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Preventative and therapeutic antihyperalgesic effects of a GABA transporter 1 inhibitor administered systemically in a mouse model of paclitaxel-induced neuropathic pain

Parvathy S Subramanian, Willias Masocha

Background: There is a dearth of drugs to manage a dose-limiting painful peripheral neuropathy induced by paclitaxel in some patients during the treatment of cancer. Gamma-aminobutyric acid transporter-1 (GAT-1) whose expression is increased in the brain and spinal cord during paclitaxel-induced neuropathic pain (PINP) might be a potential therapeutic target for managing PINP. Thus, our aim was to evaluate if systemic administration of a GAT-1 inhibitor ameliorates PINP.

Methods: The reaction latency to thermal stimuli (hot plate test; at 55 °C) of female BALB/c mice was recorded before and after intraperitoneal treatment with paclitaxel, its vehicle, and/or a selective GAT-1 inhibitor NO-711. The effects of NO-711 on motor coordination were evaluated using the rotarod test at a constant speed of 4 rpm.

Results: No motor deficits were observed with NO-711 at a dose of 3 mg/kg, whereas a higher dose 5 mg/kg caused motor impairment and reduced mean time spent on the rotarod. The coadministration of paclitaxel with NO-711 3 mg/kg prevented the development of paclitaxel-induced thermal hyperalgesia at day 7 after drug treatment. NO-711 at 3 mg/kg produced antinociceptive effects against thermal nociception in mice with established paclitaxel-induced thermal hyperalgesia.

Conclusions: These results show that systemic administration of the GAT-1 inhibitor NO-711 has preventative and therapeutic activity against paclitaxel-induced thermal hyperalgesia at doses that do not impair motor activity. Thus, low doses of GAT-1 inhibitors could be useful for the prevention and treatment of PINP.

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Abstract

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Introduction

Chemotherapy-induced neuropathic pain (CINP) limits the use of some chemotherapeutic drugs, such as paclitaxel, oxaliplatin and vincristine, in the management of various types of cancer. The incidence of chemotherapy-induced peripheral neuropathy in patients treated with some chemotherapeutic drugs is very high, for example in patients treated with paclitaxel it is around 70.8% (95% CI = 43.5–98.1) (Seretny et al. 2014). In another study it was found that 64% of patients experienced chemotherapy-induced peripheral neuropathy during paclitaxel treatment and 27% of these patients were diagnosed with neuropathic pain (Reyes-Gibby et al. 2009). Unfortunately, there is a dearth of drugs to prevent or manage this type of pain. Currently, only duloxetine has a moderate recommendation for the management of CINP, whilst other drugs used for other neuropathic pain conditions may be given because of the limited CINP treatment options (Hershman et al. 2014). Thus, studies on the pathophysiology of CINP and the development of new treatment options are essential.

Using an animal model of paclitaxel-induced neuropathic pain (PINP) we recently observed an increased expression of gamma-aminobutyric acid transporter 1 (GAT-1) transcripts in the anterior cingulate cortex (ACC) (Masocha 2015); which is an area involved in pain perception and modulation (Seminowicz et al. 2009; Steenland et al. 2006; Xie et al. 2009). GAT-1 is responsible for most of the GABA uptake from the synaptic cleft in the brain (Borden 1996; Conti et al. 1998; Jensen et al. 2003). In the same year (2015) another group also reported increased expression of GAT-1 in the spinal cord of an animal model of PINP (Yadav et al. 2015). In addition, they observed that intrathecal injection of a GAT-1 inhibitor ameliorates

PINP (Yadav et al. 2015). Thus, suggesting that GAT-1 plays an important role in the pathophysiology of PINP and is a potential therapeutic target.

Tiagabine, a GAT-1 inhibitor, is used for the treatment of epilepsy as an add-on therapy in the treatment of partial seizures (Bialer et al. 2007). It has various adverse effects of which the most common include fatigue, dizziness, psychomotor slowing, ataxia, somnolence, insomnia, nausea/GI upset and weight change (Bialer et al. 2007; Vossler et al. 2013). Tiagabine has also been shown to have some beneficial effects for the treatment of neuropathic pain (Novak et al. 2001). In animal models of neuropathic pain GAT-1 inhibitors have been reported to have antiallodynic and antihyperalgesic activity (Daemen et al. 2008; Yadav et al. 2015). However, in one study it was suggested that the observed antinociceptive activity of tiagabine could be attributed to sedative and motor-impairing properties, as these properties can produce false positive effects in some pain tests (Salat et al. 2015).

