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## INFLUENCE OF VORTIOXETINE AND PIOGLITAZONE ON NEUROPHYSIOLOGICAL MANIFESTATIONS IN A RAT MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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The purpose of the study was to evaluate the effects of vortioxetine and pioglitazone on the neurophysiological manifestations of chemotherapy-induced peripheral neuropathy in rats induced by paclitaxel, cisplatin, or their combination. The study investigated the neurophysiological manifestations of peripheral neuropathy induced by paclitaxel, cisplatin, or their combination, and evaluated the efficacy of vortioxetine (5 mg/kg) and pioglitazone (20 mg/kg) in their correction in a rat model. Von Frey (mechanical allodynia), hot plate (thermal hyperalgesia), and Rotarod (motor impairment) tests were conducted in 12 groups of animals (n=30) over 120 days. Chemotherapy induced an expressed allodynia and hyperalgesia, peaking at day 7. Prophylactic and therapeutic administration of vortioxetine and pioglitazone significantly reduced these manifestations by days 14–28. Motor impairments remained unaffected. The results suggest the potential of vortioxetine and pioglitazone for neuropathy correction.

**Key words:** peripheral neuropathy, paclitaxel, cisplatin, vortioxetine, pioglitazone, mechanical allodynia, thermal hyperalgesia, rats.

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## ВПЛИВ ВОРТІОКСЕТИНУ ТА ПІОГЛІТАЗОНУ НА НЕЙРОФІЗІОЛОГІЧНІ ПРОЯВИ ХІМІОТЕРАПЕВТИЧНО-ІНДУКОВАНОЇ ПЕРИФЕРІЙНОЇ НЕЙРОПАТІЇ В ЕКСПЕРИМЕНТІ НА ЩУРАХ

Метою дослідження було визначення впливу вортіоксетину та піоглітазону на нейрофізіологічні прояви периферичної нейропатії у щурів, які були спричинені хіміотерапією, викликаному введенням паклітакселу, цисплатини або їх комбінацій. Досліджено нейрофізіологічні прояви периферійної нейропатії, спричиненої паклітакселом, цисплатином або їх комбінацією, та оцінено ефективність вортіоксетину (5 мг/кг) і піоглітазону (20 мг/кг) у їх корекції на моделі щурів. Використано тести фон Фрея (механічна алодинія), гарячої пластинки (термічна гіпералгезія) та Rotarod (рухові порушення) у 12 групах тварин (n=30) протягом 120 днів. Хіміотерапія викликала виражену алодинію та гіпералгезію з піком на 7-у добу. Профілактичне та лікувальне застосування вортіоксетину й піоглітазону достовірно знижувало ці прояви на 14–28-у добу. Рухові порушення залишилися без змін. Результати свідчать про перспективність вортіоксетину та піоглітазону для корекції нейропатії.

**Ключові слова:** периферійна нейропатія, паклітаксел, цисплатин, вортіоксетин, піоглітазон, механічна алодинія, термічна гіпералгезія, щури.

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Chemotherapy-induced peripheral neuropathy (CIPN) is a serious complication of oncological therapy, affecting 30–70 % of patients receiving paclitaxel, cisplatin, or their combination [4]. CIPN manifests as sensory disturbances, such as mechanical allodynia (pain from non-noxious stimuli), thermal hyperalgesia (increased sensitivity to heat), paresthesia, and motor impairments, significantly impairing quality of life [4, 13]. Paclitaxel, a taxane that stabilizes microtubules, induces neurotoxicity through damage to sensory neurons, disruption of axonal transport, and neuroinflammation [15]. A platinum-based drug Cisplatin causes platinum accumulation in dorsal root ganglia, leading to oxidative stress and neuronal apoptosis [14]. The combination of these drugs exacerbates neurotoxicity, complicating treatment. Despite the prevalence of CIPN, current methods for its prevention and treatment remain limited, necessitating new pharmacological approaches.

Recent studies on CIPN focus on pathogenic mechanisms, such as microglial activation, release of pro-inflammatory cytokines (IL-1 $\beta$ , TNF- $\alpha$ ), and mitochondrial dysfunction [4]. Traditional drugs, such as gabapentin, pregabalin, and duloxetine, are used for symptomatic relief of CIPN, but their efficacy varies, and side effects (sedation, dizziness) limit their use [12]. Alternative approaches include the use of antioxidants, neuroprotectors, and anti-inflammatory agents that can modulate neuroinflammation and oxidative stress. For example, acetyl-L-carnitine and alpha-lipoic acid have shown moderate efficacy in

reducing sensory symptoms, but data remain controversial [2]. Vortioxetine, a multimodal antidepressant with serotonergic activity, has demonstrated neuroprotective properties in models of neurodegenerative disorders by reducing neuroinflammation and modulating serotonin receptors [12]. Pioglitazone, a PPAR- $\gamma$  agonist, reduces oxidative stress and inflammatory responses, which may be beneficial for CIPN correction [3]. Preliminary studies suggest the potential of these drugs in modulating CIPN pathogenic pathways, but their efficacy in preventing and treating neuropathy induced by paclitaxel and cisplatin requires further investigation.

