

THE ANTIHYPERALGIC EFFECT OF MEMANTINE IN A RAT MODEL OF PACLITAXEL INDUCED NEUROPATHIC PAIN

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Abstract

Chemotherapy-induced neuropathic pain continues to pose significant challenges to therapeutic attempts. Memantine, a N-methyl D-aspartate (NMDA) receptor blocker, is currently used in the treatment of patients with moderate to severe Alzheimer's disease. NMDA receptors are suspected to be involved in chronic pain pathogenesis by lowering sensitivity thresholds to mechanical and thermal stimuli. The aim of this study was to evaluate the analgesic effect of memantine in a paclitaxel-induced neuropathic pain model in rats. Sensitivity thresholds decreased for the paclitaxel treated group, and returned to normal values for the memantine treated ones. Results indicate antiallodynic and antihyperalgesic properties for paclitaxel induced neuropathic pain.

Rezumat

Durerea neuropată indusă de medicația antitumorală este în continuare greu de abordat terapeutic. Memantina, un blocant al receptorilor N-metil-D-aspartat (NMDA), este utilizată în tratamentul pacienților cu boală Alzheimer, de intensitate moderată până la severă. Receptorii NMDA pentru glutamat sunt suspectați a fi implicați în patogeneza durerii cronice prin diminuarea pragurilor de sensibilitate la stimuli mecanici și termici. Scopul acestui studiu este de a analiza efectul analgezic al memantinei într-un model de durere neuropată indusă de paclitaxel la șobolan. Pragul de sensibilitate la stimuli mecanici a scăzut pentru animalele tratate cu paclitaxel, fenomen atenuat de administrarea de memantină. Rezultatele arată potențialul antihiperalgic și antialodinic al memantinei în neuropatia periferică generată de paclitaxel, la șobolan.

Keywords: paclitaxel, neuropathic pain, memantine

Introduction

Paclitaxel, a chemotherapeutic drug from the taxane group, produced semi-synthetically from the European yew tree (*Taxus baccata*), is currently used for the treatment of breast cancer, cervical cancer, ovarian cancer and Kaposi sarcoma [9, 10]. Its antitumour activity is due to microtubule assembly promotion; as a consequence, paclitaxel treatment can lead to a dose-limiting side effect, painful peripheral neuropathy. Symptoms include numbness and tingling, followed by burning, shooting, throbbing and stabbing pain, primarily in the hands and feet, as reported by most patients [2]. Paclitaxel can potentially damage all peripheral nerves, although the large myelinated ones are more vulnerable, such as those innervating the extremities [1]. Taxane induced neuropathy (TIN) is currently unresponsive to normal analgesic treatment, and patients' options are most often restricted to a limited spectrum of drugs [5]. The mechanisms for TIN pathophysiology remain uncharted; among

several hypotheses being investigated, alteration of glutamate activity is also being explored. Glutamate is the main neurotransmitter present in the terminals of afferent nerves involved in pain signalling, and exaggerated activity in this transmission is registered in painful neuropathies [7]. Thus, knowing that NMDA receptors influence glutamate signalling, and are partially responsible for the development of hyperexcitability, the role of memantine in the treatment of paclitaxel-induced neuropathic pain should be significant.

Materials and Methods

Animals

Adult male Wistar rats (207 ± 45,65 g; N = 40) were supplied by the rodent farm of the University of Medicine and Pharmacy "Carol Davila" and housed in groups of eight on sawdust bedding in plexiglas cages, having free access to water. All animals were habituated to the testing environment. The temperature and relative humidity were continuously monitored

using an electronic hygro-thermometer. The recorded temperature was between 20°C and 22°C and the relative humidity was generally maintained at 40 - 70%. Experiments were carried out between 8:00 a.m. and 14:00 p.m. All the procedures were carried out in accordance to the bioethics norms concerning laboratory animal research, as stipulated by the 43/2014 law and the 2010/63/UE Directive from the European Parliament and 22 September Council about lab animal protection. The experimental protocol was approved by the Faculty of Pharmacy, "Carol Davila" Medicine and Pharmacy University, Bucharest, Ethics Committee.

Rat model of paclitaxel-induced peripheral neuropathy
Paclitaxel, 2 mg/kg bw: Paclitaxel, 2 mg/kg bw: Sindaxel® (Actavis) - 6 mg/mL paclitaxel in dehydrated citric acid, Cremophor El and dehydrated ethanol), was diluted with saline solution. Paclitaxel was injected intraperitoneally (i.p.) in a dose of 2 mg/kg bw once daily, for 4 consecutive days, following the method described by Flatters in 2006 and modified [6, 11].