The aim of this study was to evaluate whether systemic administration of a GAT-1 inhibitor can prevent the development of PINP and also if it has therapeutic effects against established PINP at doses that do not impair motor activity.

Materials and Methods

Animals

Animals used in this study were handled in compliance with the Kuwait University, Health Sciences Center (HSC), Animal Resources Centre (ARC) guidelines and in compliance with Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. All animal experiments were approved by the Ethical Committee for the use of Laboratory Animals in Teaching and in Research, HSC, Kuwait University. Female BALB/c mice (8 to 12 weeks old; 20 – 30 g; n = 115) supplied by the ARC at the HSC, Kuwait University were used in this study. The animals were kept in temperature controlled (24 ± 1°C) rooms with food and water given ad libitum.

Administration of paclitaxel to induce thermal hyperalgesia

Paclitaxel (Tocris, Bristol, UK) was dissolved in a solution made up of 50% Cremophor EL and 50% absolute ethanol to a concentration of 6 mg/ml and then diluted in normal saline (NaCl 0.9%), to a final concentration of 0.2 mg/ml just before administration. Vehicle or paclitaxel 2 mg/kg were injected intraperitoneally (i.p.) for 5 consecutive days, the cumulative dose of paclitaxel was 10 mg/kg (the paclitaxel administration schedule is depicted in Figure 1). This paclitaxel treatment regimen produces painful neuropathy and thermal hyperalgesia in mice (Nieto et al. 2008; Parvathy & Masocha 2013).

Administration of NO-711

1-[2-[[(diphenylmethylene)imino]oxy]ethyl]-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid hydrochloride (NO-711) (Sigma, St. Louis, MO, USA) was dissolved in normal saline and administered to mice i.p. at a volume of 10 ml/kg body mass.

For the rotarod test NO-711 was administered once at doses of 3 and 5 mg/kg to naïve mice.

Two treatment regimens were used to treat paclitaxel-treated mice; the first was a preventative/prophylactic treatment (Figure 1A) and the second a therapeutic treatment (Figure 1B).

For the preventative treatment NO-711 3 mg/kg was co-administered daily with paclitaxel, as described above, for 5 consecutive days (Figure 1A). The mice were assessed for the development of thermal hyperalgesia on day 7 and those that received paclitaxel plus NO-711 were compared with the mice treated with the paclitaxel plus vehicle (for NO-711) only.

For the therapeutic treatment, NO-711 at doses of 1 and 3 mg/kg, was administered once at 7 days after first administration of paclitaxel (Figure 1B), when mice had developed thermal hyperalgesia as previously described (Parvathy & Masocha 2013).

Assessment of thermal nociception

Reaction latencies to the hot-plate test were measured, before (baseline latency), at day 7 after first injection of paclitaxel alone or together with NO-711 (preventative treatment), and at various times on day 7 starting at 30 minutes after treatment with NO-711 (therapeutic treatment). Briefly, mice were individually placed on a hot plate (Panlab SL, Barcelona, Spain) with the temperature adjusted to 55 ± 1 °C. The time to the first sign of nociception, paw licking,

106 flinching or jump response to avoid the heat was recorded and the animal immediately removed
107 from the hot plate. A cut-off period of 20 seconds was maintained to avoid damage to the paws.

108 **Assessment of motor coordination**

109 Motor coordination was evaluated using the rotarod apparatus (Panlab SL, Barcelona, Spain).
110 The rotation of the rod was set at a constant speed of 4 rpm. All animals were trained for 3 days
111 until they could remain on the rod for 300 s (5 min) without falling. On the test day, mice
112 received single injections of NO-711 3 and 5 mg/kg or its vehicle (normal saline) before the test.
113 The latency (in seconds) for the first fall was recorded at 30 min, 1 hr and 2 hrs after
114 administration of NO-711. The cut-off time was set at 300 s.

115 **Statistical analyses**

116 Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by
117 Dunnett's multiple comparison test or two-way repeated measures ANOVA followed by
118 Bonferroni post-tests using GraphPad Prism software (version 5.0). The differences were
119 considered significant at $P < 0.05$. The results in the text and figures are expressed as the means
120 ± S.E.M.

