

THE EFFECT OF NIMODIPINE ON A RAT MODEL OF PACLITAXEL – INDUCED PERIPHERAL NEUROPATHY

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Manuscript received: October 2015

Abstract

Patients undergoing chemotherapy often exhibit painful peripheral neuropathy as a side effect. Most neurons, including sensory neurons [6] express multiple types of voltage-gated calcium channels (VGCC), thus suggesting a modulatory role of VGCC in pain transmission. We evaluated the effect of nimodipine, a L-type calcium channel blocker, on a paclitaxel-induced peripheral neuropathy model in rats. We used male Wistar rats (N = 60), divided into 6 equal groups: control (C), paclitaxel (P), paclitaxel and nimodipine 30, 45 and 60 mg/kgbw (N30, N45 and N60), and paclitaxel and gabapentine (G). We assessed the mechanical sensitivity for hyperalgesia and allodynia conditions, and also recorded the motor activity. Starting from day 10 of the treatment, paclitaxel induced statistically different decreases in sensitivity to mechanical stimuli, effect neutralized in the nimodipine and gabapentine groups. The registered results indicate nimodipine's effectiveness in reversing neuropathic hyperalgesia and allodynia produced by paclitaxel.

Rezumat

Pacienții tratați cu medicamente antitumorale raportează des ca efect secundar neuropatii periferice dureroase. Majoritatea neuronilor, inclusiv neuronii senzoriali [6] exprimă canale de calciu voltaj dependente, sugerând un rol important al acestora în transmisia durerii. Am evaluat efectul nimodipinei, blocant al canalelor de calciu de tip L, asupra neuropatiei periferice induse de paclitaxel la șobolan. Am utilizat șobolani masculi albi, sușa Wistar (N = 60), grupați în 6 loturi egale, control (C), paclitaxel (P), paclitaxel și nimodipină 30, 45 și 60 mg/kgc (N30, N45 și N60), paclitaxel și gabapentină (G). Am evaluat activitatea motorie și sensibilitatea la stimuli mecanici pentru condițiile de hiperalgezie și alodinie. Din ziua 10 de tratament, paclitaxel a scăzut semnificativ statistic pragul de sensibilitate la stimuli mecanici, scădere neutralizată în loturile tratate cu nimodipină și gabapentin. Rezultatele arată eficacitatea nimodipinei în reducerea hiperalgeziei și alodinie generate de paclitaxel, la șobolan.

Keywords: paclitaxel, von Frey, nimodipine, gabapentine

Introduction

Pain is a survival mechanism that serves as a warning sign of ongoing or impending tissue damage. The NeuPSIG (Special Interest Group on Neuropathic Pain) defines neuropathic pain as “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”, and contrary to nociceptive pain, it seems to have no beneficial effect. This common type of pain is generally underdiagnosed and undertreated, and suffering disability and impaired quality of life can be correlated with it. Chemotherapy-induced peripheral neuropathy is frequently noted as a side effect of chemotherapeutic agents, more often those which target microtubules, such as platinum salts, vinca alkaloids (vinorelbine and vincristine) and taxanes (paclitaxel and docetaxel) [4]. Taxanes are frequently used as treatment for one of the most

widespread types of tumours, namely breast cancer [18], and more than 80% of patients undergoing taxane therapy develop neuropathies, sometimes leading to its early discontinuation [8, 19]. They can also damage axons, the myelin sheath and dorsal root ganglions, they can stabilize microtubules and disrupt mitosis and intracellular transport between axons and cells [1], thus leading to apoptosis.

Taxane induced neuropathy (TIN) is a distal sensory neuropathy characterized by the stock and glove distribution. The symptoms begin symmetrically from the toes, due to their tips being innervated with the longest nerves. The continuation of treatment leads the neuropathy towards the ankles and lower legs and also to the wrists and arms [20].

