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Gabapentin and its salicylaldehyde derivative alleviate allodynia and hypoalgesia in a cisplatin-induced neuropathic pain model

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Abstract

Cisplatin is an effective chemotherapeutic agent indicated in cancer chemotherapy. However, its clinical use is associated with peripheral neuropathy that invariably impairs patient quality of life. Gabapentin (GBP) is an effective analgesic for neuropathic pain conditions but its clinical efficacy in cisplatin-induced neuropathic pain (CINP) is limited, in addition to generating unwanted side-effects. In this study, a gabapentin-salicylaldehyde derivative [gabapentsal (GPS)] was synthesized and evaluated to explore any potential benefit in comparison with GBP in a rat model of CIPN. Administration of cisplatin (3.0 mg/kg/week, i.p.) for five consecutive weeks generated reproducible mechanical-allodynia (decreased paw withdrawal threshold to von Frey filament application; PWT, g) and thermal hypoalgesia (increased nociceptive reaction latency in the hot plate paradigm; s). Treatment with GBP or its derivative on the 37^{th} day of the experimental protocol, dose dependently attenuated cisplatin-induced nocifensive behaviors. Accordingly, doses of GBP (50-100 mg/kg, i.p.) and GPS (25-100 mg/kg, i.p.) suppressed the expression of CINP by normalizing the PWT and hot plate response latency 1 h and 3 h post administration. In the rotarod paradigm, GBP at all doses markedly impaired motor performance, whilst GPS was devoid of motor incoordination except at the highest dose, when a mild impairment occurred. Salicylaldehyde alone had no effect on CINP or rotarod performance and neither was there any synergism when coadministered with GBP. These findings suggest that both GBP and GPS have beneficial effects in the neuropathic pain model though GPS may be potentially more useful in the management of CINP.

Key words Chemotherapy induced neuropathic pain; peripheral neuropathic pain; gabapentsal; motor incoordination; rat neuropathic pain model.

Chemical compounds

Gabapentin (PubChem CID: 3446)

Salicylaldehyde (PubChem CID:6998)

1. Introduction

The clinical use of anticancer agents is associated with a number of unwanted side effects including neuropathic pain, which is intolerable for the majority of patients (Markman, 2006; Stillman and Cata, 2006; Van Cutsem and Arends, 2005). These adverse effects are dose-dependent and reduce the therapeutic outcome of chemotherapeutic agents worsening patient quality of life (Ocean and Vahdat, 2004; Warner, 1995). Cisplatin and its analogues (oxaliplatin and carboplatin) are used clinically world-wide as a first-line treatment alone or in combination with other antineoplastic agents for malignant tumors including for example, those associated with lung, breast and colorectal cancer (He et al., 2016; Lynch et al., 2012; Marschner et al., 2015; Zhang et al., 2015). The neuropathy caused by cisplatin may persist for years (Chaudhry et al., 2003; Markman, 2003). In addition, approximately 20% of patients are unable to complete their course of treatment due to the intolerable neuropathic pain arising from the use of antineoplastic agents. The neuropathy caused by such drugs is managed by reduction of the cumulative dose, individual doses or sometimes by complete cessation of chemotherapy upon the appearance of neuropathic symptoms (Ocean and Vahdat, 2004).

Despite cisplatin combined treatment with a variety of drugs including calcium and magnesium infusions, amifostine, antioxidants (glutathione, vitamin E, α-lipoic acid and *N*-acetylcysteine) and anticonvulsant or antidepressant drugs (carbamazepine, lamotrigine, gabapentin, pregabalin or venlafaxine), clinical outcomes have not been totally satisfactory (Albers et al., 2014; Cavaletti and Marmiroli, 2010; Kaley and DeAngelis, 2009). There is therefore a need for an effective treatment to prevent or limit the occurrence and severity of chemotherapy-induced peripheral neuropathy.

Gabapentin (Fig.1 A) was first approved by the Medicines and Healthcare products Regulatory Agency (MHRA) in 1993 as an oral add-on therapy for the treatment of partial seizures and later in 2000 for peripheral neuropathy. It is probably one of the most effective agents of the newer generation of antiepileptics used for the treatment of neuropathic pain. The efficacy of gabapentin has been demonstrated in clinical trials and in several preclinical models of neuropathic pain (Ali et al., 2015; Field et al., 1997; Rowbotham et al., 1998). However, there is meager scientific evidence demonstrating gabapentin efficacy against cisplatin-induced preclinical neuropathic pain although a previous study has demonstrated that it does produce a dose dependent relief of mechanical allodynia in cisplatin-induced

neuropathic rats (Han et al., 2014). Moreover, gabapentin has been shown to be effective in the treatment of cancer-related neuropathic pain, by producing a meaningful reduction in pain scores in patients receiving radiotherapy, surgery or chemotherapy (Ross et al., 2005). Unfortunately, the effectiveness of systemic GBP in patients suffering from neuropathic pain may be limited by the occurrence of side-effects such as dizziness, somnolence, ataxia, lethargy and convulsions (Rose and Kam, 2002). Accordingly, the discovery of new drugs with enhanced neuropathic pain alleviating propensity associated with a reduced side effect profile is warranted.

In this context, a rationale was adopted in this investigation using a GBP derivative (See supplementary file) based on the premise of combining the reported antineuropathic effect of GBP (Rosenberg et al., 1997; Serpell and Group, 2002) with the antinociceptive and anti-inflammatory properties of salicylaldehyde (Rainsford, 2016) in a single molecular entity to explore any potential benefit over GBP. The study further investigated the cisplatin-induced neuropathic pain de-escalating efficacy of gabapentin in addition to its salicylaldehyde derivative (gabapentsal (GPS), Fig.1B) (Mallesha et al., 2012), synthesized herein via an exclusively novel method by incorporating a salicylaldehyde moiety at its amine functionality. Additionally, a motor coordination assessment (rotarod) was performed to evaluate its activity in comparison to GBP.

2. Materials and methods

2.1. Animals

Sprague-Dawley rats of either sex (150-200 g) bred in the Animal House of the Department of Pharmacy, University of Peshawar, Peshawar, Pakistan, were used in this study. All the experimental procedures on animals were performed in compliance with the UK Animals (Scientific Procedures) Act 1986 and according to the rules and ethics of the Institutional Ethical Committee. Approval for the study was granted with the registration number: 10/EC-15/Pharm. To eliminate bias, all animal groups were coded and both the experimental behavior assessors and statistical analysts were blind to the treatments.

2.2. Acute toxicity test

For the determination of acute toxicity of GPS, mice were injected intraperitoneally (i.p) with GPS at doses ranging from 25-1000 mg/kg (n = 6 mice for each dose) and their behavior was observed initially for 2 h and then up to for 24 h post drug administration. The animals were

observed for spontaneous activity, aggressiveness, cyanosis, ataxia, tail pinch response, righting reflex, writhing, convulsions, catalepsy and bizarre behavior (OECD guidelines, 2001)

2.3. Induction of cisplatin-induced neuropathic nociception

Animals were administered cisplatin (3.0 mg/kg i.p., once a week for 5 week, i.e. cumulative dose of 15mg/kg). Sterile saline solution (2.0 mL) was administered subcutaneously (s.c.) to prevent renal damage via hyperhydration before each cisplatin dose (Authier et al., 2003a; Han et al., 2014; McKeage, 1995) and animals received less than 5.0 mL of dosing solution into the peritoneal cavity.

2.3.1. Treatment groups

2.3.1.1. Experiment 1 (Treatment with GBP)

GBP was dissolved in normal saline (Zeesol NS,Shahzaib Pharmaceuticals [Pvt.] ltd. Haripur Pakistan) and was intraperitoneally administered in doses of 50, 75 and 100 mg/kg on the 37th day of the treatment protocol. The animals were randomly distributed into the following treatment groups (n = 8 rats per group).

Group 1: Saline (10 mL/kg/week for 5 weeks) + Saline (10 mL/kg on day 37)
Group 2: Cisplatin (3.0 mg/kg/week for 5 weeks) + Saline (10 mL/kg on day 37)
Group 3: Cisplatin (3.0 mg/kg/week for 5 weeks) + GBP (50 mg/kg on day 37)
Group 4: Cisplatin (3.0 mg/kg/week for 5 weeks) + GBP (75 mg/kg on day 37)
Group 5: Cisplatin (3.0 mg/kg/week for 5 weeks) + GBP (100 mg/kg on day 37)
Group 6: Saline (10 mL/kg/week for 5 weeks) + GBP (100 mg/kg on day 37)

2.3.1.2. Experiment 2 (Treatment with GPS)

The GBP derivative (GPS) was dissolved in a vehicle comprising of DMSO, Tween-80 and normal saline in a ratio of 5:1:94. The animals were randomized into the following treatment groups (n = 8 rats per group), during which they were intraperitoneally administered GPS in doses of 25, 50, 75 and 100 mg/kg on the 37th day.

Group 1: Vehicle (10 mL/kg/week for 5 weeks) + Vehicle (10 mL/kg on day 37)
Group 2: Cisplatin (3.0 mg/kg/week for 5 weeks) + Vehicle (10 mL/kg on day 37)
Group 3: Cisplatin (3.0 mg/kg/week for 5 weeks) + GBP (100 mg/kg on day 37)
Group 4: Cisplatin (3.0 mg/kg/week for 5 weeks) + GPS (25 mg/kg on day 37)

Group 5: Cisplatin (3.0 mg/kg/week for 5 weeks) + GPS (50 mg/kg on day 37)
Group 6: Cisplatin (3.0 mg/kg/week for 5 weeks) + GPS (75 mg/kg on day 37)
Group 7: Cisplatin (3.0 mg/kg/week for 5 weeks) + GPS (100 mg/kg on day 37)
Group 8: Vehicle (10 mL/kg/week for 5 weeks) + GPS (100 mg/kg on day 37)

2.3.1.3. Experiment 3 (Treatment with salicylaldehyde alone and coadministration of salicylaldehyde plus GBP

Salicylaldehyde (50 and 100 mg/kg) and salicylaldehyde plus GBP (100 + 100 mg/kg, coadministeration) were dissolved in normal saline (Zeesol NS, Shahzaib Pharmaceuticals [Pvt.] ltd. Haripur Pakistan) and administered intraperitoneally on the 37th day of the treatment protocol. The animals were randomly distributed into the following treatment groups (n = 8 rats per group).