121

Results

Motor coordination

Side effects of GAT-1 inhibitors such as tiagabine include sedation and impairment of motor coordination (Salat et al. 2015). Impairment of motor coordination and sedation affects the results of behavioural tests, including the hot plate test. Thus the effect of NO-711 on motor coordination was evaluated using the rotarod test. No significant differences of the mean time spent on the rotarod were observed between mice treated with vehicle (300 s) and the mice treated with NO-711 3 mg/kg (265 ± 35 s and 269 ± 30 s at 30 minutes and 1 hour after drug administration, respectively $P > 0.05$; Figure 2). However, mice treated with a higher dose of NO-711, 5 mg/kg, had a significant decrease in the mean time spent on the rotarod from 300 s to 123 ± 52 s and 185 ± 35 s at 30 minutes and 1 hour after drug administration, respectively ($P < 0.01$; Figure 2), indicating motor impairment. Thus, NO-711 was used at a dose of 3 mg/kg or lower for other experiments.

NO-711 prevents the development of paclitaxel-induced thermal hyperalgesia

Mice treated with paclitaxel had significant lower reaction latency times (about 34% lower) to the hot plate on day 7 compared to vehicle-treated mice, 6.3 ± 0.3 s versus 9.5 ± 0.4, respectively ($P > 0.05$; Figure 3), similar to what we described previously (Parvathy & Masocha 2013). On the other hand, mice treated with paclitaxel plus NO-711 (3 mg/kg) consecutively for 5 days had reaction latency times similar to the vehicle-only treated control mice, 8.7 ± 0.4 s versus 9.5 ± 0.4, respectively ($p > 0.05$), which were significantly higher than those of the mice treated with paclitaxel only ($P < 0.01$; Figure 3).

143 **NO-711 alleviates established paclitaxel-induced thermal hyperalgesia**

144 Mice with paclitaxel-induced thermal hyperalgesia (i.e. mice with significantly lower reaction
145 times after treatment with paclitaxel compared to pretreatment values; Figure 4A) were treated
146 with two doses of NO-711, 1 and 3 mg/kg.

147 The intraperitoneal administration of vehicle or a lower dose of NO-711 (1 mg/kg) did not
148 change the reaction latency to thermal stimulation in mice with paclitaxel-induced thermal
149 hyperalgesia compared to before vehicle or NO-711 1 mg/kg administration at day 7 ($P > 0.05$;
150 Figure 4B). However, No-711 at a dose of 3 mg/kg produced significant increase in reaction
151 latency in mice with paclitaxel-induced thermal hyperalgesia at 30 minutes and 1 hour post drug
152 administration compared to mice treated with vehicle and before NO-711 administration at day 7
153 ($P < 0.01$; Figure 4B). The mice with paclitaxel-induced thermal hyperalgesia treated with NO-
154 711 at a dose of 3 mg/kg had reaction latency of 10.1 ± 0.6 s, which was similar to the reaction
155 latency before paclitaxel administration to mice 10.5 ± 0.3 s ($P > 0.05$).

156

157 # Discussion

158 The results of this study show that systemic administration of NO-711, a GAT-1 inhibitor,
159 prevents the development of paclitaxel-induced thermal hyperalgesia and also has
160 antihyperalgesic activity in mice with established paclitaxel-induced thermal hyperalgesia at a
161 low dose that does not cause motor impairment.

162 GAT-1 inhibitors have anti-seizure activities and one of them, tiagabine, is used for the treatment
163 of epilepsy as an add-on therapy in the treatment of partial seizures (Bialer et al. 2007). They
164 have also been shown to have antinociceptive and antiallodynic activities (Daemen et al. 2008;
165 Ipponi et al. 1999; Li et al. 2011; Yadav et al. 2015). However, in one study it was suggested that
166 the observed antinociceptive activity of tiagabine could be attributed to sedative and motor-
167 impairing properties, as these properties can produce false positive effects in some pain tests
168 (Salat et al. 2015). In the rotarod test we observed that NO-711 at a dose of 3 mg/kg did not
169 cause motor impairment/sedation, similar to what has been described before for that dose (Kubo
170 et al. 2009). However a higher dose, 5 mg/kg, caused motor impairment/sedation, similar to what
171 has been observed with other high doses of NO-711 (Kubo et al. 2009; Suzdak et al. 1992).
172 Thus, NO-711 has a dose-dependent motor impairment/sedation effect as described previously
173 (Suzdak et al. 1992). In order to separate antinociceptive or antihyperalgesic effects of NO-711
174 from motor impairment/sedation effect a dose of 3 mg/kg was used for evaluation of the activity
175 of the compound against paclitaxel-induced thermal hyperalgesia.