Mechanical sensitivity

The mechanical sensitivity was assessed using a Dynamic Plantar Aesthesiometer (Ugo Basile, Italy), an automated version of the von Frey pain assessment filaments, as described in [3]. Animals were placed on an elevated wire mesh bottomed cage (22 x 16.5 x 14 cm) and responded to mechanical puncture stimulation. The unit raised a metal rod (0.5 mm in diameter) until it touched the plantar surface of the hind paw and began to exert an upwards force until the paw was withdrawn or the pre-set cut-off was reached. For the mechanical hyperalgesia, we measured the response to mechanical stimulation (force increasing rate: 4 g/s, cut-off force: 40 g, cut-off time: 90 s). One reading was taken on each paw at each time point. Each left and right hind paw was tested three times with a 3 - 5 min. interval between trials. We recorded the time of hind paw retraction upon stimulus application and the maximum force tolerated. The sensitivity to mechanical stimuli was evaluated before the treatment started and on days 4, 10, 17 and 24 [3].

The individual data are presented as the mean of six readings. The force and the time required to elicit a withdrawal response is measured, respectively, in grams and seconds.

As an expression of mechanical allodynia, previously mentioned [3], we measured the 50% response threshold using the up and down Dixon method, as mentioned by Chaplan *et al.* [4]. Using the same setting as indicated above, we applied the stimulus perpendicularly until the filament bent and we kept it in position for 6-8 seconds; brisk retraction of the

paw was a positive response, while absence of retraction was considered a negative response. Ambulation was deemed as an ambiguous response and required repetition of the application. A negative response required the use of the next stronger stimulus, and a positive response required the use of the next weaker stimulus, thus creating a sequence; this sequence is converted into the 50% response threshold.

Motor activity

The rats were individually inserted into the Activity Cage (Ugo Basile, Italy) and their movements were recorded both vertically and horizontally [8], for 3 minutes, with intermediate 1 minute checkpoints. Motor activity was evaluated before the treatment started, and on days 9, 16 and 23 [3].

Experimental protocol

After initial testing, animals were divided into five groups of 8 rats each, and were administered daily, for 24 days, as follows: Group 1: Control (C): distilled water 1 mL/100 g bw, p.o. 24 consecutive days and saline solution 0.1 mL/100 g bw, i.p., 4 consecutive days; Group 2: Paclitaxel (P): distilled water 1 mL/100 g bw, p.o. 24 consecutive days and paclitaxel 0.2 mg/kg bw, i.p., 4 consecutive days; Group 3: Memantine 10 mg/kg bw (M10): Memantine 10 mg/kg bw, p.o. 24 consecutive days and paclitaxel 0.2 mg/kg bw, i.p., 4 consecutive days; Group 4: Memantine 20 mg/kg bw (M20): Memantine 20 mg/kg bw, p.o. 24 consecutive days and paclitaxel 0.2 mg/kg bw, i.p., 4 consecutive days; Group 5: Memantine 30 mg/kg bw (M30): Memantine 30 mg/kg bw, p.o. 24 consecutive days and paclitaxel 0.2 mg/kg bw, i.p., 4 consecutive days.

Results and Discussion

Mechanical hyperalgesia

Results show a reduction of paw withdrawal time and also of maximum tolerated force for animals treated with paclitaxel, as compared to the control group. Mean values decreased from baseline levels (6.9 s, and 29.5 g) after the first 4 days of paclitaxel administration and reached their lowest levels on the 24th day (4.74 s and 19.2 g, $p < 0.01$). All doses of given memantine improved mechanical sensitivity throughout the administration period, although the most significant antihyperalgesic effect was obtained using 10 mg/kg bw, at the 17th measuring day (7.6 s and 29.7 g, $p < 0.01$ and $p < 0.001$, respectively) (Figure 1).

Treatment with 20 and 30 mg/kg bw of memantine improved the mechanical sensitivity, however, without resolving into being dose dependent.

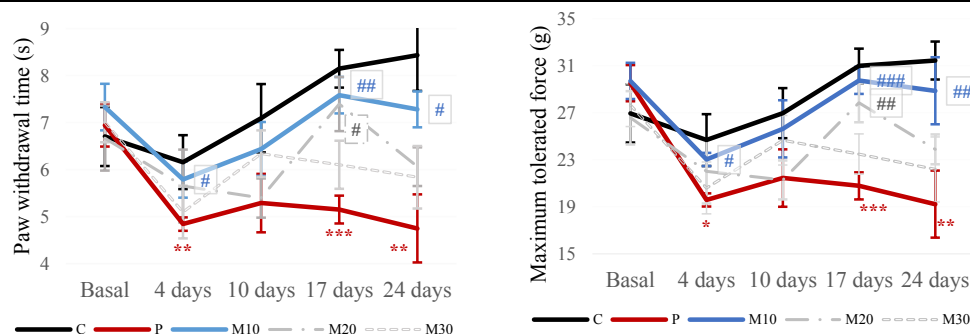


Figure 1.

The mean $\pm$ SEM of the paw withdrawal time (left-side graph) and maximum tolerated force (right-side graph), to mechanical stimulation with 4 g/s. Comparisons were performed using the unpaired t-test or Mann Whitney test in relation to the control group (C) * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ and # $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$ to the paclitaxel group (P).

