Original Article

The Analgezic Effect of Vortioxetine on somatic and visceral Pain using the mouse models

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Abstract

Aim: Experimental investigation of the vortioxetine effects on somatic and visceral nociception in mice.
Material and method: We used healthy, non-genetically modified white Swiss mice (20-25 g), randomly assigned in 3 groups of 6 animals each, treated orally 14 consecutive days, as follows: Group I (DW): distilled water 0,1 ml/10 g body weight, Group 2 (VRT): 20 mg/kbw vortioxetine. In the 14th day of the experiment the Group 3 (KET) received the positive control drug ketoprofen (15 mg/kbw), 15 minutes before the performing the tests. The somatic pain testing was performed using hot plate assay. The writhing test based on chemical peritoneal irritation induced by diluted acetic acid (0,6%) was used as standardized visceral pain model. The data were statistically analyzed using SPSS variant 17.0 for Windows and ANOVA one-way method. The research protocol was approved by the Grigore T. Popa University`s Committee for Research and Ethical Issues.
Results: Ketoprofen showed a substantial and progressive prolongation in reaction time in hot plate test, respectively a decrease in the behavioral manifestations in writhing test. The administration of vortioxetine resulted in a considerable increase in the time response to thermal noxious paws stimulation in hot plate test, and a diminution in the number of writhes in writhing test.
Conclusion: Using the mouse models of acute pain hot plate analgesia meter and acid acetic writhing test, we revealed that the oral administration of vortioxetine during 14 days resulted in significant somatic and visceral analgesic effects, but less intense than of ketoprofen.

Keywords: vortioxetine, ketoprofen, hot plate, writhing test, nociception, mice.

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INTRODUCTION

The 1-[2-(2,4-dimethyl-phenylsulfanyl)-phenyl]-piperazine, hydrobromide derivative vortioxetine (VRT), is a relatively new antidepressant and an atypical antipsychotic drug. It was approved for use in the treatment of depression in the fall of 2013 in the United States, at the end of the same year in the European Union, and few months later in Canada, South Africa, Australia, Mexico and South Korea [1].

Compared to other therapeutic agents for major depressive disorder, VRT has a unique mechanism of action with a distinct clinical profile, making it effective as a first-line alternative or subsequent therapy for patients with documented failure of other antidepressants [2,3]. It is thought to be a modulator and simulator of serotonin, because it has a multimodal mechanism of action to the neurotransmitter system of serotonin through which it simultaneously modulates one or more serotonin receptors and inhibits the serotonin reuptake. It has two types of actions: blocking the serotonin transporter and a strong affinity for several serotonergic receptors. Its combined action on the serotonin transporter and on four subtypes of serotonergic receptors increases the extracellular concentration of serotonin, dopamine and norepinephrine [4,5,6].

The actions of VRT on 5-HT receptors may be involved in increasing the levels of dopamine, norepinephrine, acetylcholine, and extracellular histamine evoked by the drug in the brains of rats, given the multiple and reciprocal interactions between these neurotransmitter systems. This mechanism may also explain

the higher increases in extracellular 5-HT produced by VRT compared to selective serotonin reuptake inhibitors at a certain occupancy of the serotonin transporter [7].

Experimental research shows that chronic VRT administration improved memory disorders induced by exposure of rats to various types of chronic stress, suggesting that it has beneficial effects in ameliorating cognitive impairments induced by stress associated with prefrontal cortex disorders [3,8,9,10].

Besides its antidepressant properties proved in several short-and long-term studies [11], VRT demonstrated pro-cognitive effects in preclinical studies, favorable influencing the learning and memory processes (enhancing hippocampal synaptic plasticity and augmenting the output of pyramidal cells) [12,13]. Moreover, different researches have shown that VRT significantly reduces the autonomic, affective, behavioral and motor disturbances in animal models of anxiety and experimental-induced seizure in rats [14,15], as well as it decreases clinical manifestations in patients with panic disorders [16,17].

Positive results on cognitive function (memory and executive functioning), mood disorders, anxiety, sleep disturbances and chronic neuropathic orofacial pain were also highlighted in clinical trials [5,18,19,20,21,22].

The **purpose** of our study was the experimental investigation of the vortioxetine effects on the somatic and visceral nociceptive sensitivity in mice.