176 Intrathecal administration of NO-711 has recently been shown to attenuate established paclitaxel
177 induced mechanical and thermal hyperalgesia in rats (Yadav et al. 2015). In the current study
178 systemic (intraperitoneal) administration of NO-711 at a dose of 3 mg/kg attenuated established
179 paclitaxel-induced thermal hyperalgesia in mice. No other studies have reported the effects of a

systemically administered GAT-1 inhibitor on CINP in general or PINP in particular. However, systemic administration of GAT-1 inhibitors has been reported to attenuate thermal hyperalgesia and allodynia in other models of neuropathic pain such as chronic constriction injury of the sciatic nerve, spinal nerve ligation (Daemen et al. 2008; Giardina et al. 1998). Thus, systemic administration of NO-711 has antihyperalgesic activities against PINP similar to the systemic administration of another GAT-1 inhibitor, tiagabine, in other models of neuropathic pain.

Our findings and those Yadav and colleagues showing increased GAT-1 expression in the CNS, in the ACC and spinal cord, respectively, suggests an important role of GAT-1 in the pathophysiology of PINP (Masocha 2015; Yadav et al. 2015). Thus, we explored whether preventative treatment by inhibiting GAT-1 activity could be potentially useful to prevent the development of PINP. Our findings show that coadministration of NO-711 at a dose of 3 mg/kg with paclitaxel for five consecutive days prevented the development of paclitaxel-induced thermal hyperalgesia. Thus, indicating that increased GAT-1 expression and activity play a role in the development of PINP.

Conclusions

In conclusion our results show that systemic administration of a GAT-1 inhibitor, NO-711, at doses that do not cause motor impairment/sedation prevents the development of paclitaxel-induced thermal hyperalgesia and alleviates established paclitaxel-induced thermal hyperalgesia. The antihyperalgesic activity of the GAT-1 inhibitor is independent of its motor impairment/sedative activities. Thus, low doses of GAT-1 inhibitors have potential therapeutic activity to prevent or manage PINP and CINP in general. Therefore the possible clinical use of

GAT-1 inhibitors, which are already in the clinics such as tiagabine, against CINP warrants further research.

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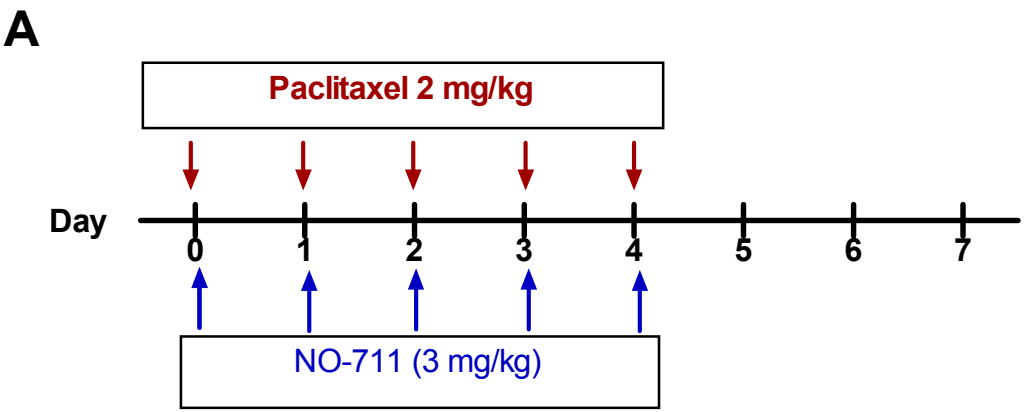
273

Figure 1(on next page)

Drug administration schedule for preventative and therapeutic treatment with NO-711 against paclitaxel-induced thermal hyperalgesia.

(A) Treatment with NO-711 to prevent the development of paclitaxel-induced thermal hyperalgesia (B) Therapeutic treatment with NO-711 to alleviate established paclitaxel-induced thermal hyperalgesia. The arrows indicate the days when the drugs were intraperitoneally administered.


