pain (4). These effects of antidepressants offer a significant advantage in treating depression and chronic pain (5). Tricyclic antidepressants and serotonin and/or norepinephrine reuptake inhibitors (SNRIs) are recommended as first-line therapy for neuropathic pain (6). How antidepressants are effective in treating pain, and the exact mechanisms underlying their effects remain unclear. In nociception, the stimulus is received by specialized nerve endings (nociceptors), transmitted to the central nervous system, integrated into neuronal structures, the noxious stimulus is perceived, and action against it is activated (7). Antidepressants likely affect nociception in some way. Recent neuropathic pain studies in animal models have shown that norepinephrine is significant in inhibiting neuropathic pain. This inhibition can take place in two forms. First, the elevation of norepinephrine directly inhibits neuropathic pain by inhibiting reuptake in the spinal cord via α^2-adrenergic receptors. Second, the elevation of norepinephrine acts on the locus coeruleus and improves the impaired descending noradrenergic inhibitory system (8). Moreover, serotonin and dopamine may enhance noradrenergic effects to prevent neuropathic pain (8). Serotonergic pathways extending from the rostral ventromedial medulla (RVM) to the spinal cord have an inhibitory influence on pain, according to studies on the effect of serotonin on pain (9, 10). However,

the impact of serotonin on pain varies depending on the receptor subtype that is activated. Regarding the effects of serotonin on pain at the receptor level, 5-Hydroxytryptamine $(5HT)_{2A}$, $5\text{-}HT_4$ was found to increase pain, $5\text{-}HT_{1A}$, $5\text{-}HT_{1B/1D}$, $5\text{-}HT_{2C}$, $5\text{-}HT_7$ decreased pain, while $5HT_3$ increased it in some studies and decreased it in others (11). Despite numerous studies demonstrating that serotonin is efficient in pain regulation, particularly in the spinal cord, the precise mechanism is unclear.

Vortioxetine, a serotonin transporter protein inhibitor (SERT) and 5-HT1A agonist, is the first antidepressant with this target combination to be commercialized. In 2013, the US Food and Drug Administration approved it to treat major depressive disorder (12). In Turkey, the Turkish Medicines and Medical Devices Agency granted an antidepressant license in 2014. Vortioxetine has been examined in vitro using recombinant cell lines expressing human and rat targets in various binding and functional assays. Vortioxetine was discovered to be a $5HT_3$, $5\text{-}HT_7$, and $5\text{-}HT_{1D}$ receptor antagonist, a 5-HT1B receptor partial agonist, a $5\text{-}HT_{1A}$ receptor agonist, and a SERT inhibitor in these analyses (13). Furthermore, an acute dose of vortioxetine increased norepinephrine in the nucleus locus coeruleus (14). It was suggested that this increase was not due to a direct effect of vortioxetine but via $5HT_3$ receptor antagonism (15). Vortioxetine has also been demonstrated to raise dopamine levels in the prefrontal cortex (14), which is thought to be owing to a $5\text{-}HT_{1A}$ agonist effect (16).

As a multimodal antidepressant, vortioxetine is likely associated with nociception, both by inhibiting SERT and interacting with various 5- HT receptors and increasing norepinephrine. This study aimed to investigate the possible effect of vortioxetine on pain threshold in mice.

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METHOD

The Local Ethics Committee of Kafkas University approved this experimental animal study on October 25, 2017, with decision number 2017/95. The study was conducted in the Laboratory of Experimental Animal Production and Research of Kafkas University.

Experimental Animals

The study used 40 male Swiss albino mice weighing 25-30 grams bred in Kafkas University's Laboratory of Experimental Animal Production and Research. The mice were divided into four groups and housed in appropriate cages in the animal laboratory, at a temperature of 23 +/-1 degrees Celsius, in an environment with a light-dark cycle of 12 hours and 40-5% humidity.

Process

The animals were divided into four groups, including the control group. In distilled water, vortioxetine was dissolved (17). The control group received distilled water, the first group received 5 mg/kg, the second group received 10 mg/kg, and the third group received 0.3 ml vortioxetine (20 mg/kg) intraperitoneally.

Hot-plate Test

The hot-plate test is a test to evaluate the nociceptive response to heat. Usually, 52 or 55°C is used, less commonly 48°C. To prevent the animal from jumping off the plate, a clear plastic cylinder is placed around it. After a while, as the surface temperature rises, the mouse will lick its paw or lift a paw. Higher temperatures are less desirable because of the risk of burns. The measured parameter is usually the delay time for licking the paw or lifting the claw (18). Our study determined the time elapsed after subjects licked or jumped their hind paws after lying down on the 55°C hot plate surface. The measurements were taken 30 and 90 minutes after the injection. 30 seconds was set as the cut-off point to avoid tissue damage, and 30 seconds was accepted as the reaction time. The hot plate was cleaned with 20% ethanol after each mouse.

Statistical Evaluation

The effect of vortioxetine on the pain threshold of mice was evaluated using a paired sample t-test, followed by one-way analysis of variance (ANOVA) to assess the influence of dose on pain threshold, and finally Dunnett's t-test.

RESULTS

A paired-samples t-test was performed to evaluate the pain thresholds of the groups after 30th and 90th minutes. There was no significant decrease in response times in the control group at 30th and 90th minutes (p=0.68). However, in the vortioxetine groups, there was a significant decrease in reaction times from the 30th to the 90th minute (p<0.01) (Table 1).

A single-factor analysis of variance was performed between groups for the 30th and 90th-minute measurements to examine the effect of dose on pain threshold. There was a p<0.001 difference between doses at both the 30th minute (df=3, F=124.53), p<0.001 and the 90th minute (df=3, F=159.77) (Table 2).