CIPN studies in experimental rat models allow the assessment of neurophysiological changes using standardized tests, such as von Frey (mechanical allodynia), hot plate (thermal hyperalgesia), and Rotarod (motor impairments) [5, 12, 13]. These methods provide quantitative evaluation of sensory and motor impairments, reflecting clinical manifestations of CIPN in humans. Investigating new pharmacological agents, such as vortioxetine and pioglitazone, in these models may open new therapeutic perspectives for CIPN, particularly given their ability to target neuroinflammatory and oxidative mechanisms.

**The purpose** of the study was to evaluate the effects of vortioxetine and pioglitazone on the neurophysiological manifestations of chemotherapy-induced peripheral neuropathy in rats induced by paclitaxel, cisplatin, or their combination.

**Materials and methods.** The study was conducted on 360 male Wistar rats (weight 180–220 g), housed in standard vivarium conditions (temperature  $22\pm 2^\circ\text{C}$ , relative humidity 50–60 %, 12-hour light cycle) in plastic cages with free access to food (standard feed) and water. The experiment was conducted according to current bioethical principles (Directive 2010/63/EU). To reduce stress, animals underwent a 7-day adaptation to vivarium conditions before the experiment began. Euthanasia was performed by overdose of sodium thiopental (50 mg/kg, i.p.) after the experiment's completion.

Animals were randomized on 12 groups (n=30 per group):

1. Intact: No interventions, sample collection on days 1, 7, 14, 28, 60, 90, and 120.
2. Control (Paclitaxel, “Actavis”, Romania-Iceland) at 2 mg/kg i.p. every other day, 4 injections (total dose 8 mg/kg, diluted in 0.5 ml saline).
3. Control (Cisplatin, “Pfizer”, USA) at 2 mg/kg i.p. twice weekly for 4 weeks (total dose 16 mg/kg, diluted in 1 ml saline).
4. Control (combination): Cisplatin (2 mg/kg, i.p.) and paclitaxel (5 mg/kg, i.p.) via tail vein once weekly for 6 weeks (diluted in 0.5 ml saline).
- 5–6. Paclitaxel + vortioxetine (“Brintellix”, H. Lundbeck A/S, Denmark) at 5 mg/kg *per os* (diluted in 1 ml distilled water) 5 days before (group 5) or on day 3 after chemotherapy (group 6) for 14 days.
- 7–8. Cisplatin + pioglitazone (“Glutazon”, Berlin Chemie AG, Germany) at 20 mg/kg *per os* (diluted in 1 ml distilled water) 5 days before (group 7) or on day 3 after chemotherapy (group 8) for 14 days.
- 9–10. Cisplatin + vortioxetine: As per groups 5–6.
- 11–12. Paclitaxel + pioglitazone: As per groups 7–8.

Pain threshold (g) was measured using von Frey monofilaments (“Stoelting”, USA; force range 0.4–60 g, calibrated before each session) on the hind paws [4]. The test was performed three times with 5-minute intervals, using the “up-down” method to determine the 50 % threshold. Latency of pain response (s) was measured on a hot plate ( $52\pm 0.5^\circ\text{C}$ , “Ugo Basile”, Italy). Animals were placed on the plate, and the time to response (paw licking, jumping) was recorded. Maximum exposure time was 30 s to avoid burns. Time to fall (s) was assessed using the Rotarod test (“Ugo Basile”, Italy; speed 10–20 rpm). Animals underwent prior training (3 sessions of 180 s).

Analyses were conducted using SPSS 26.0. Data are presented as mean $\pm$ standard deviation. Normality was assessed using the Shapiro-Wilk test. Group comparisons were performed using the Student's t-test for pairwise comparisons and one-way ANOVA with Tukey's post-hoc test for multiple comparisons. A level of  $p < 0.05$  was considered significant.

**Results of the study and their discussion.** In control groups (2–4) receiving paclitaxel, cisplatin, or their combination, profound mechanical allodynia was observed, clinically manifested as increased sensitivity of the hind paws to light touch. As early as day 1 after chemotherapy, animals exhibited avoidance of contact with von Frey monofilaments, flinching, or paw withdrawal at minimal pressure. The peak of allodynia occurred on day 7, when animals responded to stimuli that normally did not elicit reactions, indicating significant sensory processing impairment (Table 1). Sensitivity remained high until day 14, but gradual recovery began by day 28, with complete normalization of the threshold observed only by day 120, suggesting the prolonged nature of neuropathic changes.