Group 1: Saline (10 mL/kg/week for 5 weeks) + Saline (10 mL/kg on day 37)
Group 2: Cisplatin (3.0 mg/kg/week for 5 weeks) + Saline (10 mL/kg on day 37)
Group 3: Cisplatin (3.0 mg/kg/week for 5 weeks) + GBP (100 mg/kg on day 37)
Group 4: Cisplatin (3.0 mg/kg/week for 5 weeks) + GPS (100 mg/kg on day 37)
Group 5: Cisplatin (3.0 mg/kg/week for 5 weeks) + Salicylaldehyde (50 mg/kg on day 37)
Group 6: Cisplatin (3.0 mg/kg/week for 5 weeks) + Salicylaldehyde (100 mg/kg on day 37)
Group 7: Cisplatin (3.0 mg/kg/week for 5 weeks) + GBP + Salicylaldehyde [(100 +100) mg/kg, coadministration, on day 37]

2.4. Assessment of static mechanical allodynia

Animals were tested for static mechanical-allodynia on the 37th day before and after 1 h and 3 h post-administration of gabapentin, GPS, salicylaldehyde alone or salicylaldehyde plus gabapentin (coadministration). A series of 8 von Frey filaments (0.4, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g) (Stoelting, Wood Dale, Illinois, USA) were applied perpendicularly to the mid-plantar surface of the left hind paw to an extent that caused the filaments to bend (Chaplan et al., 1994). Each von Frey filament was applied for a period of up to 6 s as a cut-off time or until a positive response occurred. Lifting of the paw or flinching immediately upon removal of the filament was recorded as a positive response and a succeeding von Frey filament of lower force was applied for the following recording. In the case of an absence of response, the subsequent von Frey filament of higher force was applied. This procedure was continued until four measurements were taken after the first change in direction (positive

response) or five consecutive negative responses (2.00, 3.63, 5.50, 8.50 and 15.10-g force) or four consecutive positive responses (2.00, 1.20, 0.70, 0.4-g force). A force of 15.10 g was selected as the cut-off force at which point further application ceased. von Frey filaments were applied at intervals of several seconds to avoid any influence of previous stimuli on behavior. Any ambulation was noted as an indefinite response, and the stimulus was repeated. The paw withdrawal threshold (PWT, g) was measured in both hind paws with an interval of 5 min and the mean was calculated from an average of three readings. The pattern of each response was converted to the 50% PWT (Shahid et al., 2016).

2.5. Assessment of thermal nociception

On the 2nd day of final cisplatin injection (i.e. the 37th protocol day), the animals were tested for thermal nociceptive response latency on a hot plate (Harvard apparatus, USA) before, and 1 h and 3 h post-administration of GBP, GPS, salicylaldehyde alone or salicylaldehyde plus GBP (coadministration). The metallic testing plate was maintained at 52.0 ± 0.2 ^{0}C and the response latency in seconds was recorded to the onset of escape jumping or lifting/licking of hind paws. In order to avoid tissue injury, a cut-off time limit of 60 seconds was imposed. Each response latency was measured in triplicate and the mean value from the three measurements was taken as the heat thermal nociceptive response latency (Huang et al., 2004).

2.6. Assessment of motor coordination

An individually designed accelerating rotarod was used in which the animals were placed on a rotating drum with the speed increasing from 4 to 40 rpm over 300 s (Shahid et al., 2016). The endurance latency for the animals to fall off the rod was measured using a stop watch. Animals were trained for 4 days, with three trials per day with a daily increment in the maximum time spent on the rotarod from 70 s each trial on day 1 to 300 s per trial on day 4, in order to allow rats to memorize the task. During training, any animal that fell off prematurely was gently placed back on the rotating drum. On the 4th day, the animals were randomly distributed into various groups (n = 8 rats per group) and the baseline was recorded by measuring the initial latency to fall off (dismount) the rotating rod. Then the average time spent on the rotarod across three trials was measured before (pre-dose) and after 1 h and 3 h of treatment with GBP (50, 75 and 100 mg/kg) or GPS (25, 50, 75 and 100 mg/kg), salicylaldehyde alone (50 and 100 mg/kg) or salicylaldehyde plus GBP (100 + 100) mg/kg, coadministration).

2.7. *Statistical analysis*

Results are presented as mean ± S.E.M. The data in the neuropathic experiments were analyzed using two-way repeated measures analysis of variance (ANOVA) followed by *post hoc* Bonferroni test using GraphPad Prism 5 (GraphPad Software Inc. San Diego CA, USA). A value of $P \leq 0.05$ was accepted as significant.

3. Results

3.1. Acute toxicity test

No overt behavioral changes were observed during at an observation time of 2h post GPS administration and no mortality occurred at doses up to 1000 mg/kg.

3.2. *Activity of GBP on cisplatin-induced neuropathic static mechano-allodynia*

Weekly administration of cisplatin (3.0 mg/kg) for five weeks resulted in a significant reduction ($P< 0.001$) in the mechanical nociceptive threshold to the innocuous von Frey filaments throughout the experimental period. The paw withdrawal threshold decreased from 13.4 ± 1.2 g (day -1) to 3.8 ± 1.4 g (37^{th} day). Treatment with GBP had a significant main dose effect on cisplatin-induced static mechanical allodynia [time = (F (3, 140) = 26.92, $P< 0.0001$), treatment = (F (5, 140) = 31.69, $P< 0.0001$), interaction = (F (15, 140) = 6.73, $P< 0.0001$)]. On the 37^{th} day (2^{nd} day after the last cisplatin injection), GBP significantly attenuated cisplatin-induced nociception to gentle static pressure stimuli when tested1 h and 3 h post-doses of 50 mg/kg ($P< 0.05$, $P< 0.01$), 75 mg/kg ($P< 0.01$, $P< 0.001$) and 100 mg/kg ($P< 0.001$, $P< 0.001$) respectively. Administration of GBP (100 mg/kg) reversed the cisplatin-induced decrease in the nociceptive threshold since a significant increase ($P< 0.001$) in the PWT was observed, compared to cisplatin alone treatment (Fig 2).

3.3. Effect of the gabapentin derivative (GPS) on cisplatin-induced neuropathic static mechano-allodynia

A single intraperitoneal administration of cisplatin (3.0 mg/kg/week) for 5 consecutive weeks generated static mechanical allodynia bilaterally in the hind paws whereby a significant decrease ($P< 0.001$) in the threshold to elicit the paw withdrawal response in response to von Frey filaments was observed. The PWT decreased from a baseline PWT value of 14.3 ± 0.7 g

(day -1) to 1.5 ± 0.5 g on the 37th day. A significant attenuated effect was afforded by the treatment on the expression of cisplatin-induced static mechanical allodynia [time = (F (3, 196) = 72.52, P< 0.0001), treatment = (F (7, 196) = 41.36, P< 0.0001), interaction = (F (21, 196) = 9.93, P< 0.0001)]. GPS effectively reversed the cisplatin-induced reduction in mechanical nociceptive threshold upon application of gentle pressure with von Frey filaments to the mid-plantar surface of the bilateral hind paws on the 37th day. Hence *post hoc* comparisons revealed significant anti-allodynic activity at doses of 25 mg/kg (P< 0.01, P< 0.05), 50 mg/kg (P< 0.001, P< 0.001), 75 mg/kg (P< 0.001, P< 0.001) and 100 mg/kg (P< 0.001, P< 0.001) when the allodynia paradigm was tested 1 h and 3 h post drug administration, respectively. The systemic dose of the positive control, GBP (100 mg/kg), allayed the cisplatin-induced neuropathic allodynia by significantly suppressing (P< 0.001) the evoked nociceptive response, 1 h and 3 h post drug injection. Additionally, injection of GPS to non-cisplatin treated animals had no effect on the nociceptive behavior and when compared to the cisplatin positive control, it produced a robust antinociceptive effect (P< 0.001) throughout the whole testing time-period (Fig 3).

3.4. Effect of GBP on cisplatin-induced neuropathic thermal hypoalgesia

Administration of cisplatin (3.0 mg/kg per week) for five consecutive weeks produced extensive changes in the perception of thermal nociception such that significant thermohypoalgesia (P< 0.001) was observed during testing on the 37th protocol day. Consequently, cisplatin increased the nociceptive response latency in the hot plate paradigm from a pre-experimental value of 15.6± 2.8s (day -1) to 37.3 ± 4.0s on the concluding experimental day (37th day). GBP produced a significant main dose effect on cisplatin-induced thermohypoalgesia [time = (F (3, 140) = 20.97, P< 0.0001), treatment = (F (5, 140) = 15.24, P< 0.0001), interaction = (F (15, 140) = 4.04, P< 0.0001)]. Correspondingly, the apparent thermal antinociceptive action of cisplatin on the hot plate was significantly diminished by GBP on the37th protocol day 1 h and 3 h post-dosing with 50 mg/kg (P< 0.05, P< 0.01), 75 mg/kg (P< 0.01, P< 0.01) and 100 mg/kg (P< 0.001, P< 0.001) respectively. GBP (100 mg/kg) by itself did not modify thermal nociceptive behavior as no change in the response latency was observed but it intrinsically produced a significant antinociceptive response (P< 0.001) versus cisplatin-induced neuropathic thermohypoalgesia (Fig 4).

3.5. Effect of the gabapentin derivative (GPS) on cisplatin-induced neuropathic thermal hypoalgesia

Cisplatin significantly augmented ($P< 0.001$) the baseline nociceptive threshold evoked by the hot plate thermal stimulus on protocol day 37. In consequence, the thermal response threshold increased from a pre-experimental latency of 16.6 s to 38.6 s (pre-dose) then up to 39.8 s (1 h) and 40.8 s (3 h) post-dose on the test day. GPS produced a significant dose effect on cisplatin-induced thermal hypoalgesia [time = (F (3, 196) = 48.63, $P< 0.0001$), treatment = (F (7, 196) = 20.36, $P< 0.0001$), interaction = (F (21, 196) = 3.26, $P< 0.0001$)]. Thus GPS reversed cisplatin hypoalgesia in a dose dependent fashion 1 h and 3 h after administration of 50 mg/kg ($P< 0.05$, $P< 0.01$), 75 mg/kg ($P< 0.01$, $P< 0.001$) and 100 mg/kg ($P< 0.05$, $P< 0.01$) respectively on the 37th day. Likewise, the positive control, GBP (100 mg/kg), had a significant alleviating action ($P< 0.01$) on cisplatin escalated thermohypoalgesia. It was also noteworthy that on its own, GPS even at the highest dose (100 mg/kg) did not modify the baseline threshold nociceptive reaction latency (Fig 5).