The reaction times of both the 5 mg/kg, 10 mg/kg, and 20 mg/kg vortioxetine groups at 30th and 90th minutes were significantly less than the reaction times of the control group, which was given distilled water, according to posthoc comparisons performed applying Dunnett's t-test (p<0.001) (Table 3).

Table 1. 30th and 90th minute reaction times (t-test)

Group	Number of animals	Vortioxetine dose	30th minute reaction time (sec)	90th minute reaction time (sec) ± sd	P value
Control	10	0 mg/kg	24.85±1.14	24.13±1.18	0.68
Group 1	10	5 mg/kg	19.49±1.82	17.47±1.55	0.006
Group 2	10	10 mg/kg	16.92±1.58	14.32±1.78	0.003
Group 3	10	20 mg/kg	12.25±1.33	9.65±1.52	0.003
Mean			18.38±4.85	16.40±5.53	<0.001

sd, standard deviation; sec, second.

Table 2. Dose-reaction time relationship (one-way Anova)

		Sum of squares	Df	Mean square	F	P
30th minute	Between groups	828.66	3	276.22	124.53	<0.001
	In-group	79.85	36	2.22		
	Total	908.52	39			
90th minute	Between groups	1107.45	3	369.15	159.77	<0.001
	In-group	83.18	36	2.31		
	Total	1190.63	39			

Table 3. Comparison of the reaction times of the groups treated with vortioxetine and the control groups (Dunnett t-test).

Dependent variable	(I) dose	(J) dose	Mean difference	Standard error	P
30th minute	5	0	-5.36*	0.67	<0.001
	10	0	-7.93*	0.67	<0.001
	20	0	-12.61*	0.67	<0.001
90th minute	5	0	-6.66*	0.68	<0.001
	10	0	-9.81*	0.68	<0.001
	20	0	-14.48*	0.68	<0.001

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DISCUSSION

The hot-plate test was used in this study to explore the effect of vortioxetine on the nociceptive system. As a result of the study, vortioxetine decreased reaction time and increased pain sensitivity at 5 mg, 10 mg, and 20 mg.

In this study, which investigated the effect of vortioxetine on the nociceptive system, it was found that although vortioxetine was supposed to increase pain threshold, i.e., reaction time, on the contrary, it decreased pain threshold.

As far as we know, no study has used the hot-plate approach to assess the effect of vortioxetine on the nociceptive system. It has been observed that vortioxetine has an analgesic effect on chronic neuropathic pain induced by the chronic constriction method. In contrast, it does not affect neuropathic pain induced by inflammation (19). The difference in experimental animal models could explain the analgesic effect in this study and the increase in pain sensitivity in our study. Again, the hot-plate test is a model that can be used to study the differences in nociception between different mouse species (20). The difference in the effect of the two studies on pain could also be due to the difference in the species of mice used.

The $5HT_3$ subtype is the only ligand-gated cation channel with excitatory function among serotonin receptors. It is expressed in both the dorsal horn of the spinal cord and primary afferent neurons. It plays an essential role in the regulation of pain hypersensitivity by decreasing serotoninergic neurons (21). Furthermore, Guo et al. demonstrated that selective stimulation of the neuronal $5HT_3$ receptor causes hyperactivation of microglia and astrocytes, leading to spinal sensitization and pain hypersensitivity (22). However, some studies have also shown that pain is reduced by activating $5HT_3$ receptors (11). Vortioxetine's $5HT_3$ receptor antagonist effect may increase pain sensitivity and reduce reaction time.

The dorsal horn of the spinal cord contains a silent circuit of low-threshold afferent fibers and projection neurons that, when triggered, convert touch sensation into pain. Excitatory interneurons expressing the protein kinase C gamma (PKC-γ) isoform are essential for the function of this circuit (23). Because PKC-γ interneurons are inhibited by glycinergic and gamma-aminobutyric acidergic (GABAergic) interneurons, information transmitted in this pathway does not reach projection neurons in the more superficial dorsal horn (24). It was also discovered that blocking glycine receptors in animal experiments with intrathecal strychnine resulted in premature activation of projection neurons and, more critically, in mechanical hypersensitivity (25). Thus, touch can cause pain by activating or sensitizing a normally silent dorsal horn circuit containing PKC-γ interneurons. Vortioxetine suppresses inhibitory GABAergic neurotransmission by its potent antagonist effects at $5HT_3$ receptors, according to electrophysiological evidence (26). The possible efficacy of this mechanism may have raised pain sensitivity due to GABA suppression.

Pain sensitivity may be reduced due to impaired attention and decreased stress response (27). On the other hand, vortioxetine is a highly effective antidepressant for cognitive functions such as executive functions, processing speed, working, and episodic memory (28). The increased attention and stress response associated with these cognitive effects may have led to a reduction in reaction time in the experiment.

We believe that our study can be helpful in several areas. The mechanism of pain is not yet fully understood. Because vortioxetine reduces, enhances, or does not affect pain in different models, it may pave the way for new research because it may act through different receptors and pathways. If our study is supported by the results of animal experiments and clinical observational studies involving the depression and pain model, it may also serve as a guide for the choice of medication in cases where pain and depression coexist.

Our study has limitations. Only the hot-plate test was used in our research. However, unlike other models, the hot-plate test measures a more complex behavior consisting of lifting and licking a paw in response to acute heat, which necessitates neurological processing in the brain (17). Because vortioxetine can influence neurological processes within the brain, the hot-plate test was used in this research. The second limitation is that we used only one species of mice. Other species may give different results. Third, because we experimented on mice, it may not provide the same results in humans.

As a result, vortioxetine at doses of 5 mg, 10 mg, and 20 mg was found to decrease reaction time and thus increase pain sensitivity. However, further studies are needed to determine the mechanism of action of vortioxetine on nociception.

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