MATERIAL AND METHODS

2.1. Substances

The used substances: vortioxetine, ketoprofen, saline solution and acetic acid were obtained from Sigma Chemical Co (Steinheim, Germany). Substances were dissolved in saline solution (0,9%), prepared immediately before use.

2.2. Animals

The experiments were performed using healthy, non-genetically modified white Swiss mice (20–30g) procured from the 'Cantacuzino' National Medical-Military Institute for Research and Development, Baneasa Station, Bucharest, Romania, and brought to the bio-base of the University of Medicine and Pharmacy 'Grigore T. Popa' Iasi, within the CEMEX ("Centre for Advanced Research and Development in Experimental Medicine") Laboratory.

The animals were housed in standard conditions of temperature (23±1°C), 12 hours light per day cycle (light period, 07:00-19:00), relative humidity of 45-55 % in special cages. Animals had water ad libitum and were maintained at 85% of their free feeding weight by controlling the quantity of their single daily meal following the experimental session during 45 minutes. Standard laboratory pellets of food and tap water were freely available, except during the time of the experiments.

Before the experimental investigations, mice were placed on a raised wire mesh, under a clear plastic box, and allowed 2 hours to acclimate to the laboratory environment.

2.3. Experimental protocol

Animals were randomly distributed in to three groups (n=6), treated orally (using an eso-gastric device), as follows:

Group I (DW): distilled water 0,1 ml/10 g body weight - 14 consecutive days,

Group 2 (VRT): 20 mg/kg body weight (kbw) vortioxetine - 14 consecutive days.

In the 14th day of the experiment, the Group 3 (KET) received oraly a single dose of 15 mg/kbw ketoprofen, 15 minutes before the performing of the nociceptive tests. This was used as a positive control agent, with recognized antinociceptive effects on both pain models.

2.3.1. Hot plate test

Hot plate test performs rapid and precise screening of analgesic drug properties on small-laboratory animals. The hot-plate test consists in a thermal pain measurement using the hot plate apparatus (Ugo-Basile, Gemonio, Italy). In this test, the animal is placed on a heated-plate (55+0.3^{o}C) to measure his thermal pain reflexes which are characterized either by withdrawal or by licking of the paw. This experimental model was used to determine the latency of nocifensive reactions evoked by thermal heat stimuli applied on the paw, in order to evaluate the central mechanism of analgesic activity [23]. The latency to first sign of hind paw licking or jump response to avoid thermal noxious paw stimulation was recorded. Briefly, each animal was placed in a restrainer, 2 min before treatment, and baseline reaction time was measured. The baseline latency (before drug injection) in the hot plate test was 4,2 ± 0.2 seconds (mean ± standard error of mean). The recommended cut-off time of 12

seconds was used to prevent tissue damage. Measurements of reaction time are given with a 0.01 s precision. Differences between the experimental and baseline latencies are interpreted as an index of analgesia.

In this experimental model, which evaluate the paw`s animal reactivity to thermal noxious stimulation, it was considered that: the increases in the latency for the mouse to avoid the thermal noxious paw stimulation are indicative of analgesia, while decreases of the latency response are indicative of hyperalgesia [23,22,25]. The latency time response (seconds) was measured before administration of any drug or vehicle (baseline) and 15, 30, 60, 90, 120 minutes after the substances administration. The latency time response from each group was calculated as mean ± standard deviation (S.D.) of mean.

Response latency values measurements were converted to per cent of maximum possible effect (% MPE) according to the formula:

% MPE = (Observed latency – Baseline latency) x 100 / (Cut off time – Baseline latency)

2.3.2. Writhing test

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice. This model of visceral pain consists of chemical peritoneal irritation induced by diluted acetic acid (0,6%) in volume of 0.1 ml/10g body weight [22,25]. The mice are placed individually into glass beakers and five minutes are allowed to elapse. The animals are observed and the behavioral manifestations were registered. Pain responses were scored by counting the number of stretches or writhes per animal, every 5 minutes, during 30 minutes period after intraperitoneal injection of diluted acetic acid. Stretches or writhes (arching of the back, development of tension in the abdominal muscles, elongation of the body and extension of the forelimbs) are viscero-somatic reflex responses to noxious peritoneal irritation [26,27].