3.6. Activity of GBP and its derivative (GPS) on rotarod performance

There was a significant main dose effect on motor coordination in rotarod endurance latency observed 1 h and 3 h post dosing [time = (F (3, 140) = 94.32, $P< 0.0001$), treatment = (F (7, 140) = 30. 64, $P< 0.0001$), interaction = (F (21, 140) = 10.55, $P< 0.0001$)]. Hence, GBP impaired motor coordination as manifested by a decrease in the rotarod dismount latency at 50 mg/kg ($P< 0.01$) and this activity was progressively more pronounced ($P< 0.001$) at 75 and 100 mg/kg (i.e. dose related). In comparison, there was no detectable motor incoordination on the rotarod to GPS over the dose range 25-75 mg/kg. However, at 100 mg/kg, GPS did instigate a mild impairment of performance, whereby the animals showed decreased dismount latency at 1 h and 3 h. However, this effect was transient ($P< 0.05$) compared to saline treated animals (Fig 6)

3.7. Activity of salicylaldehyde alone on cisplatin-induced neuropathic static mechano-allodynia

Consecutive weekly administration of cisplatin (3.0 mg/kg) for five weeks resulted in a significant reduction ($P < 0.001$) in the mechanical nociceptive threshold to the innocuous von Frey filaments (force) throughout the experimental period. The paw withdrawal threshold decreased from 14.13 g (day -1) to 2.3 g (37th day). Treatment with salicylaldehyde alone had

a significant main dose effect on cisplatin-induced static mechanical allodynia [time = (F (3, 224) = 121.7, P< 0.0001), treatment = (F (7, 224) = 47.15, P< 0.0001), interaction = (F (21, 224) = 8.6, P< 0.0001)]. On 2nd day of last cisplatin injection (37th protocol day), salicylaldehyde did not attenuate cisplatin-induced antinociception to gentle static von Frey stimuli when tested 1 h and 3 h post-doses of 50 mg/kg (P> 0.05, P> 0.05), and 100 mg/kg (P > 0.05, P > 0.05) respectively. Coadministration of salicylaldehyde plus GBP (100mg + 100 mg/kg) resulted in an increased paw withdrawal threshold (PWT) at 1 h (P < 0.01) and 3 h (P < 0.001) post dosing. Administration of GBP and GPS (100 mg/kg) reversed (P< 0.001) the cisplatin-induced fall in the nociceptive threshold since a significant boost (P< 0.001) in the PWT was observed, compared to cisplatin alone treatment (Fig 7).

3.8. *Effect of salicylaldehyde alone on cisplatin-induced neuropathic thermal hypoalgesia*

Treatment with cisplatin (3.0 mg/kg per week) for five successive weeks resulted in extensive changes in the perception of thermal nociception such that significant thermohypoalgesia (P< 0.001) was observed during testing on the 37th protocol day. Consequently, cisplatin increased the nociceptive response latency in the hot plate paradigm from a pre-experimental value of 17.0 s (day -1) to 38.9 s on the concluding experimental day (37th day). Salicylaldehyde by itself did not produce any significant effect on cisplatin-induced thermohypoalgesia at doses of 50 and 100 mg/kg 1 h (P > 0.05) and 3 h (P > 0.05) post administration on the final experimental day. Coadministration of salicylaldehyde and GPS (100 + 100 mg/kg) normalized the cisplatin enhanced thermal threshold considerably at 1 h (P < 0.05) and 3 h (P < 0.01) post injection. On the other hand, GBP and GPS at doses of 100 mg/kg shortened the thermal antinociception response time (s) at 1 h (P < 0.05, P < 0.001) and 3 h (P < 0.01, P < 0.001) post administration, respectively (Fig 8).

3.9. Activity of salicylaldehyde alone and salicylaldehyde plus gabapentin on rotarod performance

A significant main dose effect on motor coordination on rotarod dismount latency was observed 1 h and 3 h post dosing [time = (F (3, 168) = 114.3, P< 0.0001), treatment = (F (7, 168) = 75.07, P< 0.0001), interaction = (F (15, 168) = 25.48, P< 0.0001)]. Salicylaldehyde alone on the other hand, manifested no modifying effect on rotarod latency at doses of 50 (P> 0.05) and 100 mg/kg (P>0.05). Coadministration of GBP and salicylaldehyde (100 + 100 mg/kg) induced a significant decline in the rotarod endurance time (P < 0.001) which was comparable to GBP 100 mg/kg (P < 0.001) at 1 h and 3 h post administration. In contrast,

GPS, at the highest dose used in the experiment, (100 mg/kg) instigated a mild impairment in performance ($P< 0.05$) 1 h and 3 h post administration when compared to saline treated animals (Fig 9).

4. Discussion

The present study was carried out to evaluate the neuropathic pain alleviating effectiveness of GBP and its salicylaldehyde derivative, designated as gabapentsal (GPS), using a refined model of cisplatin-induced neuropathic pain in rats (Authier et al., 2003a; Han et al., 2014). Chemotherapy-induced peripheral neuropathy (CIPN) is a complication in patients who have received a chemotherapeutic agent that is known to be neurotoxic. The platinum compounds (cisplatin, carboplatin, oxaliplatin) belong to a commonly used class of neurotoxic chemotherapeutic agents which are associated with a length dependent neuropathy that primarily affects distal sites, and as doses increase cumulatively, symptoms progress in severity to more proximal areas. The sensory signs and symptoms (mild to moderate numbness and tingling of hands and feet) typically develop before motor symptoms (rare weakness with high doses) and a subset of patients develop painful CIPN (Grisold et al., 2012).

Several antineoplastic drugs have been reported to induce peripheral neuropathy in experimental animals and these include vincristine (Authier et al., 2003b), paclitaxel (Polomano et al., 2001) and cisplatin (Authier et al., 2003a). In this respect, animal models have been used to evaluate anticancer drug-induced neuropathies in a search for potential therapies to prevent or at least minimize nerve damage (Aley et al., 1996; Authier et al., 2003a; Nozaki-Taguchi et al., 2001; Polomano et al., 2001). The cisplatin-induced rat model of CIPN is currently regarded as being suitable for analgesic efficacy profiling of novel compounds and for comparative investigation of the pathobiology of this condition relative to that of CIPN induced by other cancer chemotherapy agents (Han et al., 2014). In this study, weekly injection of cisplatin (3.0 mg/kg i.p.) for 5 weeks (cumulative dose = 15mg/kg) produced reliable mechanical allodynia and thermal hypoalgesia in rat hind paws, which were consistent with previous reports concerning the cisplatin-induced neuropathic pain model (Authier et al., 2003a; Han et al., 2014).

It has been demonstrated that cisplatin causes axonal degeneration, particularly in subcutaneous tissue of the hind paw and to lesser extent, within the sciatic nerve and lumber region of the rat spinal cord in addition to a reduction in peripheral nerve conduction velocity

(Authier et al., 2003a). Furthermore, chronic administration of cisplatin has been reported to be associated with development of mechanical allodynia and hyperalgesia, cold thermal allodynia and hyperalgesia, as well as heat thermal hypoalgesia in rats (Authier et al., 2003a; Han et al., 2014). In the current study, mechanical allodynia and thermal hypoalgesia were selected as the parameter to assess the development of peripheral neuropathic pain because low animal stress levels are associated with this model (Vera et al., 2007). Moreover, cisplatin dosing is known to be associated with thermal hypoalgesia in the rat hind paw which is thought to reflect clinical reports of CIPN in patients. In this instance, development of mechanical allodynia and loss of noxious heat sensitivity may well arise from damage to myelinated αβ fibers with concurrent depletion of C-fibers (Han et al., 2014). In our study, treatment with GBP and GPS limited the expression of mechanical allodynia and thermohypoalgesia caused by chronic cisplatin administration. An important issue is to view the alterations in the observed nociceptive behaviors (mechanical hyperalgesia and thermal hypoalgesia) in relation to the changes in the axons. Mechanical hyperalgesia could be attributed to lesions in large fibers that usually exert an inhibitory feedback on small fibers (Melzack and Wall, 1967; Wall, 1978) which process mechanical hyperalgesia. Other studies have also ascribed the development of mechanical allodynia and loss of noxious heat sensitivity to damaged myelinated αβ fibers with concurrent depletion of C-fibers (Cavanaugh et al., 2009; Dougherty et al., 2004). GBP has been indicated for the treatment of neuropathy, though the exact mechanism through which it inhibits neuropathic pain still remains unclear. Evidence suggests that it potentially operates by increasing the level of GABA (Kocsis and Honmou, 1994) by acting as a non-NMDA receptor antagonist (Chen et al., 2000; Kaneko et al., 2000) and/or by inhibiting the α2δ1 subunit of voltage gated calcium channels (Gee et al., 1996; Shimoyama et al., 2000). GBP (and possibly GPS) are multi-target CNS modulators, acting predominantly on spinal and supraspinal sites of the CNS. GBP is a novel anticonvulsant agent that may have a unique effect on voltage dependent Ca^{++} channel currents at postsynaptic dorsal horn neurons. Furthermore, GBP may interrupt an entire series of events, not just a single process that leads to the development of neuropathic pain. Preclinical models of anti-inflammatory and neuropathic pain indicate that GBP effectively antagonizes the maintenance of this pain (Nicholson, 2000). Several studies advocate that GBP does not bind to either $GABA_A$ or $GABA_B$ receptors (Dooley et al., 1986; Suman-Chauhan et al., 1993) nor is it transformed metabolically into GABA (Vollmer et al., 1986; Taylor et al., 1998; Prakash, 2014). *In vitro* at higher concentrations, GBP is a mixed type inhibitor of GABA-transaminase (Goldlust et al., 1995) and enhances the activity of

partially purified glutamic acid decarboxylase enzyme (Taylor et al., 1992). In turn, NMR spectroscopic data have shown that GABA concentrations *in vivo* are increased in patients receiving GBP (Petroff et al., 1996; Taylor et al., 1992). It is possible therefore that multiple mechanisms are responsible for the anticonvulsant, anxiolytic, neuroprotective (Baydas et al., 2005) and antinociceptive activity observed in different animal models. In the context of the GBP effect on thermal hypoalgesia, a neuroprotective action mediated by glutamate receptor-independent mechanisms has been reported (Popescu et al., 2013). There is also evidence that GBP improves nerve remyelination after chronic sciatic nerve constriction injury (Câmara et al., 2015). However, such neuroprotective or regenerative processes mayor may not be invoked sufficiently rapidly to modify thermal hypoalgesia within the timeframe of our experiments. An alternative explanation therefore may merely implicate motor incoordination and/or sedation as an impediment to the nociceptive stimulus response.