Table 1

**Influence of vortioxetine and pioglitazone on mechanical pain threshold in the von Frey test in rats with chemotherapy-induced peripheral neuropathy**

| Group   | The values of the mechanical pain threshold, g (M±σ) |             |             |             |             |             |
|---|--|-------------|-------------|-------------|-------------|-------------|
|   | Day 1  | Day 7       | Day 14      | Day 28      | Day 60      | Day 120     |
| Intact  | 56.12±6.89   | 56.12±6.89  | 56.12±6.89  | 56.12±6.89  | 56.12±6.89  | 56.12±6.89  |
| Paclitaxel (gr. 2)                            | 24.89±3.01*  | 16.78±2.12* | 20.45±2.89* | 27.56±4.23* | 45.67±7.12* | 69.34±10.78 |
| Cisplatin (gr. 3)                             | 27.34±3.45*  | 19.56±2.67* | 22.67±3.12* | 30.45±5.12* | 48.23±8.34* | 72.89±12.34 |
| Paclitaxel + vortioxetine (gr. 5, prevention) | 30.12±3.67*  | 25.45±3.01* | 34.56±3.45† | 55.23±7.89† | 60.12±8.45  | 70.45±11.23 |
| Cisplatin + pioglitazone (gr. 7, prevention)  | 31.23±3.89*  | 26.78±3.23* | 35.12±3.67† | 56.45±8.12† | 61.34±9.01  | 71.67±11.89 |

Notes. \* p<0.05 compared to the intact group; † p<0.05 compared to the respective control group.

Mice in experimental groups (5–12) demonstrated significantly lower levels of clinical manifestations of allodynia. For example, in group 5 (paclitaxel + vortioxetine, prophylaxis), animals showed reduced responses to stimuli on day 14, with fewer instances of paw withdrawal compared to control group 2. By day 28, animal behavior approached normal, with minimal signs of discomfort during testing. In group 7 (cisplatin + pioglitazone, prophylaxis), faster recovery of sensory function was noted, evidenced by stable contact with monofilaments without visible pain responses. By days 60 and 120, animals in experimental groups showed no behavioral differences from the intact group, indicating the efficacy in long-term allodynia correction of suggested treatment regimen.

In control groups (2–4), thermal hyperalgesia manifested as increased sensitivity to thermal stimuli, clinically expressed as rapid paw licking or jumping on the hot plate as early as day 1 after chemotherapy. The most significant manifestations were observed on day 7, when animals responded to the temperature much faster than the intact group, showing clear discomfort and anxious behavior (Table 2). Hyperalgesia persisted until day 14, impairing normal interaction with the surface. From day 28, sensitivity gradually decreased, but complete restoration of response latency occurred only by day 120, indicating prolonged sensory dysfunction.

Table 2

**Influence of vortioxetine and pioglitazone on reaction latency time in the hot plate test in rats with chemotherapy-induced peripheral neuropathy**

| Group   | The values of reaction latency time, s (M±σ) |             |             |             |             |            |
|---|--|-------------|-------------|-------------|-------------|------------|
|   | Day 1  | Day 7       | Day 14      | Day 28      | Day 60      | Day 120    |
| Intact  | 16.89±0.87                                   | 16.89±0.87  | 16.89±0.87  | 16.89±0.87  | 16.89±0.87  | 16.89±0.87 |
| Paclitaxel (gr. 2)                            | 11.89±1.12*                                  | 9.45±0.67*  | 9.89±0.78*  | 12.67±0.89* | 14.23±1.12* | 15.12±1.34 |
| Cisplatin (gr. 3)                             | 12.45±1.34*                                  | 10.01±0.89* | 10.45±0.97* | 13.12±1.12* | 15.01±1.45* | 16.78±1.78 |
| Paclitaxel + vortioxetine (gr. 5, prevention) | 13.67±1.45*                                  | 12.34±1.01* | 16.45±1.56† | 16.89±1.01  | 17.12±1.23  | 17.01±1.45 |
| Cisplatin + pioglitazone (gr. 7, prevention)  | 13.89±1.56*                                  | 12.67±1.12* | 16.67±1.67† | 17.01±1.12  | 17.34±1.45  | 17.23±1.56 |

Notes: \* – p<0.05 compared to the intact group; † – p<0.05 compared to the respective control group.

In experimental groups (5–12), vortioxetine and pioglitazone significantly attenuated thermal hyperalgesia. In group 5, animals showed calmer behavior on the hot plate by day 14, with prolonged response times compared to control group 2, indicating reduced pain sensitivity. By day 28, animal responses were close to normal, with no signs of anxiety or excessive sensitivity. In group 7, a reduction in hyperalgesia was noted by day 14, with more controlled movements and less visible pain responses. By days 60 and 120, animals in experimental groups exhibited no clinical signs of hyperalgesia, behaving similarly to the intact group, confirming the therapeutic effect of the suggested therapeutic intervention.