A
Paclitaxel 2 mg/kg
Day
0
1
2
3
4
5
6
7
NO-711 (3 mg/kg)

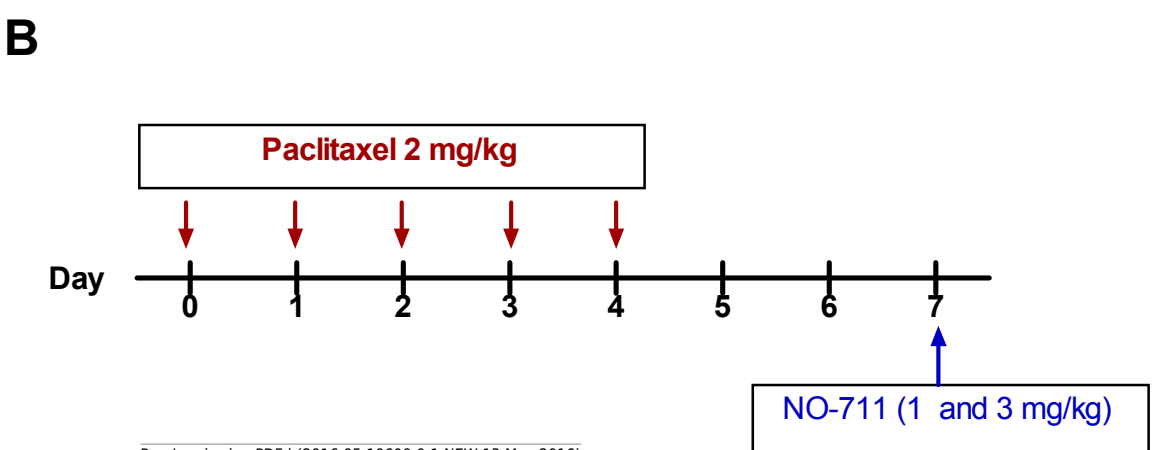

B
Paclitaxel 2 mg/kg
Day
0
1
2
3
4
5
6
7
NO-711 (1 and 3 mg/kg)

Figure 2(on next page)

Time course of the mean time spent on the rotarod (s) for two doses of NO-711 (3 and 5 mg/kg) in a rotarod test in naïve BALB/c mice.

Each point represents the mean ± S.E.M of values obtained from 8 animals. ** $P < 0.01$ compared to drug vehicle at the same time point after treatment (two-way repeated measures ANOVA followed by Bonferroni post-test).