For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Hand-opereted counters and stopwatches were employed to score writhing frequency of the mice placed in glass cages. All trials were video-taped and monitored by an observer unfamiliar with treatment condition. The tasks were performed between 8:00 and 13:00 a.m. Data for each measure were evaluated separately by analysis of variance for repeated determinations.

The percentage inhibition of writhing was calculated as follows:

% Inhibition = (1 - No. writhes in treated mice/ No. writhes in control mice) ×100

2.4. Statistical analysis of the results

Results from each test were reported as median with its corresponding confidence limits (95%) and were presented in graphs as means ± S.D. (standard deviation of mean, n=6) for six mice in a group. The values were processed using SPSS version 17.0 for Windows and one-way ANOVA method. P-values (probability) below =.05 were considered statistically significant versus control.

2.5. Ethical aspects

Experimental protocol of tests used, was approved by the Ethics Commission of University of Medicine and Pharmacy ‚Grigore T. Popa' from Iași (Certificate No. 25/14.07.2020), according to the National and International Standards [28,29]. Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical considerations all the animals were sacrificed at the end of the experiment [30].

RESULTS AND DISCUSSION

Using the standardized somatic pain model, hot plate test, we obtained information regarding the effects of VRT on the cutaneous nociceptive reactivity in mice.

The administration of KET (15 mg/kbw) resulted in a rapidly and statistically significant (**P<0.01) increasing of latency period of the response to thermal stimulus, effect maintained at all moments of time in hot plate test (Fig. 1). This observed effect was in concordance with the communicated literature data, regarding the effects of this analgesic-antipyretic drug in this experimental somatic pain model in rodents [31,32].

The treatment with VRT (20 mg/kbw) (**P<0.01) was associated with a significant reduction of the response to thermal noxious tail stimulation in each moment of the determination in hot plate test (Fig. 1). The effects intensity of VRT on the latency time response was inferior to KET in all moments of time determinations in this cutaneous nociceptive model in mice (Fig. 1).

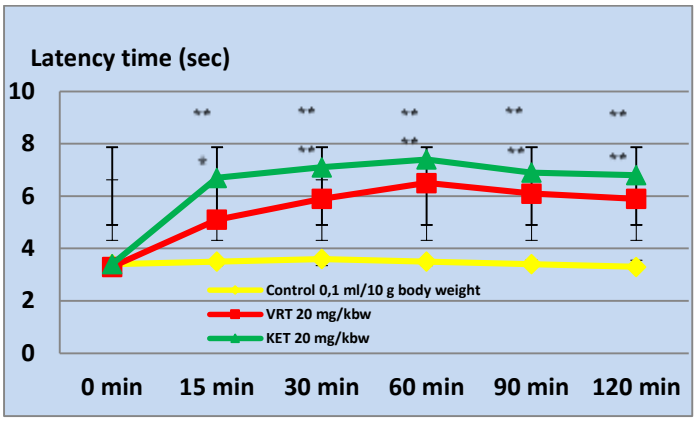

Fig. 1. The effects of VRT (20 mg/Kbw) in hot plate test in mice.

Test drugs: significant from normal control, * P < 0.05; ** P < 0.01.
Each point is the mean ± standard deviation (S.D.) of mean of latency time response (seconds) for six mice in a group.

The most intense antinociceptive effect was observed for KET after 60 minutes in the experiment (60.6±0.8%) (Fig. 2).

The maximum antinociceptive effects of the antidepressant agent was manifested at 60 minutes ($\%MPE_{60}$ of RBX = 47.8±1.3%), in this cutaneous pain model in mice (Fig. 2).

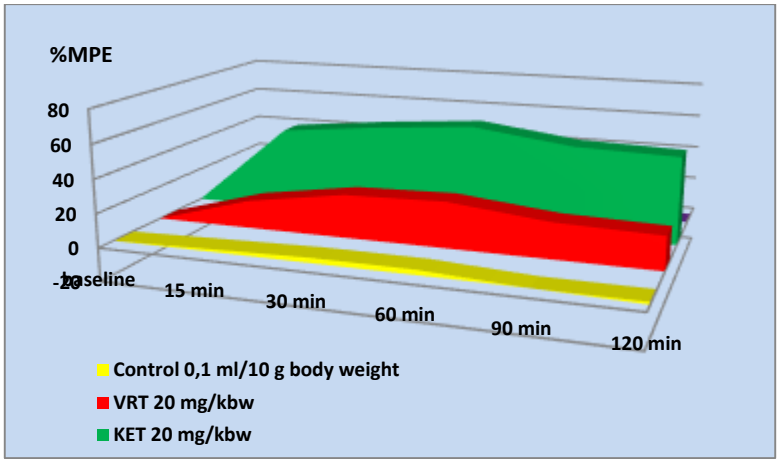

Fig. 2. The maximum possible effect of VRT (20 mg/Kbw) in hot plate test in mice.