Mechanical allodynia

Installation of mechanical allodynia was noticeable by the 10th day and statistically confirmed by the 17th day after the first paclitaxel dose. This is reflected by a continuous decrease of the 50% response threshold of the paclitaxel group following the application of von Frey hairs. The effect plateaued between the 17th and 24th day, when the difference from the control group could be quantified by a greater than 40% increase in sensitivity ($p < 0.01$). The 10 mg/kg bw of memantine was again the most effective by restoring the 50% response threshold to control values (9.15 g after 10 days, $p < 0.05$; 10.57 g after 17 days, $p < 0.01$; and 9.39 g after 24 days, $p < 0.05$). Also, giving 20 mg/kg bw of memantine also proved to be beneficial for reducing mechanical allodynia (with a maximum effect after 17 days: 50.9%, $p < 0.01$) (Figure 2).

In comparison to basal levels, the motor activity significantly decreased constantly in each group, but with no statistically significant differences between the 5 groups. This could most likely be explained by the animals' habituation to the testing environment; these results strongly suggest that

neither memantine, nor paclitaxel, affect motor activity in comparison to the control, and that the treatments do not significantly influence ambulation (Figure 3).

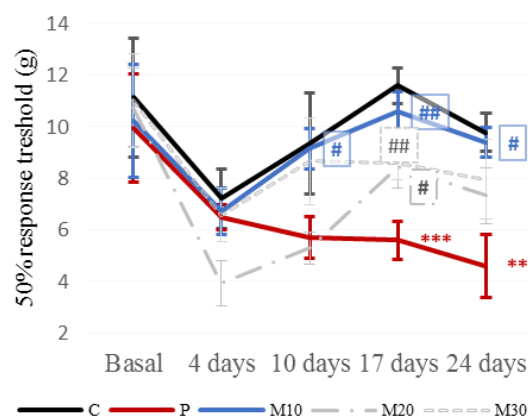


Figure 2.

The mean $\pm$ SEM of the 50% response threshold to von Frey hairs. Comparisons were performed using the Mann Whitney test in relation to the control group (C) ** $p < 0.01$; *** $p < 0.001$ and # $p < 0.05$; ## $p < 0.01$ to the paclitaxel group (P).

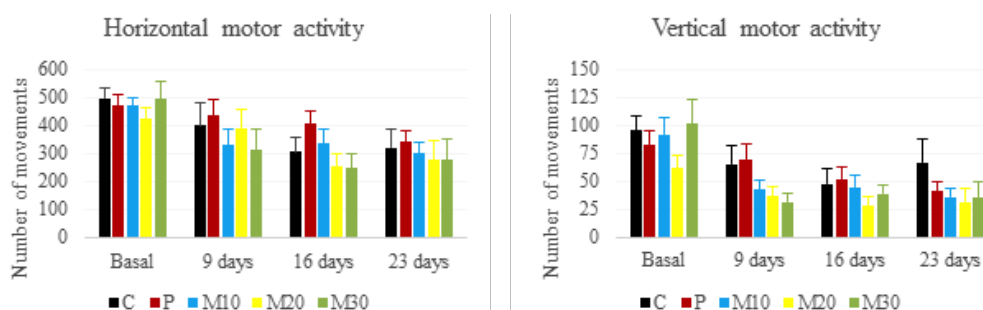


Figure 3.

Motor activity - horizontal and vertical (mean number of movements $\pm$ SEM) - for each of the 5 groups. Groups were compared using the one-way ANOVA or Kruskal-Wallis test, followed by the Tukey or Dunn's post-test, respectively.

Weight

The average weight for each group displayed a consistent tendency to increase, without a significant difference between groups at any given time point (Figure 4). As such, neither paclitaxel, nor memantine influenced weight gain, as expected.

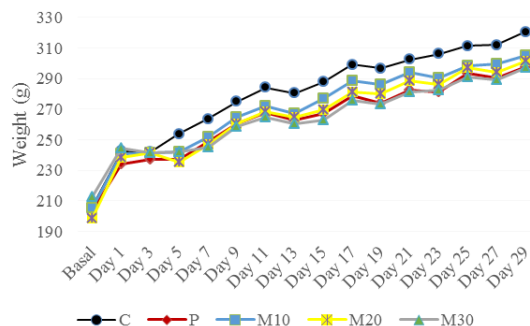


Figure 4.

Weight evolution for each of the 5 groups (Mean/group). Group comparisons were performed using one-way ANOVA test, followed by the Tukey post-test.

Conclusions

In this experiment, we provided evidence for the utility of memantine treatment in TIN, due to its antiallodynic and antihyperalgesic effects. Paclitaxel produced neuropathy symptoms in rats in our study, which were reduced, interestingly, mostly by the 10 mg/kg bw dose, and to a lesser extent, by the 20 mg/kg bw and 30 mg/kg bw doses. Thus, a concentration - effect relation could not be established. Moreover, the treatment did not show any decreases in the motor activity in comparison with the control or the paclitaxel group. Further research will target the combination of analgesic effect of drugs with complex or different mechanism.

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