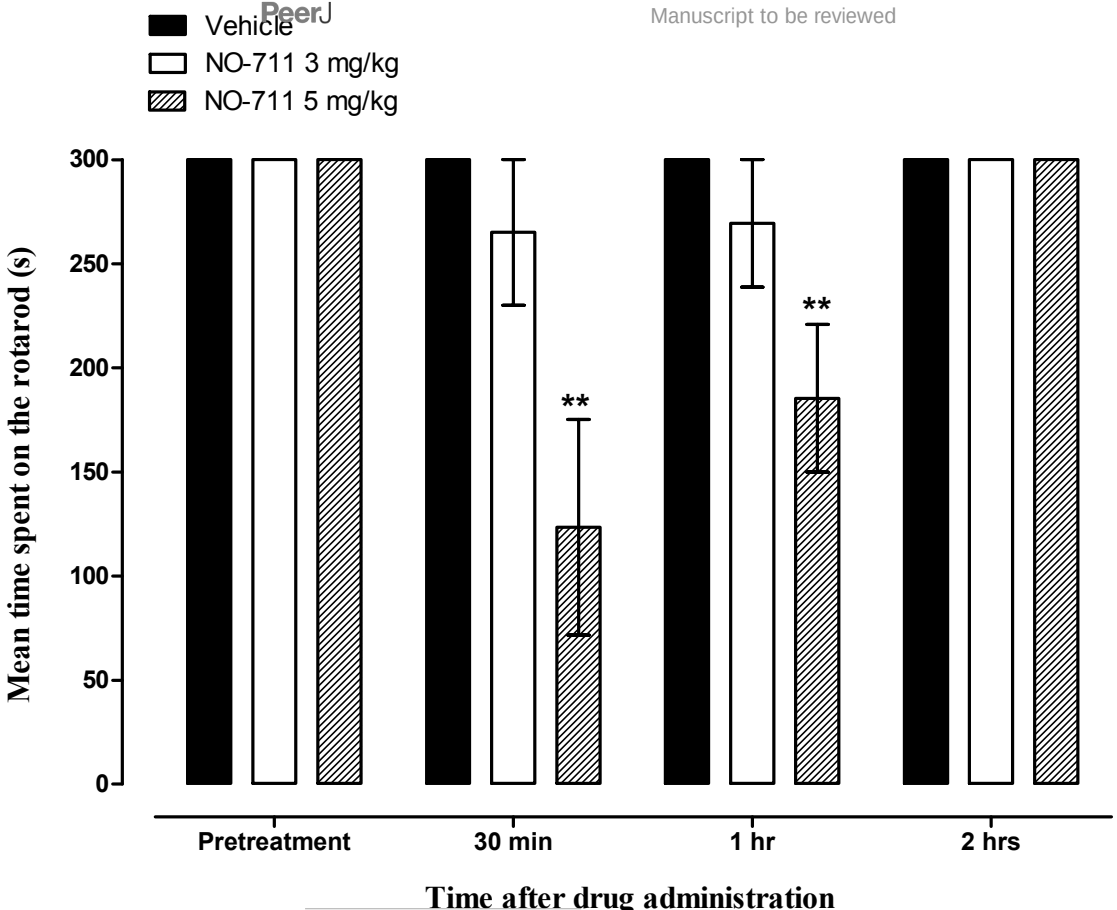

Vehicle
NO-711 3 mg/kg
NO-711 5 mg/kg
Mean time spent on the rotarod (s)
300
250
200
150
100
50
0
**
**
Pretreatment
30 min
1 hr
2 hrs
Time after drug administration

Figure 3(on next page)

Coadministration of NO-711 with paclitaxel protects against the development of paclitaxel-induced thermal hyperalgesia in a hot-plate test.

Effects of coadministration of paclitaxel with NO-711 on the development of paclitaxel-induced thermal hyperalgesia in BALB/c mice . Each point represents the mean ± S.E.M of the values obtained from 8 animals. ** $p < 0.01$ compared to control mice (treated with vehicles only) and # # $p < 0.01$ compared to mice treated with paclitaxel + vehicle (two-way repeated measures ANOVA followed by Bonferroni post-test).