Each point is the mean ± S.D. of maximum possible effect for six mice in a group.

Using the standardized visceral pain model, writhing test, we obtained information regarding the effects of the studied antidepressant drug VRT (20 mg/kbw) on the visceral nociceptive reactivity in mice.

The administration of KET (15 mg/kbw) resulted in a statistically significant (**P<0.01) rapid and persisting decrease of number of behavioral manifestations due to chemical noxious peritoneal irritation, effect maintained in all time intervals in this visceral experimental model (Fig. 3). The results are congruent with the data existent in the literature, regarding the effects of this analgesic-antipyretic drug writhing test in rodents [31,33,34].

Intraperitoneal administration of VRT 20 mg/kbw resulted in a reduction of the behavioral visceral manifestations, statistically significant comparing with control group in all moments of determination in writhing test (Fig. 3). The effects of VRT on the diminution of the number of writhes were less intense than those induced by KET, in all time intervals of the evaluation, in this visceral pain model in mice (Fig. 3).

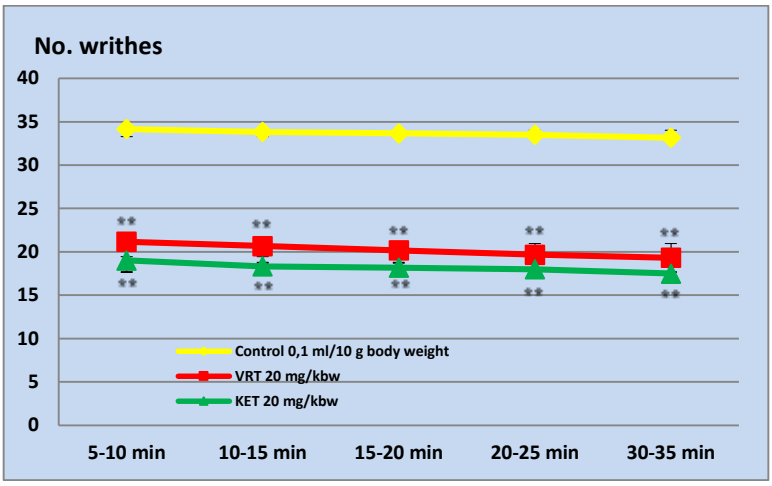

Fig. 3. The effects of VRT (20 mg/kbw) in writhing test in mice.

Test drugs: significant from normal control, ** $p < 0.01$.
Each point is the mean ± S.D. of writhes number for six mice in a group.

The biggest percentage of inhibition (48±0.3%) was observed for KET in the interval 25-30 minutes in this visceral pain model (Fig. 4).

The maximum percentage inhibition produced by VRT was achieved in the interval between 25 and 30 minutes (42±0.8%) in writhing test in mice (Fig. 4).

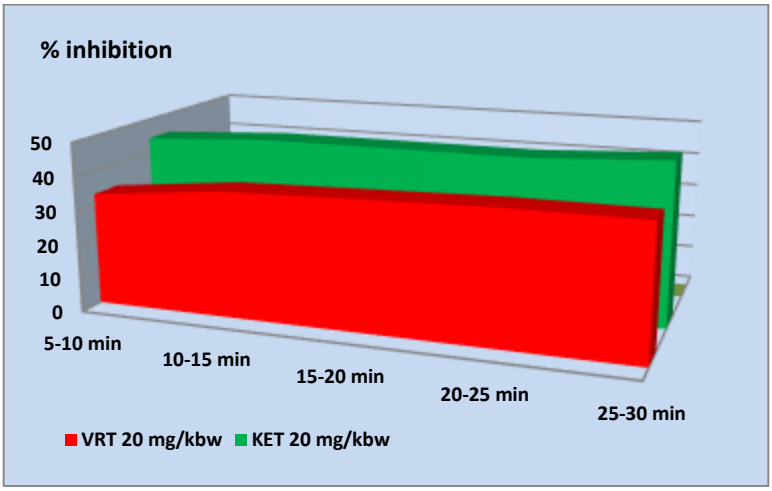

Fig. 4. The pain inhibition percent of VRT in writhing test in mice.