GBP has been demonstrated in several clinical trials to be beneficial against various neuropathies including those associated with diabetes, postherpetic neuralgia, and other aetiologies (Gilron and Flatters, 2006). However, in a large Phase 3 randomized control trial, GBP did not possess positive clinical effects against chemotherapy-induced peripheral neuropathy (CIPN) even though a smaller trial had described some efficacy (Rao et al., 2007). There is therefore, a contrast between the current GBP data in animals and the inconsistencies seen clinically. Other animal studies have reported that an up-regulation of the α2δ1 subunit of voltage-dependent calcium channels in dorsal root ganglia does not occur to nerve injury caused by chemotherapy, but it also does when induced by other insults (Luo et al., 2002). Moreover, CIPN patients express a loss of sensation to light touch, pin prick and temperature rather than mechanical allodynia (Rao et al., 2007). This may explain why GBP appears to be clinically ineffective against CINP in oncology patients and yet it reverses mechanical allodynia in rats.

In this study, a GBP-salicylaldehyde derivative (GPS) was synthesized and its antinociceptive effectiveness was assessed in relation to GBP in the cisplatin-induced neuropathic pain model in rats. Salicylaldehyde(2-hydroxybenzaldehyde) Schiff base derivatives exhibit a broad range of biological activities. They possess promising anti-inflammatory, analgesic (Júnior et al., 2011), antiradical (Horackova et al., 2000), antidiabetic (Vančo et al., 2008), antiviral (Wang et al., 1990), nematicidal (Caboni et al., 2013), antitumor (Hodnett and Dunn, 1970), antifungal (Backes et al., 2014) and antibacterial (Kalaivani et al., 2012) activities. The lack of adverse effects up to 1000 mg/kg of GPS indicates that it is safe to the highest tested dose used in the *in vivo* behavioral studies. Treatment with GPS had an attenuating action on

cisplatin-induced nocifensive behaviors as evidenced by alleviation of mechanical allodynia and thermal hypoalgesia at a relatively low dose (25 mg/kg) as well as at higher doses equivalent to those of GBP. Moreover, GPS was devoid of any observable side effects commonly encountered with the use of GBP such as asthenia, ataxia, (McLean et al., 1999) or motor incoordination (Shahid et al., 2016). This was confirmed using the rotarod paradigm in which GBP induced considerable impairment of motor coordination. These unwanted motoric effects were ostensibly avoided with GPS, though at the highest dose, a transient decrease in endurance dismount latency was observed along with retention of antinociceptive activity. Similarly, salicylaldehyde, when tested at doses of 50 and 100 mg/kg, it did not display any significant effect on cisplatin induced static mechano-allodynia or thermal hypoalgesia. Salicylaldehyde was also found to be devoid of any activity on rotarod performance. Nevertheless, coadministration of GBP with salicylaldehyde (100 + 100 mg/kg) resulted in a decline of the paw withdrawal threshold (static mechano-allodynia) which was comparable to that of GBP alone (Fig.7). Also, there was a normalizing effect of the GBP plus salicylaldehyde combination on cisplatin induced thermohypoalgesia. The activity of the combination was most likely to be attributable to the presence of GBP rather than salicylaldehyde which did not manifest any effect on either of the parameters when it was administered alone. Performance of animals treated with the combination) on the rotarod also expressed motor incoordination analogous to that previously seen with GBP (Shahid et al.,2017). CIPN can be extremely painful and disabling, causing considerable loss of functional abilities liable to decrease quality of life. The treatment of CIPN remains largely ineffective and even though different pharmacological strategies have been attempted, no agent has yet been shown to be advantageous. As a result, many patients are forced to dose-reduce or discontinue potentially curative neurotoxic drugs (Park et al., 2013; Wolf et al., 2008). This emphasizes the need for new pharmacotherapy with an improved therapeutic profile that could prove to be beneficial in the treatment of CIPN.

5. Conclusion

Weekly injections of cisplatin (3.0 mg/kg) for five weeks evoked nociceptive sensitivity to non-painful tactile stimuli (static mechanical allodynia) and also increased heat nociceptive perception (thermohypoalgesia) expressed on the 37^{th} protocol day. GBP and its salicylaldehyde derivative (GPS) both suppressed these cisplatin-induced nocifensive behaviors as a manifestation of a neuropathic pain alleviating propensity in this rodent model of CIPN.

GPS did not markedly impair the motor performance, as the motor incoordination detected with it was of a much lower magnitude than that with GBP in the rotarod assay, demonstrating that the observed antinociception was unlikely to have occurred due to motor abnormalities. Therefore, GPS may be advantageous in the management of CINP, though further clinical and pre-clinical studies are warranted.

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Conflict of interest

The authors have no conflicts of interest to declare.

References

Albers, J.W., Chaudhry, V., Cavaletti, G., Donehower, R.C., 2014. Interventions for preventing neuropathy caused by cisplatin and related compounds. The Cochrane Library, Art. No.: CD005228.005221-005297.

Aley, K., Reichling, D., Levine, J., 1996. Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. Neuroscience 73, 259-265.

Ali, G., Subhan, F., Abbas, M., Zeb, J., Shahid, M., Sewell, R.D.E, 2015. A streptozotocin-induced diabetic neuropathic pain model for static or dynamic mechanical allodynia and vulvodynia: Validation using topical and systemic gabapentin. Naunyn. Schmiedebergs Arch. Pharmacol. 388, 1129–1140.

Authier, N., Gillet, J.-P., Fialip, J., Eschalier, A., Coudore, F., 2003a. An animal model of nociceptive peripheral neuropathy following repeated cisplatin injections. Exp. Neurol. 182, 12-20.

Authier, N., Gillet, J.-P., Fialip, J., Eschalier, A., Coudore, F., 2003b. A new animal model of vincristine-induced nociceptive peripheral neuropathy. Neurotoxicology 24, 797-805.

Backes, G.L., Neumann, D.M., Jursic, B.S., 2014. Synthesis and antifungal activity of substituted salicylaldehyde hydrazones, hydrazides and sulfohydrazides. Biorg. Med. Chem. 22, 4629-4636.

Baydas, G., Sonkaya, E., Tuzcu, M., Yasar, A., Donder, E., 2005. Novel role for gabapentin in neuroprotection of central nervous system in streptozotocine-induced diabetic rats. Acta Pharmacologica Sinica 26, 417-422.

Caboni, P., Aissani, N., Cabras, T., Falqui, A., Marotta, R., Liori, B., Ntalli, N., Sarais, G., Sasanelli, N., Tocco, G., 2013. Potent nematicidal activity of phthalaldehyde, salicylaldehyde, and cinnamic aldehyde against Meloidogyne incognita. J. Agric. Food Chem. 61, 1794-1803.

Câmara, C.C., Araújo, C.V., de Sousa, K.K., Brito, G.A., Vale, M.L., Raposo R. da S, Mendonça, F.E., Mietto, B.S., Martinez, A.M., Oriá, R.B.,2015. Gabapentin attenuates neuropathic pain and improves nerve myelination after chronic sciatic constriction in rats.Neurosci. Lett. 607,52-8.

Cavaletti, G., Marmiroli, P., 2010. Chemotherapy-induced peripheral neurotoxicity. Nat. Rev. Neurol. 6, 657-666.

Cavanaugh, D.J., Lee, H., Lo, L., Shields, S.D., Zylka, M.J., Basbaum, A.I., Anderson, D.J., 2009. Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. PNAS. 106, 9075-9080.

Chaplan, S., Bach, F., Pogrel, J., Chung, J., Yaksh, T., 1994. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Meth. 53, 55-63.

Chaudhry, V., Chaudhry, M., Crawford, T., Simmons-O'Brien, E., Griffin, J., 2003. Toxic neuropathy in patients with pre-existing neuropathy. Neurology 60, 337-340.

Chen, S.-R., Eisenach, J.C., McCaslin, P.P., Pan, H.-L., 2000. Synergistic effect between intrathecal non-NMDA antagonist and gabapentin on allodynia induced by spinal nerve ligation in rats. J. Am. Soc Anesthesiol. 92, 500-500.

Dooley, D., Bartoszyk, G., Hartenstein, J., Reimann, W., Rock, D., Satzinger, G., 1986. Preclinical pharmacology of gabapentin, Golden Jubilee Conference and Northerm European Epilepsy Meeting. Abst.

Dougherty, P.M., Cata, J.P., Cordella, J.V., Burton, A., Weng, H.-R., 2004. Taxol-induced sensory disturbance is characterized by preferential impairment of myelinated fiber function in cancer patients. Pain 109, 132-142.

Field, M., Oles, R., Lewis, A., McCleary, S., Hughes, J., Singh, L., 1997. Gabapentin (neurontin) and S-(+)-3-isobutylgaba represent a novel class of selective antihyperalgesic agents. Br. J. Pharmacol. 121, 1513-1522.

Gee, N.S., Brown, J.P., Dissanayake, V.U., Offord, J., Thurlow, R., Woodruff, G.N., 1996. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the subunit of a calcium channel. J. Biol. Chem. 271, 5768-5776.

Gilron, I., Flatters, S.J., 2006. Gabapentin and pregabalin for the treatment of neuropathic pain: A review of laboratory and clinical evidence. Pain Res. Manag. 11, 16A-29A.

Goldlust, A., Su, T.-Z., Welty, D.F., Taylor, C.P., Oxender, D.L., 1995. Effects of anticonvulsant drug gabapentin on the enzymes in metabolic pathways of glutamate and GABA. Epilepsy Res. 22, 1-11.

Grisold, W., Cavaletti, G., Windebank, A.J., 2012. Peripheral neuropathies from chemotherapeutics and targeted agents: diagnosis, treatment, and prevention. Neuro-oncol. 14, iv45-iv54.

Han, F.Y., Wyse, B.D., Smith, M.T., 2014. Optimization and pharmacological characterization of a refined cisplatin-induced rat model of peripheral neuropathic pain. Behav. Pharmacol. 25, 732-740.

He, G., Xiao, X., Zou, M., Zhang, C., Xia, S., 2016. Pemetrexed/cisplatin as first-line chemotherapy for advanced lung cancer with brain metastases: A case report and literature review. Medicine. 95 (32):e4401.

Hodnett, E.M., Dunn, W.J., 1970. Structure-antitumor activity correlation of some Schiff bases. J. Med. Chem. 13, 768-770.

Horackova, M., Ponka, P., Byczko, Z., 2000. The antioxidant effects of a novel iron chelator salicylaldehyde isonicotinoyl hydrazone in the prevention of H_2O_2 injury in adult cardiomyocytes. Cardiovasc. Res. 47, 529-536.