Among the most commonly reported symptoms, the patients report paraesthesia, dysesthesia, numbness, electric shock-like sensation, motor impairment,

altered proprioception, temperature perception, and real neuropathic pain, severe in 25% of the cases [1, 3, 20]. Although taxanes can damage all the peripheral nerves, the large myelinated ones are the most vulnerable [1]. The symptoms may appear during therapy and can last up to 48 months after treatment cessation, or they may become chronic, without any signs of recovery [16]. Also, an acute pain syndrome following a week of paclitaxel therapy has been characterized by severe pain in the back, hips, shoulders, thighs and legs [12].

The mechanisms for TIN pathophysiology are still unknown, although some hypotheses have been investigated in animal models [14, 24]. In peripheral pain transmission, a number of different ion channels are required, such as Na^+ channels, K^+ channels and voltage-gated calcium channels (VGCC), which are the most important regulators of calcium concentration in neurons.

Therefore, they are paramount to nociception and neuropathic pain; paclitaxel increases VGCC currents in rat dorsal root ganglion neurons, proving its important role in paclitaxel-induced mechanical hyperalgesia. Also, the administration of gabapentin, an antiepileptic drug that acts as a VGCC blocker, diminishes this symptom [9].

Other potential mechanisms include the interaction with the TLR4 (Toll like Receptor) [21], the interference with the fast axonal transport [11], the activation of glial cells in the spinal cord [15], changes in the expression of glutamate receptors [22], and the increase of P substance release [13].

Nimodipine has been shown to be effective in reducing neuropathy symptoms in some cases, such as in the cisplatin-induced peripheral neuropathy [7], or to enhance pain alleviation when co-administered with morphine in heat sensitivity tests [10], so it is a good candidate for regulating the mechanical sensitivity in other neuropathies such as the one consecutive to paclitaxel treatment.

Materials and Methods

Animals

Adult male Wistar rats (288 ± 40.8 g; $N = 60$) were supplied by the rodent farm of the "Carol Davila" University of Medicine and Pharmacy and housed in groups of twelve on sawdust bedding in plexiglass cages, having free access to food and water. Experiments were carried out between 8:00 a.m. and 14:00 a.m. 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%. All the procedures were carried out in accordance to the bioethics rules 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 regarding lab animal protection. The study protocol was approved by the University Ethics Committee.

Rat model of paclitaxel-induced peripheral neuropathy

Paclitaxel, 2 mg/kgbw: Paclitaxel, 2 mg/kgbw: 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/kgbw once daily, for 4 consecutive days, following the method described by Flatters 2006 and modified [5, 23].

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. 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 assessments. 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.

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, we measured the 50% response threshold using the up and down Dixon method, as mentioned by Chaplan *et al.* [2]. Using the same setting as mentioned above, we applied the stimulus perpendicularly until the filament bent and we kept it in position for 6 - 8 seconds; a positive response was the brisk retraction of the paw, and the absence of retraction was considered a negative response. Walking 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 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 [17], for 3 minutes, with intermediate 1 minute checkpoints. Motor activity was evaluated before the treatment started, and on days 9, 16 and 23.

Experimental protocol

After initial testing, animals were divided into six groups of 10 rats each, and were administered daily, for 24 days, the following: Group 1: Control (C): distilled water 1 mL/100 gbw, p.o., 24 consecutive days and saline solution 0.1 mL/100 gbw, i.p., 4 consecutive days; Group 2: Paclitaxel (P): distilled water 1 mL/100 gbw, p.o., 24 consecutive days and paclitaxel 0.2 mg/kgbw, i.p., 4 consecutive days; Group 3: Nimodipine 30 mg/kgbw (N30): Nimodipine 30 mg/kgbw, p.o., 24 consecutive days and paclitaxel 0.2 mg/kgbw, i.p., 4 consecutive days; Group 4: Nimodipine 45 mg/kgbw (N45): Nimodipine 45 mg/kgbw, p.o., 24 consecutive days and paclitaxel 0.2 mg/kgbw, i.p., 4 consecutive days; Group 5: Nimodipine 60 mg/kgbw (N60): Nimodipine 60 mg/kgbw, p.o., 24 consecutive days and paclitaxel 0.2 mg/kgbw, i.p., 4 consecutive days; Group 6: Gabapentine (G): Gabapentine 300 mg/kgbw, p.o., 24 consecutive days and paclitaxel 0.2 mg/kgbw, i.p., 4 consecutive days.

