Pharmacological characterization of paclitaxel-induced neuropathic pain in rats

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Introduction

- Peripheral neuropathic pain is a well-documented side effect of chemotherapy in 25-50% of cancer patients undergoing treatment.
- Paclitaxel (Taxol®) is a widely-used anticancer agent for treatment of ovarian, breast, lung, head, and neck cancer. There are two well-documented side effects of this treatment: myelosuppression and peripheral sensory neurotoxicity. These side effects often necessitate the use of suboptimal doses (dose-limiting therapy), or even a complete suspension of treatment.
- In patients, paclitaxel-induced peripheral neuropathy is characterized by degeneration of sensory axons and is clinically manifested as numbness, pain, and thermohyperesthesia in hands and feet.
- This poses a growing, significant clinical problem as the average life span improves and cancer becomes more prevalent. Additionally, in some cases this neuropathy develops into a chronic problem even after cessation of treatment.
- In rodents, administration of paclitaxel causes neuropathic pain that is comprised of thermal hyperalgesia and mechanical allodynia, which can be reversed by different analgesic agents.

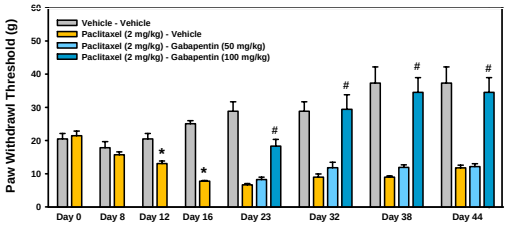
Hypothesis

Paclitaxel-induced neuropathic pain in rats can be attenuated by gabapentin, antidepressants, and opiates.

Methods

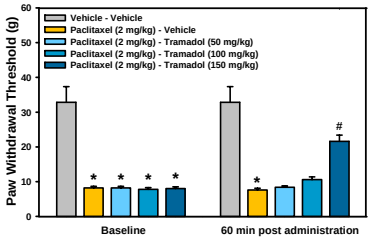
- Animals: Male Sprague Dawley rats from Harlan Laboratories were used in these studies. Upon receipt, rats were assigned unique identification numbers (tail marked) and group housed with 3 rats per cage in polycarbonate cages with micro-isolator filter tops. All rats were examined, handled, and weighed prior to initiation of the study to assure adequate health and suitability. During the course of the study, 12/12 light/dark cycles were maintained. The room temperature was maintained between 20 and 23ºC with a relative humidity maintained around 50%. Chow and water were provided *ad libitum* for the duration of the study. In each test, animals were randomly assigned across treatment groups. Animals were not disturbed between test days.
- Treatment: Rats received an intraperitoneal injection of paclitaxel (2 mg/kg) on days 0, 2, 4 and 6. Paclitaxel-induced neuropathy was assessed using von Frey filaments. The following compounds were assessed, with all compounds administered at an injection volume of 1ml/kg: gabapentin (50 and 100 mg/kg) was dissolved in 0.5% carboxymethylcellulose (CMC) and administered i.p. 30 minutes prior to testing. Tramadol (50, 100, and 150 mg/kg) was dissolved in 0.5% CMC and administered p.o. 60 minutes prior to testing. Morphine (2, 5, and 10 mg/kg; i.p.), duloxetine (3, 10, and 30 mg/kg; p.o.), and amitriptyline (3, 10, and 30 mg/kg; p.o.) were all dissolved in sterile injectable saline and administered 60 minutes prior to testing.
- Von Frey Testing: Withdrawal from a mechanical stimulus was measured by applying von Frey (VF) filaments of ascending bending force to the plantar surface of the hind paws (ipsilateral and contralateral). A positive response was defined as withdrawal from the von Frey filament. Confirmation of the paw withdrawal threshold (PWT) was tested by assessing the response to the filament above and below the withdrawal response. Rats were brought to the experimental room and allowed to habituate in the room for one hour prior to testing, and acclimated to the observation chambers for 15 minutes prior to taking all PWT measurements.
- Baseline responses: Baseline PWT responses were measured prior to paclitaxel or test compound administration. Rats were subsequently balanced and assigned to treatment groups based on baseline PWT values of left and right hind paws between washouts.
- Statistical Analysis: Left and right von Frey data were averaged together and analyzed by analysis of variance (ANOVA) followed by Fisher PLSD post-hoc comparisons. An effect was considered significant if p<0.05. Data are presented as the mean standard error of the mean (S.E.M.).