Huang, C., Hu, Z.-P., Long, H., Shi, Y.-S., Han, J.-S., Wan, Y., 2004. Attenuation of mechanical but not thermal hyperalgesia by electroacupuncture with the involvement of opioids in rat model of chronic inflammatory pain. Brain Res. Bull. 63, 99-103.

Júnior, W.B., Alexandre-Moreira, M.S., Alves, M.A., Perez-Rebolledo, A., Parrilha, G.L., Castellano, E.E., Piro, O.E., Barreiro, E.J., Lima, L.M., Beraldo, H., 2011. Analgesic and anti-inflammatory activities of salicylaldehyde 2-chlorobenzoyl hydrazone (H2LASSBio-466), salicylaldehyde 4-chlorobenzoyl hydrazone (H2LASSBio-1064) and their zinc (II) complexes. Molecules 16, 6902-6915.

Kalaivani, P., Prabhakaran, R., Ramachandran, E., Dallemer, F., Paramaguru, G., Renganathan, R., Poornima, P., Padma, V.V., Natarajan, K., 2012. Influence of terminal substitution on structural, DNA, protein binding, anticancer and antibacterial activities of palladium (II) complexes containing 3-methoxy salicylaldehyde-4 (N) substituted thiosemicarbazones. Dalton Trans. 41, 2486-2499.

Kaley, T.J., DeAngelis, L.M., 2009. Therapy of chemotherapy-induced peripheral neuropathy. Br. J. Haematol. 145, 3-14.

Kaneko, M., Mestre, C., Sánchez, E.H., Hammond, D.L., 2000. Intrathecally administered gabapentin inhibits formalin-evoked nociception and the expression of Fos-like immunoreactivity in the spinal cord of the rat. J. Pharmacol. Exp. Ther. 292, 743-751.

Kocsis, J.D., Honmou, O., 1994. Gabapentin increases GABA-induced depolarization in rat neonatal optic nerve. Neurosci. Lett. 169, 181-184.

Luo, Z., Calcutt, N., Higuera, E., Valder, C., Song, Y.-H., Svensson, C., Myers, R., 2002. Injury type-specific calcium channel α2δ-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. J. Pharmacol. Exp. Ther. 303, 1199-1205.

Lynch, T.J., Bondarenko, I., Luft, A., Serwatowski, P., Barlesi, F., Chacko, R., Sebastian, M., Neal, J., Lu, H., Cuillerot, J.-M., 2012. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non–small-cell lung cancer: results from a randomized, double-blind, multicenter phase II study. J. Clin Oncol. 30, 2046-2054.

Mallesha, L., Mohana, K.N., Veeresh, B., 2012. Synthesis and biological activities of Schiff bases of gabapentin with different aldehydes and ketones: a structure–activity relationship study. Med. Chem. Res. 21, 1-9.

Markman, M., 2003. Toxicities of the platinum antineoplastic agents. Expert Opin. Drug Saf. 2, 597-607.

Markman, M., 2006. Chemotherapy-induced peripheral neuropathy: underreported and underappreciated. Curr. Pain Headache Rep. 10, 275-278.

Marschner, N., Arnold, D., Engel, E., Hutzschenreuter, U., Rauh, J., Freier, W., Hartmann, H., Frank, M., Jänicke, M., 2015. Oxaliplatin-based first-line chemotherapy is associated with improved overall survival compared to first-line treatment with irinotecan-based chemotherapy in patients with metastatic colorectal cancer–results from a prospective cohort study. Clin.Epidemiol. 7, 295-303.

McKeage, M.J., 1995. Comparative adverse effect profiles of platinum drugs. Drug Saf. 13, 228-244.

McLean, M.J., Morrell, M.J., Willmore, L.J., Privitera, M.D., Faught, R.E., Holmes, G.L., Magnus-Miller, L., Bernstein, P., Rose-Legatt, A., 1999. Safety and tolerability of

gabapentin as adjunctive therapy in a large, multicenter study. Epilepsia 40, 965-972.

Melzack, R., Wall, P.D., 1967. Pain mechanisms: a new theory. Survey of Anesthesiology 11, 89-90.

Nicholson, B., 2000. Gabapentin use in neuropathic pain syndromes. Acta Neurol. Scand. 101(6):359-71.

Nozaki-Taguchi, N., Chaplan, S.R., Higuera, E.S., Ajakwe, R.C., Yaksh, T.L., 2001. Vincristine-induced allodynia in the rat. Pain 93, 69-76.

Ocean, A.J., Vahdat, L.T., 2004. Chemotherapy-induced peripheral neuropathy: pathogenesis and emerging therapies. Support Care Cancer 12, 619-625.

OECD guidelines, 2001. OECD (Organization for Economic Co-operation and Development) revised draft guidelines 423. OECD Guidelines for the Testing of Chemicals. Organization for Economic Co-operation and Development.

Park, S.B., Goldstein, D., Krishnan, A.V., Lin, C.S.Y., Friedlander, M.L., Cassidy, J., Koltzenburg, M., Kiernan, M.C., 2013. Chemotherapy induced peripheral neurotoxicity: A critical analysis. CA. Cancer J. Clin. 63, 419-437.

Petroff, O.A., Rothman, D.L., Behar, K.L., Lamoureux, D., Mattson, R.H., 1996. The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. Ann. Neurol. 39, 95-99.

Polomano, R.C., Mannes, A.J., Clark, U.S., Bennett, G.J., 2001. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. Pain 94, 293-304.

Popescu, B.O., Tuineag, M., Stoica, R., 2013. Gabapentin is neuroprotective through glutamate receptor-independent mechanisms in staurosporine-induced apoptosis of cultured rat cerebellar neurons. Tranl. Neurosci. 4, 429–436.

Prakash, C., 2014. Gabapentin. Handbook of Metabolic Pathways of Xenobiotics. 2, 1-2. Wiley.

Rainsford, K.D., 2004. Aspirin and related drugs. CRC Press.

Rao, R.D., Michalak, J.C., Sloan, J.A., Loprinzi, C.L., Soori, G.S., Nikcevich, D.A., Warner, D.O., Novotny, P., Kutteh, L.A., Wong, G.Y., 2007. Efficacy of gabapentin in the management of chemotherapy-induced peripheral neuropathy. Cancer 110, 2110-2118.

Rose, M., Kam, P., 2002. Gabapentin: Pharmacology and its use in pain management. Anaesthesia 57, 451-462.

Rosenberg, J.M., Harrell, C., Ristic, H., Werner, R.A., de Rosayro, A.M., 1997. The effect of gabapentin on neuropathic pain. Clin. J. Pain. 13, 251-255.

Ross, J., Goller, K., Hardy, J., Riley, J., Broadley, K., A'hern, R., Williams, J., 2005. Gabapentin is effective in the treatment of cancer-related neuropathic pain: A prospective, open-label study. J. Palliat. Med. 8, 1118-1126.

Rowbotham, M., Harden, N., Stacey, B., Bernstein, P., Magnus-Miller, L., Group, G.P.N.S., 1998. Gabapentin for the treatment of postherpetic neuralgia: a randomized controlled trial. JAMA. 280, 1837-1842.

Serpell, M., Group, N.P.S., 2002. Gabapentin in neuropathic pain syndromes: a randomised, double-blind, placebo-controlled trial. Pain 99, 557-566.

Shahid, M., Subhan, F., Ahmad, N., Ali, G., Akbar, S., Fawad, K., Sewell, R.D.E, 2017. Topical gabapentin gel alleviates allodynia and hyperalgesia in the chronic sciatic nerve constriction injury neuropathic pain model. Eur. J. Pain. 21, 668-680.

Shimoyama, M., Shimoyama, N., Hori, Y., 2000. Gabapentin affects glutamatergic excitatory neurotransmission in the rat dorsal horn. Pain 85, 405-414.

Stillman, M., Cata, J.P., 2006. Management of chemotherapy-induced peripheral neuropathy. Curr. Pain Headache Rep. 10, 279-287.

Suman-Chauhan, N., Webdale, L., Hill, D.R., Woodruff, G.N., 1993. Characterisation of [3H] gabapentin binding to a novel site in rat brain: homogenate binding studies. Eur. J. Pharmacol. 244, 293-301.

Taylor, C.P., Gee, N.S., Su, T.-Z., Kocsis, J.D., Welty, D.F., Brown, J.P., Dooley, D.J., Boden, P., Singh, L., 1998. A summary of mechanistic hypotheses of gabapentin pharmacology. Epilepsy Res. 29, 233-249.

Taylor, C.P., Vartanian, M.G., Andruszkiewicz, R., Silverman, R.B., 1992. 3-Alkyl GABA and 3-alkylglutamic acid analogues: two new classes of anticonvulsant agents. Epilepsy Res. 11, 103-110.

Van Cutsem, E., Arends, J., 2005. The causes and consequences of cancer-associated malnutrition. Eur. J. Oncol. Nurs. 9 (Suppl 2), S51-S63.

Vančo, J., Marek, J., Trávníček, Z., Račanská, E., Muselík, J., Švajlenová, O., 2008. Synthesis, structural characterization, antiradical and antidiabetic activities of copper (II) and zinc (II) Schiff base complexes derived from salicylaldehyde and β-alanine. J. Inorg. Biochem. 102, 595-605.

Vera, G., Chiarlone, A., Cabezos, P.A., Pascual, D., Martín, M.I., Abalo, R., 2007. WIN 55,212-2 prevents mechanical allodynia but not alterations in feeding behaviour induced by chronic cisplatin in the rat. Life Sci. 81, 468-479.

Vollmer, K.O., von Hodenberg, A., Kölle, E.U., 1986. Pharmacokinetics and metabolism of gabapentin in rat, dog and man. Arzneimittelforschung 36, 830-839.

Wall, P.D., 1978. The gate control theory of pain mechanisms. A re-examination and re-statement. Brain 101, 1-18.

Wang, P.H., Keck, J.G., Lien, E.J., Lai, M.M., 1990. Design, synthesis, testing, and quantitative structure-activity relationship analysis of substituted salicylaldehyde Schiff bases of 1-amino-3-hydroxyguanidine tosylate as new antiviral agents against coronavirus. J. Med. Chem. 33, 608-614.

Warner, E., 1995. Neurotoxicity of cisplatin and taxol. Int. J. Gynecol. Cancer 5, 161-169.