Results and Discussion

Mechanical hyperalgesia

Taking into account that hyperalgesia induced by antineoplastic drugs reduces the time to response to the mechanical stimuli, the experimental results shown in Figure 1 underline the increase of pain sensitivity following paclitaxel administration.

Taxanes reduce both the paw retraction time and also the maximum tolerated force; the presented experimental results underline a progressive increase of tactile pain sensitivity following paclitaxel administration, together with a statistically significant reduction in the same sensitivity as nimodipine is administered. Nimodipine treatment, in all doses given, has proved to have at any given time an analgesic effect in the mechanical hyperalgesia induced by paclitaxel (Figure 1). It seems to have an effect which improves over time, reaching the maximum after 24 days: hyperalgesia time N30: 33.11% $p < 0.001$, N45: 34.52% $p = 0.0015$, N60: 23.97% $p = 0.0024$, hyperalgesia force N30: 27.79% $p < 0.001$, N45: 32.86% $p = 0.0019$, N60: 21.19% $p = 0.0044$.

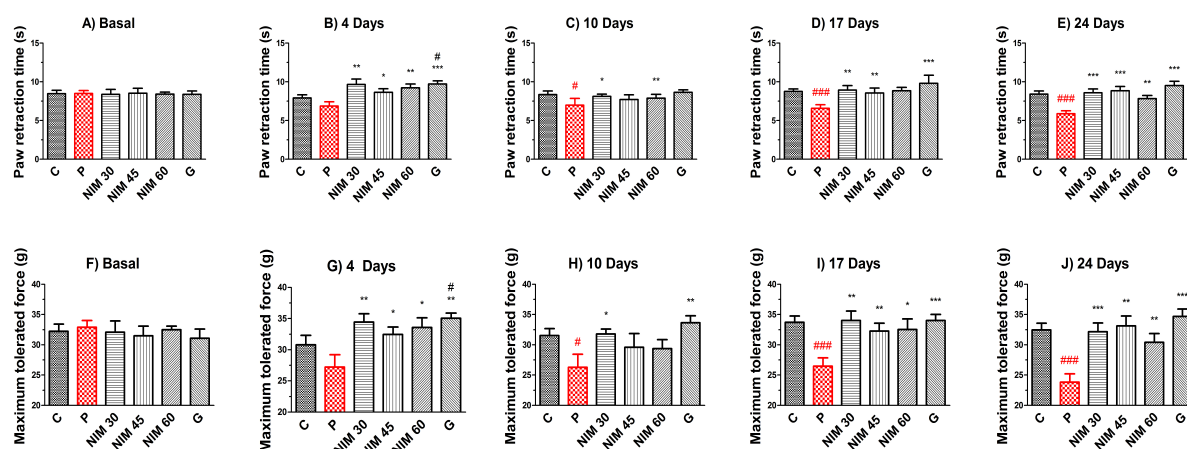


Figure 1.

The paw withdrawal time (A, B, C, D, E), presented as mean $\pm$ SEM, and maximum tolerated force (F, G, H, I, J), to mechanical stimulation 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 to the paclitaxel group (P); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Nimodipine treatment, in all used doses, has proved at any given time an analgesic effect in the mechanical hyperalgesia induced by paclitaxel (Figure 1). Also, there are no statistically significant differences between the nimodipine groups and the gabapentine group, used here as a positive reference. Allodynia seems to have an earlier onset, at the 4 day point after the first paclitaxel dose. Nimodipine counteracts the increase in sensitivity, with a moderate

but significant effect after 10 days (N30: 25.87% not significant, N45: 51.23% $p < 0.05$, N60: 14.66% not significant), and a definite, strong effect after 17 days of treatment, as compared to the paclitaxel group (N30: 55.67% $p = 0.0015$, N45: 89.87% $p < 0.001$, N60: 56.14% $p < 0.001$) (Figure 2). However, gabapentine is significantly more effective in this case (10 days 75.53% $p < 0.001$; 17 days 103.94% $p < 0.001$).