Motor impairments in control groups (2–4) based on the Rotarod test were minimal. Animals exhibited slight reductions in motor coordination, manifested as shortened time on the rotating rod, but these changes did not reach clinical significance. On days 1, 14, and 60, animals retained mobility, albeit with slightly greater difficulty compared to the intact group. By day 120, motor coordination in control groups was close to normal, with no pronounced signs of motor deficit.

The treatment in the experimental groups (5–12) had no effect on motor function. Animals in these groups, similar to controls, showed slight reductions in time on the Rotarod, but without clinically

noticeable impairments in gait or coordination. For example, in group 5 on days 14 and 60, animals behaved similarly to control group 2, with no visible changes in mobility. By day 120, motor performance in experimental groups was comparable to the intact group, indicating no effect of the drugs on motor function.

The results confirm the high neurotoxicity of paclitaxel, cisplatin, and their combination, manifested by pronounced mechanical allodynia and thermal hyperalgesia peaking on day 7. Vortioxetine and pioglitazone significantly reduced sensory impairments, likely due to their ability to modulate neuroinflammation and oxidative stress. For instance, Micov et al. (2020) demonstrated that vortioxetine reduces microglial activation in neuropathy models [9], while Khasabova et al. (2019) found that pioglitazone suppresses NF- $\kappa$ B expression, reducing inflammatory responses [7].

Compared to other drugs used for CIPN correction, vortioxetine and pioglitazone demonstrate competitive efficacy. Gabapentin, commonly used for neuropathic pain relief, reduces allodynia by 30–50 % in CIPN models but is associated with sedative effects and cognitive impairment [10]. Serotonin-norepinephrine reuptake inhibitor, particularly duloxetine, show efficacy in 40–60 % of cases but may cause nausea and dizziness [8]. Pregabalin is effective in 50–70 % of cases but is associated with drowsiness and dependency risk [6]. In contrast, vortioxetine at 5 mg/kg in this study reduced allodynia by 53.94 % and hyperalgesia by 62.55 % by day 14 without sedative effects, possibly due to its serotonergic action and neuroinflammation modulation. Pioglitazone (20 mg/kg) reduced allodynia by 45.61 % and hyperalgesia by 59.62 %, attributed to its anti-inflammatory and antioxidant properties. Unlike gabapentin and pregabalin, vortioxetine and pioglitazone did not affect motor function, which is advantageous for CIPN patients requiring preserved physical activity.

Limitations of the study include the use of fixed doses of vortioxetine (5 mg/kg) and pioglitazone (20 mg/kg) and the experiment duration (120 days), necessitating further investigation of optimal dosing regimens and long-term effects. The lack of effect on motor impairments suggests selective action on sensory neurons, consistent with data on their limited impact on motor functions. Clinical application prospects include developing combination strategies with vortioxetine and pioglitazone to enhance CIPN correction, particularly for patients with resistant neuropathy forms.

## Conclusions

1. Paclitaxel, cisplatin, and their combination reproducibly induced chemotherapy-induced peripheral neuropathy in rats, manifested as profound mechanical allodynia and thermal hyperalgesia with a peak of sensory dysfunction on day 7. These disturbances persisted for several weeks, with gradual recovery only by day 120, confirming the chronic and long-lasting nature of CIPN.

2. Preventive and therapeutic administration of vortioxetine (5 mg/kg) and pioglitazone (20 mg/kg) significantly alleviated sensory manifestations of neuropathy. By day 14, vortioxetine reduced mechanical allodynia by 53.94 % and thermal hyperalgesia by 62.55 %, while pioglitazone achieved reductions of 45.61 % and 59.62 %, respectively, compared to untreated controls.

3. Both pharmacological interventions facilitated earlier normalization of sensory thresholds: animals receiving vortioxetine or pioglitazone demonstrated near-baseline responses already by days 28–60, while untreated controls required up to 120 days for full recovery. This highlights the modulatory effects of these drugs on neuroinflammatory and oxidative stress pathways underlying CIPN.

4. Motor coordination assessed by the Rotarod test showed only minimal disturbances after chemotherapy, and neither vortioxetine nor pioglitazone exerted any additional effects on locomotor function. Preservation of motor activity without sedative or motor-suppressive actions is an important advantage over classical symptomatic drugs such as gabapentin or pregabalin.

*Prospects for further research include a detailed study of the pathogenetic mechanisms of nervous system both peripheral and central parts damage after chemotherapy, clarification their pathomorphological correlates and the development of an algorithm of pharmacocorrection and prevention.*

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