Figure 1: Gabapentin increases PWT in paclitaxel-treated rats



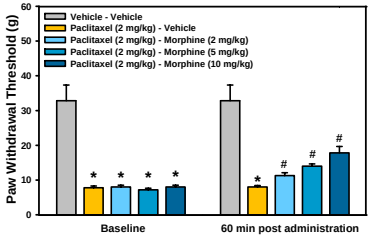
*Paclitaxel-induced neuropathy started day 12 after injection and continued throughout the study. Acute administration of gabapentin on day 23 reversed paclitaxel-induced neuropathy. The effect of gabapentin persisted with chronic administration up to day 44 when the study ended. Data represents mean ± SEM. *p<0.05 vs. Vehicle-Vehicle; #p<0.05 vs. Paclitaxel-Vehicle*

Figure 2: Tramadol increases PWT in paclitaxel-treated rats



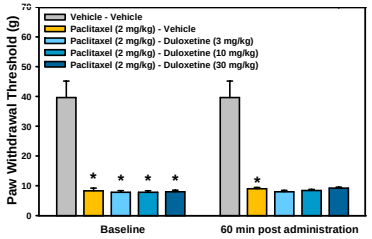
*On day 16 post-paclitaxel administration, rats were administered either vehicle or tramadol (p.o.) and PWT was assessed 60 min following injection. Data represents mean ± SEM. *p<0.05 vs. Vehicle-Vehicle; #p<0.05 vs. Paclitaxel-Vehicle*

Figure 3: Morphine increases PWT in paclitaxel-treated rats



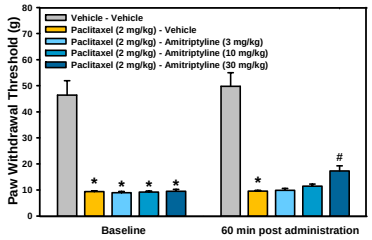
*On day 20 post-paclitaxel administration, rats were administered either vehicle or morphine (i.p.) and PWT was assessed 60 min following injection. Data represents mean ± SEM. *p<0.05 vs. Vehicle-Vehicle; #p<0.05 vs. Paclitaxel-Vehicle*

Figure 4: Duloxetine does not reverse paclitaxel-induced neuropathy in rats



*On day 22 post-paclitaxel administration, rats were administered vehicle or the serotonin-norepinephrine reuptake inhibitor duloxetine (p.o.) and PWT was assessed 60 min following injection. Data represents mean ± SEM. *p<0.05 vs. Vehicle-Vehicle*

Figure 5: Amitriptyline increases PWT in paclitaxel-treated rats



*On day 27 post-paclitaxel administration, rats were administered vehicle or the tricyclic antidepressant amitriptyline (p.o.) and PWT was assessed 60 min following injection. Data represents mean ± SEM. *p<0.05 vs. Vehicle-Vehicle; #p<0.05 vs. Paclitaxel-Vehicle*

Table 1: Summary of drugs tested in these studies

Drug	Dose (mg/kg)	Route	Vehicle	Pretreat (min)
Analgesic				
Gabapentin	50, 100	p.o.	0.5% CMC	30
Opiate Analgesics				
Tramadol	50, 100, 150	p.o.	0.5% CMC	60
Morphine	2, 5, 10	i.p.	saline	60
Antidepressants				
Amitriptyline	3, 10, 30	p.o.	saline	60
Duloxetine	3, 10, 30	p.o.	saline	60

On test days, baseline PWT was assessed. Following pretreatment with test compound, PWT was again assessed.

Summary

- In the present studies, we evaluated various analgesics and antidepressants on mechanical hyperalgesia induced by paclitaxel.
- In SD rats, administration of paclitaxel (2 mg/kg, i.p.) decreased PWT as assessed by VF filaments with an onset of neuropathy on day 12. Both acute and chronic administration of gabapentin (100mg/kg, p.o.) reversed paclitaxel-induced neuropathy.
- Acute administration of opiates such as morphine and tramadol were also effective in reversing paclitaxel-induced neuropathy. It should be noted that unlike gabapentin, neither morphine nor tramadol showed a full reversal.
- As clinical studies have shown, antidepressants may also assist in alleviating chemotherapy-induced neuropathy. In these studies we evaluated two classes of antidepressants: the serotonin-norepinephrine reuptake inhibitor duloxetine and the tricyclic antidepressant amitryptaline. Duloxetine had no effect on paw withdrawal threshold in paclitaxel-treated rats. However, amitriptyline partially reversed this neuropathy only at the highest dose tested. Doses, route of administration, frequency of administration and pretreatment times may need to be optimized to see more robust effects of these antidepressant agents.
- The paclitaxel model of chemotherapy-induced neuropathy in rats can be used as a preclinical model to evaluate novel potential therapies for peripheral neuropathy induced by cancer-treatments.
- Further studies are ongoing to evaluate the efficacy of different classes of analgesic agents in this model.

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