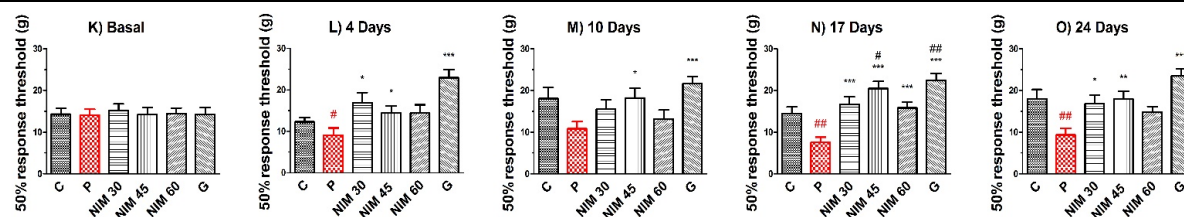


Figure 2.

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

The motor activity significantly decreased constantly in each group, in comparison to basal levels, but with no statistical significant differences between the 6 groups. This would probably be

explained by the animals' habituation to the experimental conditions; these results strongly suggest that neither nimodipine, nor paclitaxel, affect motor activity in comparison to the control.

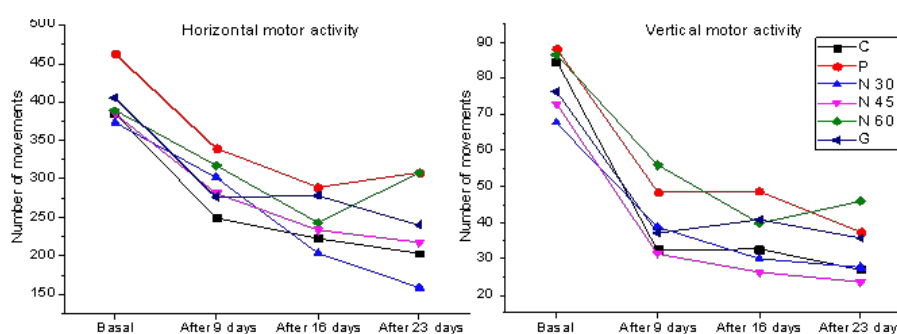


Figure 3.

Motor activity - horizontal and vertical (mean number of movements) - for each of the 6 groups. Group comparisons were performed using unpaired t test, or Mann Whitney test, in comparison to the paclitaxel group.

The average weight for each group displayed a subtle tendency to increase, without a significant difference between groups at any given time point, excepting the gabapentine group which had significantly lower weights during most of the treatment duration (-0.95 to +6.71% in comparison to the control) (Figure 4).

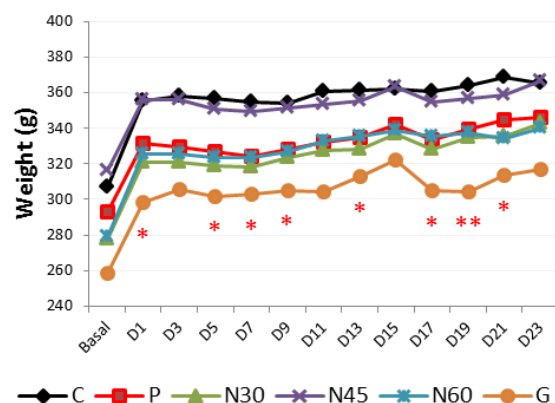


Figure 4.

Weight evolution for each of the 6 groups (M/group). Group comparisons were performed using one way ANOVA test, followed by the Tukey post-test, in comparison to the control group (* $p < 0.05$, ** $p < 0.01$).

Conclusions

These results highlight that paclitaxel induces a peripheral neuropathy characterized by hyperalgesia and allodynia and that the voltage gated calcium channel blocker nimodipine reduces these symptoms. The 30 mg/kgbw dose seems to be more effective in reducing hyperalgesia, while at 45 mg/kgbw it reduces allodynia. Moreover, the treatment did not show any decreases in 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 mechanisms of action.

Acknowledgement

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed by the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/137390.

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