Wolf, S., Barton, D., Kottschade, L., Grothey, A., Loprinzi, C., 2008. Chemotherapy-induced peripheral neuropathy: prevention and treatment strategies. Eur. J. Cancer 44, 1507-1515.

Zhang, J., Wang, Z., Hu, X., Wang, B., Wang, L., Yang, W., Liu, Y., Liu, G., Di, G., Hu, Z., 2015. Cisplatin and gemcitabine as the first line therapy in metastatic triple negative breast cancer. Int. J.Cancer 136, 204-211.

Figure legends

Fig 1: Chemical structures of gabapentin (GBP) and gabapentsal (GPS).

Figure 2: Effect of single gabapentin (GBP) treatment at 50 mg/kg (GBP-50), 75 mg/kg (GBP-75) and 100 mg/kg (GBP-100) on the expression of cisplatin-induced static mechanical allodynia [diminished von Frey filament threshold pressure; PWT (g)] in the hind paws after 5 weekly i.p. injections of cisplatin at 3.0 mg/kg (cisplatin-3) in rats. Data are expressed as mean ± S.E.M. of 50% PWT. $^{###}P < 0.001$ compared to saline treated animals, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to cisplatin alone treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. $n = 8$ rats per group.

Figure 3: Effect of a single treatment with gabapentin (GBP) at 100 mg/kg (GBP-100) and its derivative, gabapentsal at 25 mg/kg (GPS-25), 50 mg/kg (GPS-50), 75 mg/kg (GPS-75) and 100 mg/kg (GPS-100) on the expression of cisplatin-induced static mechanical allodynia [diminished von Frey filament threshold pressure; PWT (g)]in the hind paws after 5 weekly i.p. injections of cisplatin at 3.0 mg/kg (cisplatin-3) in rats. Data are expressed as mean ± S.E.M. of 50% PWT. $^{###}P < 0.001$ compared to vehicle treated animals, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to cisplatin alone treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. $n = 8$ rats per group.

Figure 4: Effect of a single gabapentin (GBP) treatment at 50 mg/kg (GBP-50), 75 mg/kg (GBP-75) and 100 mg/kg (GBP-100) on the expression of cisplatin-induced thermal hypoalgesia[increased response latency to the thermal stimulus in the hot plate paradigm (s)]after 5 weekly i.p. injections of cisplatin at 3.0 mg/kg (cisplatin-3) in rats. Data are expressed as mean ± S.E.M. of reaction latency. $^{###}P < 0.001$ compared to saline treated animals, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to cisplatin alone treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. $n = 8$ rats per group.

Figure 5: Effect of a single treatment with gabapentin (GBP) at 100 mg/kg (GBP-100) and its derivative, gabapentsal at 25 mg/kg (GPS-25), 50 mg/kg (GPS-50), 75 mg/kg (GPS-75) and 100 mg/kg (GPS-100) on cisplatin-induced thermal hypoalgesia [increased response latency to the thermal stimulus in the hot plate paradigm(s)] after 5 weekly i.p. injections of cisplatin at 3.0 mg/kg (cisplatin-3) in rats. Data are expressed as mean ± S.E.M. of reaction latency. $^{###}P < 0.001$ compared to vehicle treated animals, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to cisplatin alone treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. $n = 8$ rats per group.

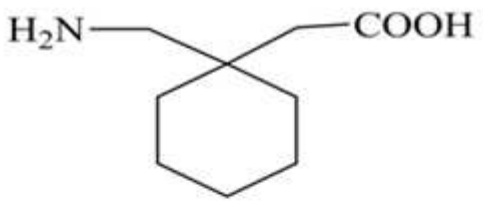
Figure 6: Effect of a single treatment with gabapentin (GBP) at 50 mg/kg (GBP-50), 75 mg/kg (GBP-75), and 100 mg/kg (GBP-100) and its derivative, gabapentsal at 25 mg/kg (GPS-25),50 mg/kg (GPS-50), 75 mg/kg (GPS-75) and 100 mg/kg (GPS-100) on rotarod performance in rats. Data are expressed as mean ± S.E.M. of endurance latency(s) across

three test trials. *P < 0.05, ***P < 0.001 compared to vehicle treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. n = 6 rats per group.

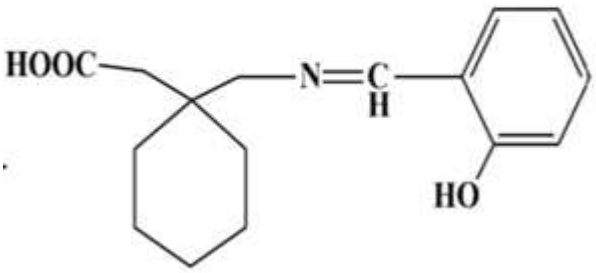
Figure 7: Effect of a single treatment with gabapentin (GBP) at 100 mg/kg (GBP-100) and its derivative, gabapentsal at 100 mg/kg (GPS-100), salicylaldehyde 50 mg/kg (Sal-50) and 100 mg/kg (Sal-100) and coadministration of gabapentin and salicylaldehyde (100 + 100) mg/kg (GBP-100 + Sal-100) on the expression of cisplatin-induced static mechanical allodynia [diminished von Frey filament threshold pressure; PWT (g)] in the hind paws after 5 weekly i.p. injections of cisplatin at 3.0 mg/kg (cisplatin-3) in rats. Data are expressed as mean ± S.E.M. of 50% PWT. $^{###}P$ < 0.001 compared to saline treated animals, *P < 0.05, **P < 0.01, ***P < 0.001 compared to cisplatin alone treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. n = 8 rats per group.

Figure 8: Effect of a single gabapentin (GBP) treatment at 100 mg/kg (GBP-100), gabapentsal 100 mg/kg (GPS-100), salicylaldehyde 50 mg/kg (Sal-50), 100 mg/kg (Sal-100) and coadministration of gabapentin 100 mg/kg plus salicylaldehyde 100 mg/kg (GBP-100 + Sal-100) on the expression of cisplatin-induced thermal hypoalgesia [increased response latency to the thermal stimulus in the hot plate paradigm (s)] after 5 weekly i.p. injections of cisplatin at 3.0 mg/kg (cisplatin-3) in rats. Data are expressed as mean ± S.E.M. of reaction latency. $^{###}P$ < 0.001 compared to saline treated animals, *P < 0.05, **P < 0.01, ***P < 0.001 compared to cisplatin alone treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. n = 8 rats per group.

Figure 9: Effect of a single treatment with gabapentin (GBP) at 100 mg/kg (GBP-100), gabapentsal at 100 mg/kg (GPS-100), salicylaldehyde 50 mg/kg (Sal-50), salicylaldehyde 100 mg/kg (Sal-100) and coadministration of gabapentin 100 mg/kg plus salicylaldehyde 100 mg/kg (GBP-100 + Sal-100) on rotarod performance in rats. Data are expressed as mean ± S.E.M. of endurance latency(s) across three test trials. *P < 0.05, ***P < 0.001 compared to saline treated animals, two-way repeated measures ANOVA followed by *post hoc* Bonferroni analysis. n = 6 rats per group.