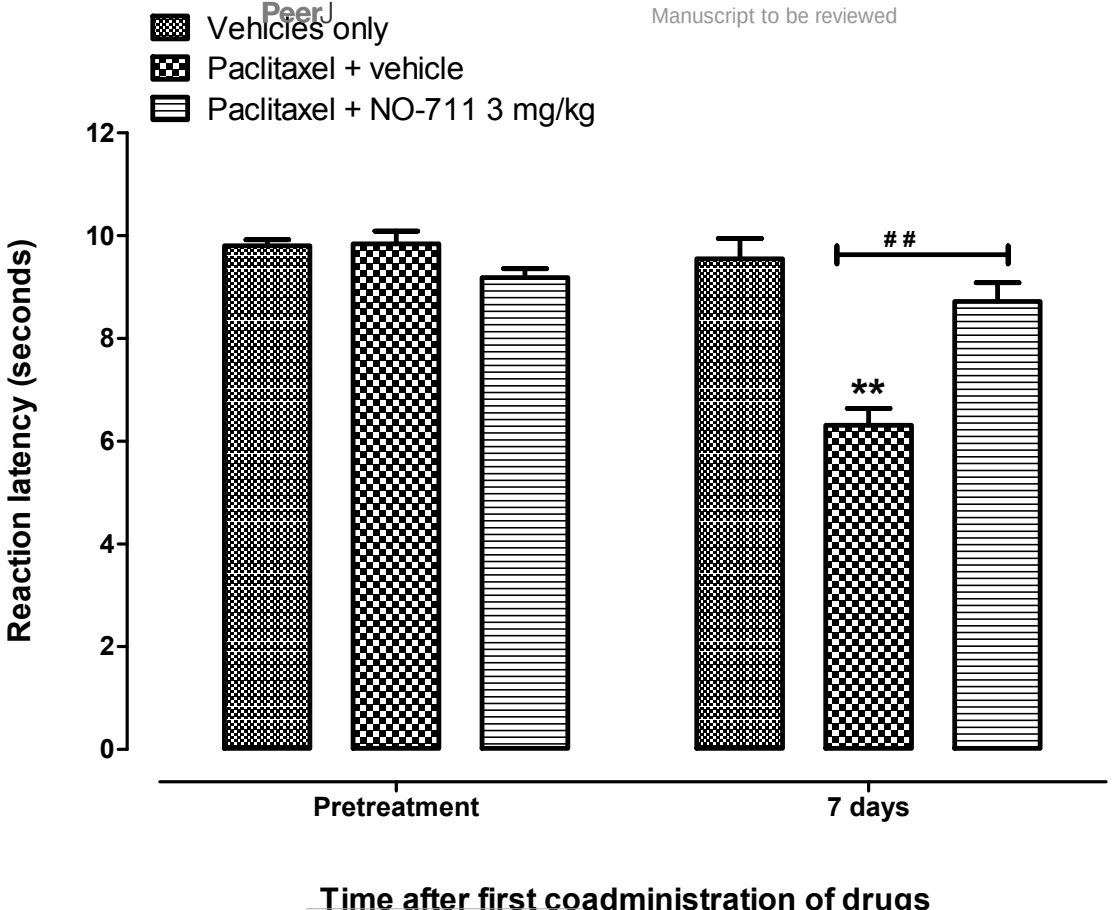
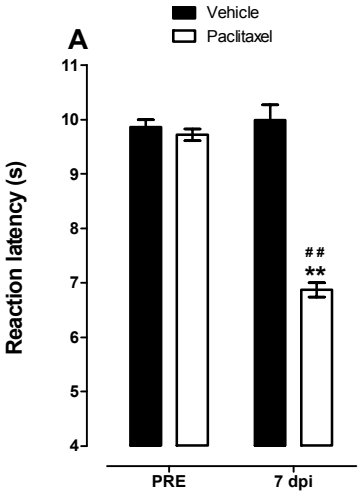
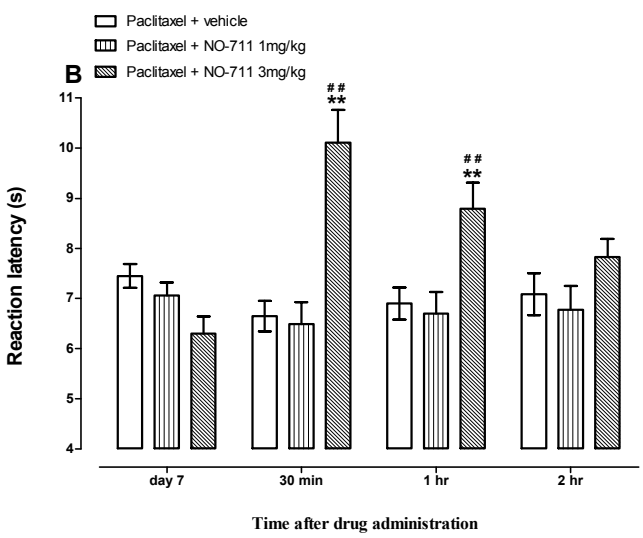

Vehicles only
Paclitaxel + vehicle
Paclitaxel + NO-711 3 mg/kg
Reaction latency (seconds)
12
10
8
6
4
2
0
##
**
Pretreatment
7 days
Time after first coadministration of drugs

Figure 4(on next page)

Antihyperalgesic effects of NO-711 on BALB/c mice with paclitaxel-induced thermal hyperalgesia in a hot-plate test.

(A) Thermal hyperalgesia in BALB/c mice at day 7 post first inoculation of paclitaxel. Each point represents the mean ± S.E.M of values obtained from 15 vehicle-treated and 52 paclitaxel-treated animals. ** $P < 0.01$ compared to drug vehicle at the same day after treatment and # # $P < 0.01$ compared to pretreatment (PRE) values (Student's t test). (B) Reaction latency times (taken at day 7 post first administration of paclitaxel) at different times after treatment with NO-711 (1 and 3 mg/kg) or its vehicle in a hot-plate test. Each bar represents the mean ± S.E.M of values obtained from 8-17 animals. ** $P < 0.01$ compared to drug vehicle at the same time point after treatment (two-way repeated measures ANOVA followed by Bonferroni test) and # # $P < 0.01$ compared to pretreatment (day 7) values (one-way ANOVA followed by Dunnett's Multiple Comparison Test).


A
Vehicle
Paclitaxel
Reaction latency (s)
11
10
9
8
7
6
5
4
##
**
PRE
7 dpi


Paclitaxel + vehicle
Paclitaxel + NO-711 1mg/kg
Paclitaxel + NO-711 3mg/kg
B
Reaction latency (s)
11
10
9
8
7
6
5
4
##
**
##
**
day 7
30 min
1 hr
2 hr
Time after drug administration