Each point is the mean ± S.D. of maximum possible effect for six mice in a group.

In our current study, the influence of VRT (2- mg/kbw) on the nociceptive reactivity, was explore in the both somatic and visceral pain models in mice, in comparison with the propionic acid derivative KET. In the hot plate test, animals received KET (15 mg/kbw) had substantially longer reaction time than mice treated with distilled water. Additionally, the MPE% displayed by the non-steroidal anti-inflammatory drug was also considerably superior compared to the control group, during the evaluation, with higher intensity noted after 60 minutes in the experiment. The use of VRT was accompanied by an analgesic activity, but less accentuated than of KET in this somatic nociceptive test in mice, effect objectified by the MPE% values, calculated for each moment of the determination in the experiment.

The writhing test was used in this research to evaluate the influence of VRT on the visceral nociception, compared with KET in the same time sessions in the experiment. The administration of KET was associated by an important diminution in the number of writhes produced after noxious chemical peritoneal irritation, exhibiting

a maximum pain inhibition percentage between 25 and 30 minutes. It was found that VRT agent increase pain threshold (with a maximum pain inhibition percentage noted in the interval 25-30 minutes), evidenced by the decreasing in the behavioral manifestation, but less intense that the effects of KET in this visceral pain model in mice.

Literature data revealed the analgesic efficacy of VRT in tail clip and tail immersion tests, the cutaneous pain models, recognized to be related with spinal transmission of painful sensitivity. These findings suggests that the antidepressant inhibit the nociceptive signals being involved in the mediation of the pain suppression central pathways [17,35, 36,37].

Recent researches revealed that VRT is also involved in the modulation of the glutamatergic and gamma-aminobutyric acid (GABA)ergic neurotransmission in the specific brain regions [38]. Moreover, animal studies demonstrated the antinociceptive effects of VRT in sciatic nerve constriction [39] and oxaliplatin-induced neuropathy [40] or reserpine-induced fibromyalgia in mice [41]. Some researches proved the antinociceptive effects of carrageenan-induced paw inflammation in mice [42], but others noted the lack of influence of this antidepressant in the inflammatory neuropathic pain [39]. The antinociceptive properties of VRT in neuropathic pain may be due to the antagonism of 5-HT3 receptors and the modulation of the 5-HT7 receptors, as well as due to its anti-inflammatory activity with favorable effects on the decreasing in neuro-inflammation associated with these types of chronic hyperalgesia [39,43].

We noted that the information about the involvement of this antidepressant agent in the mediation of pain reactivity are only a few and controversial [44], as well as the mechanisms of its antinociception is not yet completely deciphered.

Given its multimodal activity and the large number of affected receptors, as well as its multiple interconnections with other central nervous system neurotransmitter systems, we consider that extensive researches are needed to evaluate the effects of VRT in different animal pain models and on different species of laboratory animals.

CONCLUSION

Using the mouse models of acute pain hot plate analgesia meter and acid acetic writhing test, we revealed that the oral administration of VRT during 14 days resulted in significant somatic and visceral analgesic effects, but less intense than of KET.

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COMPETING INTERESTS

The authors declare no conflict of interest

AUTHORS' CONTRIBUTIONS

Conceptualization and methodology, Liliana Mititelu-Tartau, Paula Alina Fotache, Ana-Maria Pelin; Data analysis and investigation, Paula Alina Fotache, Beatrice Rozalina Buca; writing—original draft preparation, Paula Alina Fotache, Ana-Maria Pelin; writing—review and editing, Paula Alina Fotache, Ana-Maria Pelin, Beatrice Rozalina Buca; supervision and project administration, Liliana Mititelu-Tartau, Ana-Maria Pelin. All authors have read and agreed to the published version of the manuscript.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

The researches carried out followed the approval by the ethics commission of University of Medicine and Pharmacy ,Grigore T. Popa' from Iași (certificate no. 25/14.07.2020), in strict accordance with the international ethical regulations regarding animal experiments.

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