(A) Gabapentin



(B) Gabapentsal

Fig.1

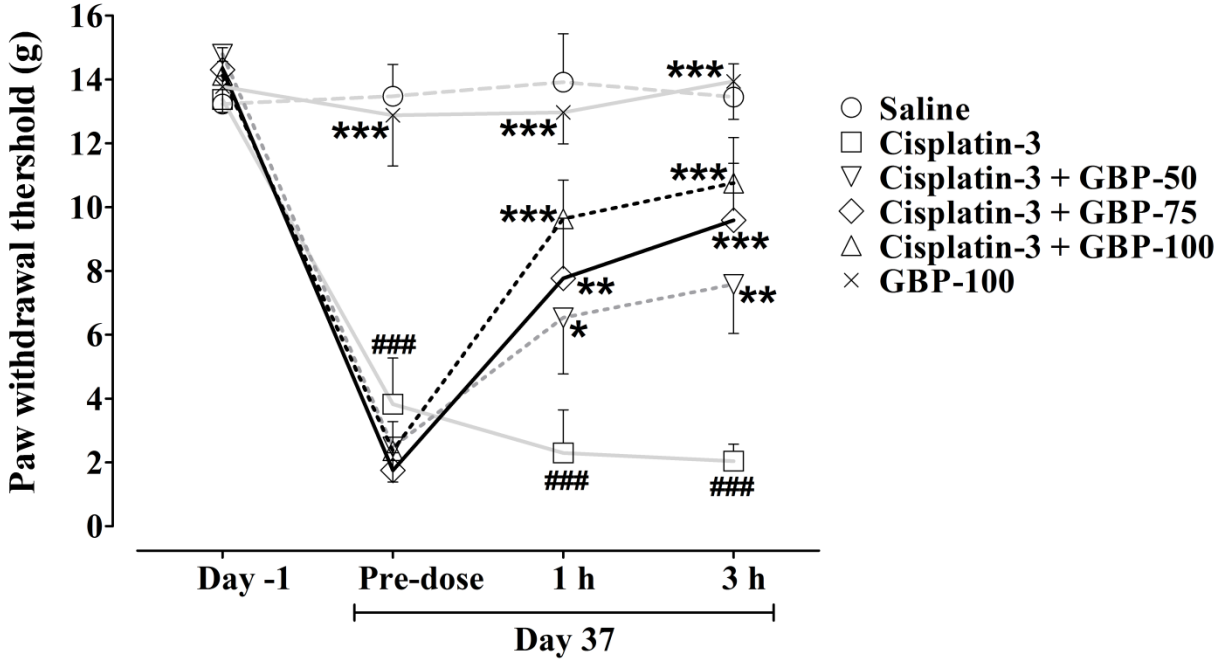


Fig.2

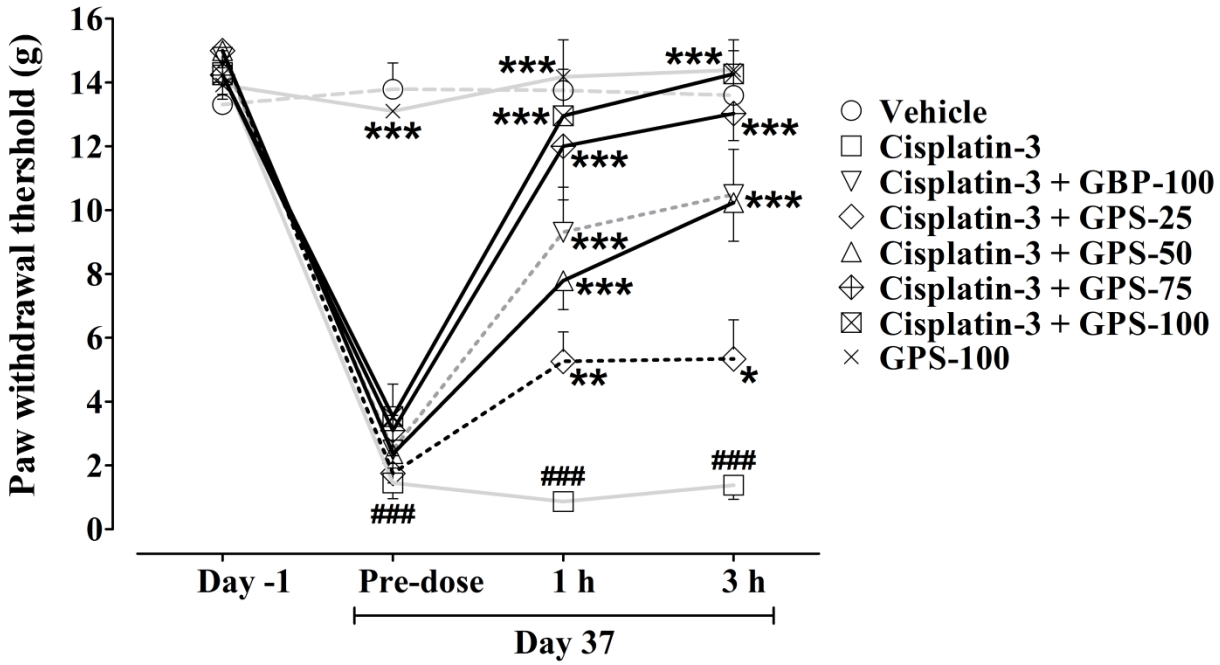

Paw withdrawal thershold (g)
16
14
12
10
8
6
4
2
0
Day -1
Pre-dose
1 h
3 h
Day 37
***
**
*
###
Vehicle
Cisplatin-3
Cisplatin-3 + GBP-100
Cisplatin-3 + GPS-25
Cisplatin-3 + GPS-50
Cisplatin-3 + GPS-75
Cisplatin-3 + GPS-100
GPS-100

Fig.3

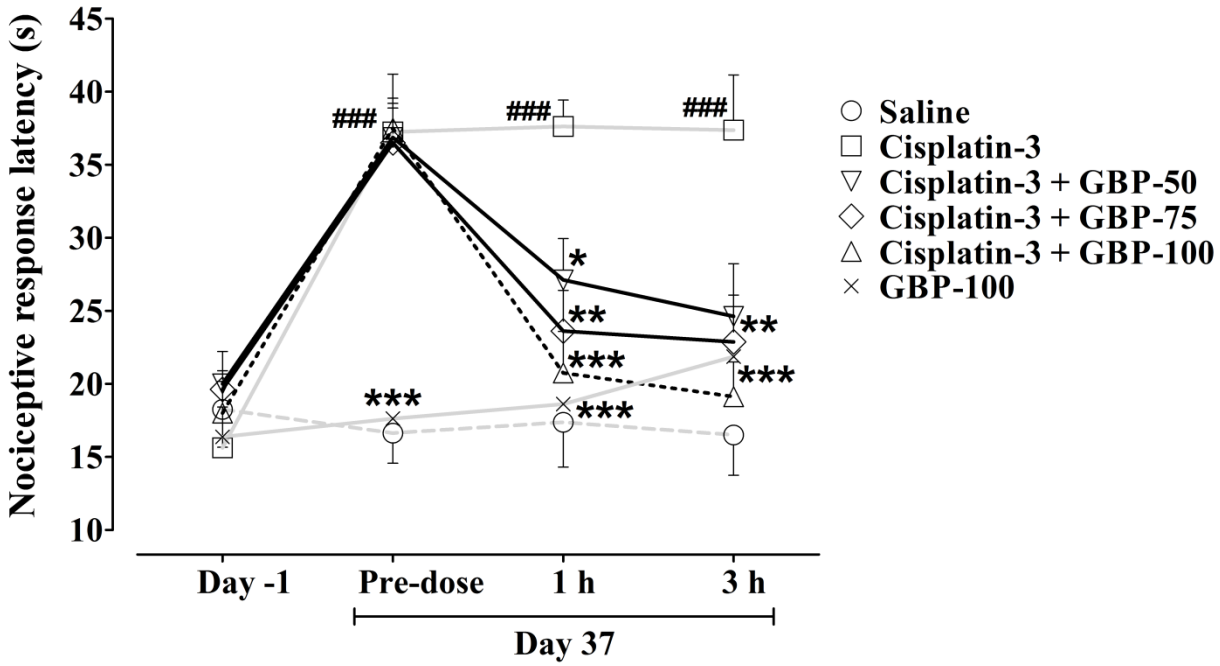

Nociceptive response latency (s)
45
40
35
30
25
20
15
10
###
###
###
*
**
**
***
***
***
***
Day -1
Pre-dose
1 h
3 h
Day 37
Saline
Cisplatin-3
Cisplatin-3 + GBP-50
Cisplatin-3 + GBP-75
Cisplatin-3 + GBP-100
GBP-100

Fig. 4

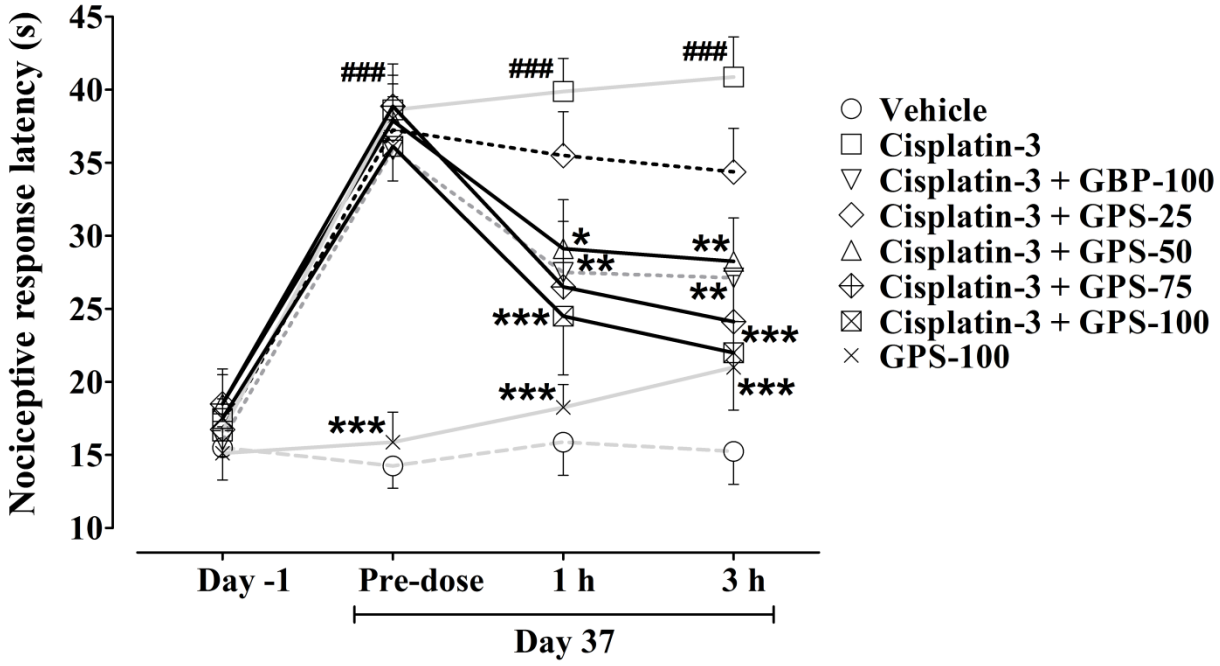

Nociceptive response latency (s)
45
40
35
30
25
20
15
10
###
###
###
*
**
**
**
***
***
***
***
Day -1
Pre-dose
1 h
3 h
Day 37
Vehicle
Cisplatin-3
Cisplatin-3 + GBP-100
Cisplatin-3 + GPS-25
Cisplatin-3 + GPS-50
Cisplatin-3 + GPS-75
Cisplatin-3 + GPS-100
GPS-100

Fig. 5

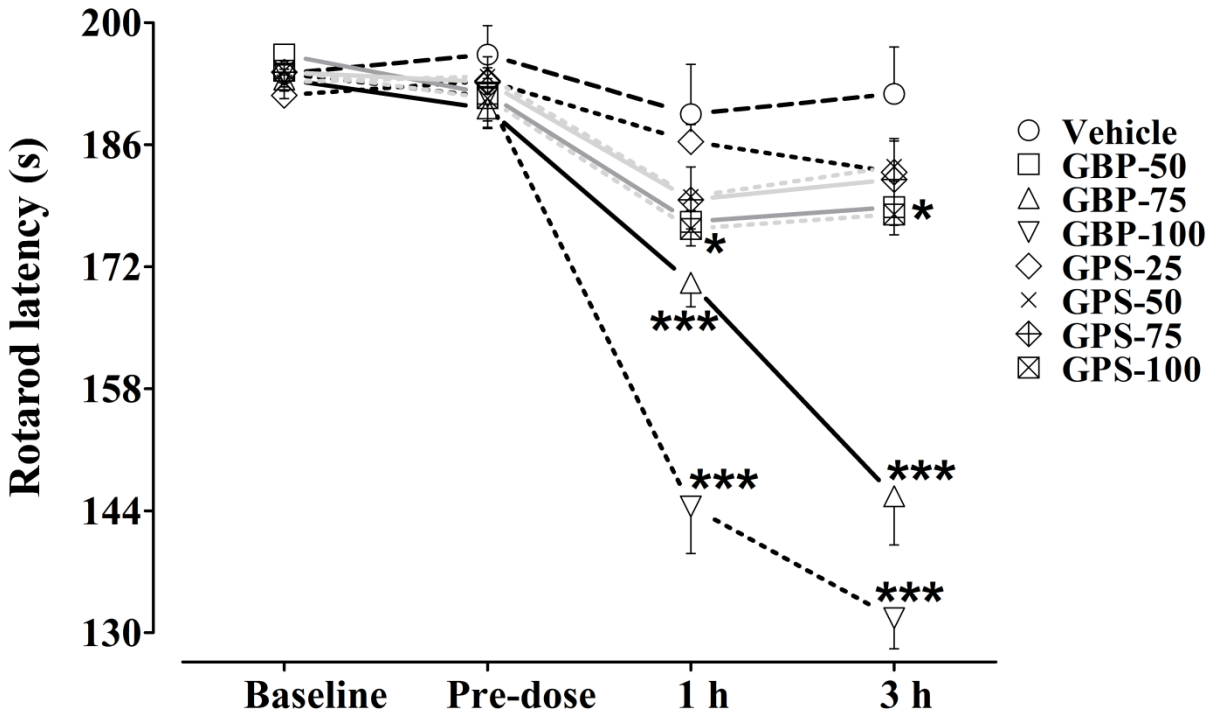

Rotarod latency (s)
200
186
172
158
144
130
Baseline
Pre-dose
1 h
3 h
*
***
***
***
*
Vehicle
GBP-50
GBP-75
GBP-100
GPS-25
GPS-50
GPS-75
GPS-100

Fig. 6

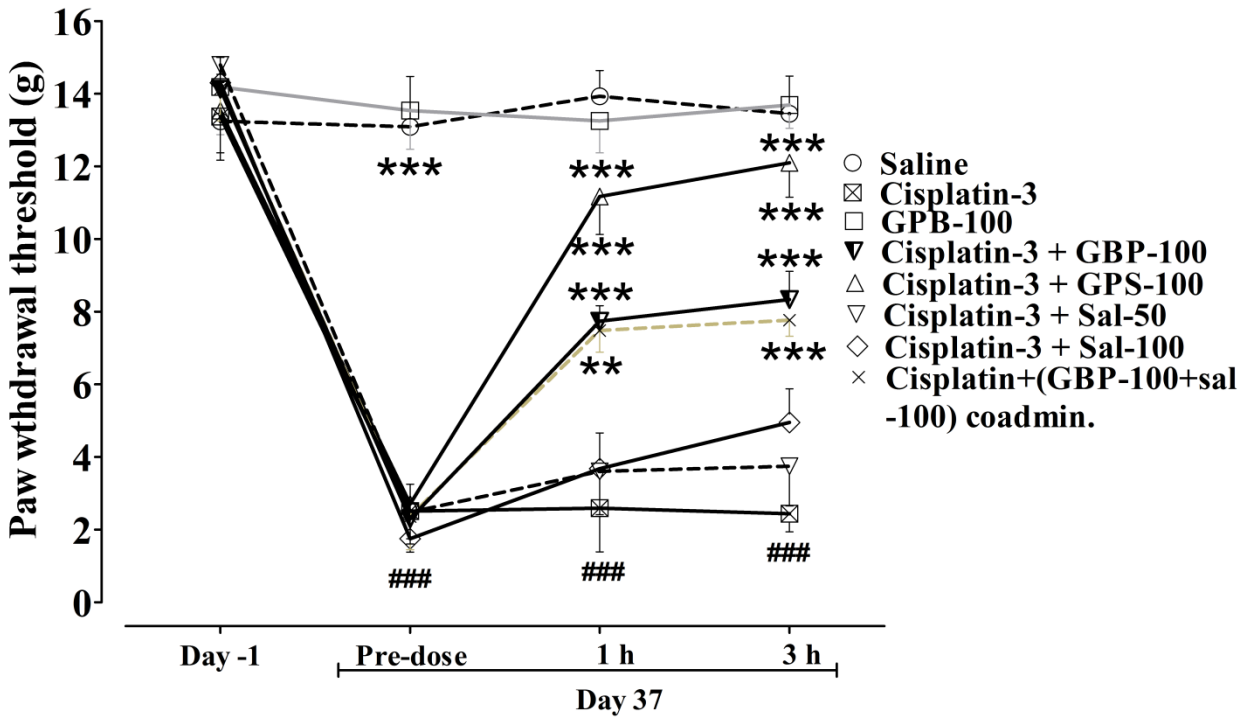

Paw wthdrawal threshold (g)
16
14
12
10
8
6
4
2
0
Day -1
Pre-dose
1 h
3 h
Day 37
***
**
###
Saline
Cisplatin-3
GPB-100
Cisplatin-3 + GBP-100
Cisplatin-3 + GPS-100
Cisplatin-3 + Sal-50
Cisplatin-3 + Sal-100
Cisplatin+(GBP-100+sal -100) coadmin.

Fig.7

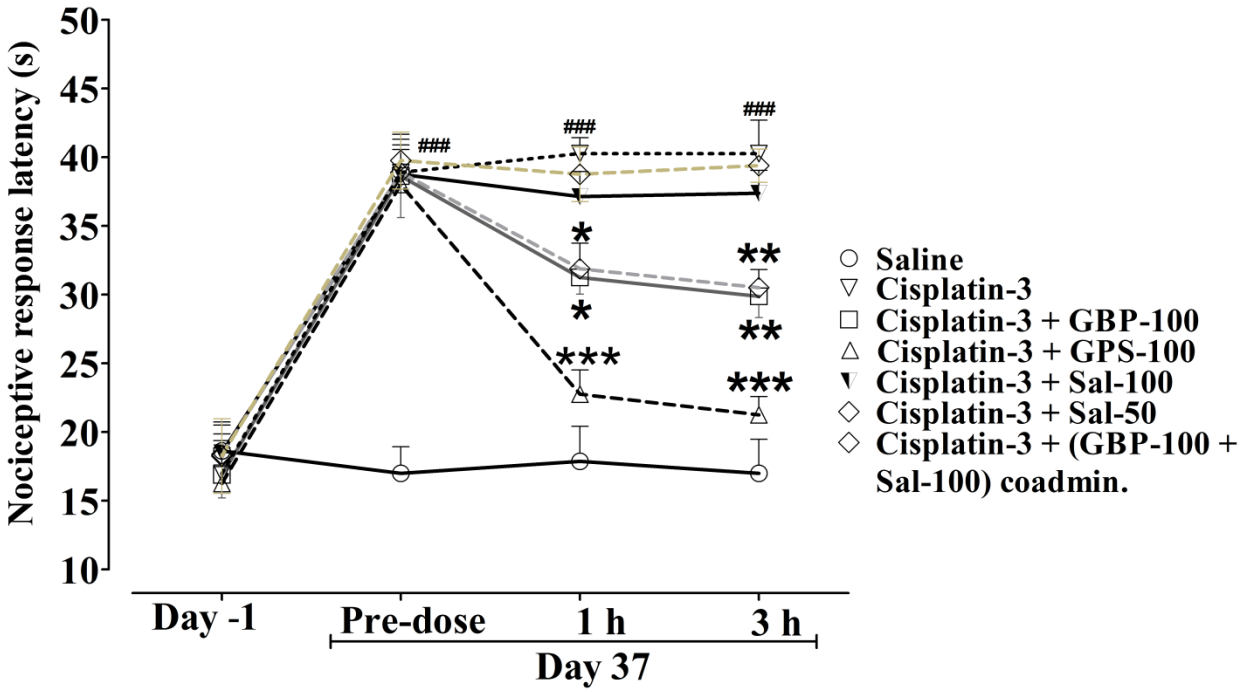

Nociceptive response latency (s)
50
45
40
35
30
25
20
15
10
###
###
###
*
*
**
**
***
***
Day -1
Pre-dose
1 h
3 h
Day 37
Saline
Cisplatin-3
Cisplatin-3 + GBP-100
Cisplatin-3 + GPS-100
Cisplatin-3 + Sal-100
Cisplatin-3 + Sal-50
Cisplatin-3 + (GBP-100 + Sal-100) coadmin.

Fig.8

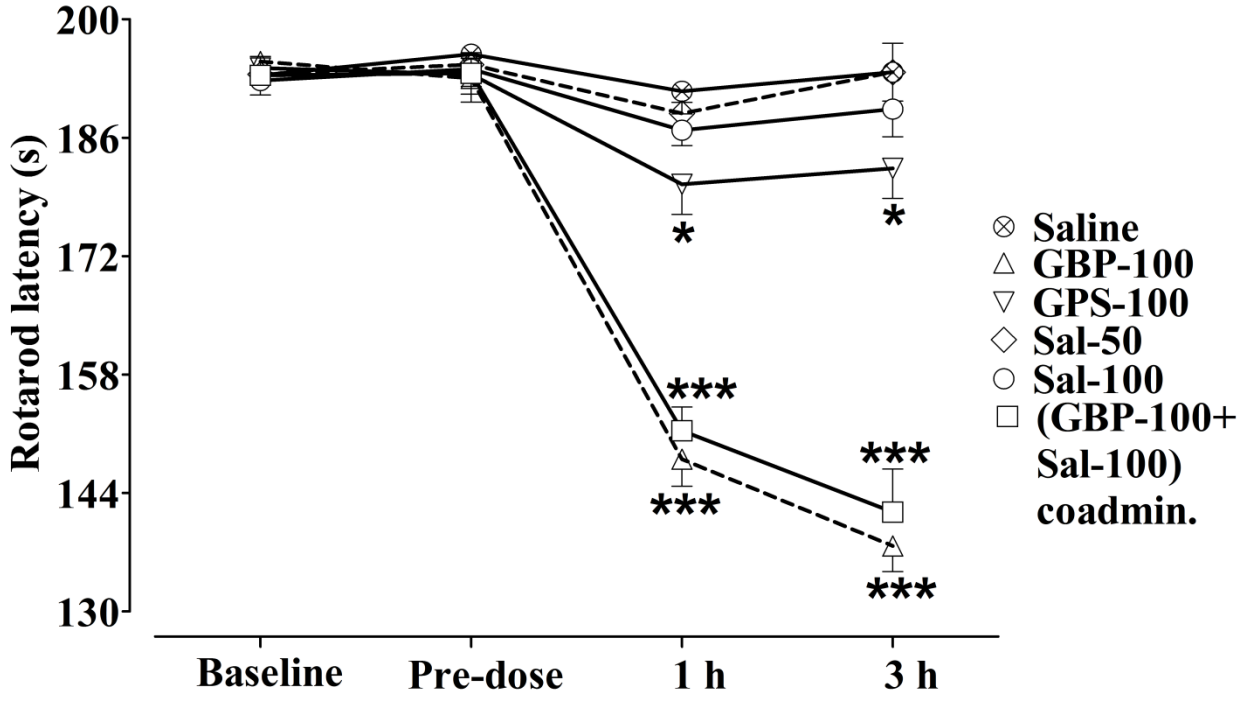

200
186
172
158
144
130
Rotarod latency (s)
Baseline
Pre-dose
1 h
3 h
*
*
***
***
***
***
Saline
GBP-100
GPS-100
Sal-50
Sal-100
(GBP-100+ Sal-100) coadmin.

Fig.9