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Antinociceptive effect of three common analgesic drugs on peripheral neuropathy induced by paclitaxel in rats

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ABSTRACT

Nowadays, there are no validated drugs to control the neuropathic pain induced by paclitaxel, one of the most effective antineoplastic drugs.
The aim was to study the involvement of opioid and NMDA receptor on established paclitaxel-induced pain, testing three common analgesics drugs morphine, ketamine and methadone.
Animals received four intraperitoneal (i.p.) injections on alternate days of paclitaxel (1 mg/kg). Three weeks later, animals showed a mechanical and heat allodynia/hyperalgesia. Morphine (1, 2.5, 5 and 10 mg/kg) abolished the reduction in the mechanical and thermal withdrawal thresholds in a dose dependent manner. This effect was blocked by naloxone. Only highest dose of ketamine (50 mg/kg) was able to increase the mechanical and thermal threshold and returned to basal values. Subanalgesic doses of morphine (1 mg/kg) and ketamine (12.5 mg/kg) produced an additive effect on heat hyperalgesia reaching an antinociceptive effect. This combination did not induce any change on tactile allodynia. Methadone (2.5 and 5 mg/kg) produced an analgesic effect that was completely antagonized by naloxone in both tests.
Our results confirm that: the activation of opioids receptor produced analgesia; the blockade of NMDA receptors produce antinociception but at high doses with motor impairments and low doses of ketamine enhancing the effect of opioids.

1. Introduction

Neuropathic pain is one of the main side effects that follow the administration of a wide number of anti-tumoral agents such as paclitaxel (Rowinsky et al., 1990). Nowadays, there is no effective treatment to prevent or reverse this chemotherapy-induced neuropathy (Quasthoff and Hartung, 2002). The mechanism(s) responsible for the pain syndrome are quite unknown (Polomano et al., 2001). Animals treated with paclitaxel present spontaneous discharge in A- and C-fibers (Xiao and Bennett, 2008) and a mitochondrial dysfunction and this could be the origin of the neuropathic pain (Flatters and Bennett, 2006).

To date, using animal models of chemotherapy-induced nerve damage potential therapeutic drugs have been tested. Flatters and Bennett (2004) reported that ethosuximide but not morphine and MK-801 reversed paclitaxel-induced painful neuropathy. Many drugs like cannabinoid (Pascual et al., 2005), gabapentin (Matsumoto et al., 2006), thalidomide and minocycline (Cata et al., 2008), neurotropin (Kawashiri et al., 2009) and acetyl-L-carnitine (Flatters et al., 2006) have been successful reducing hyperalgesia induced by paclitaxel. In 2008, Xiao et al., tramadol, topiramate, amitriptyline, baclofen and oxcarbazepine were tested as potential analgesics on this peripheral neuropathy. To date had not tested ketamine or its combination with opioids for the treatment of neuropathy induced by paclitaxel.

Morphine is a well known opioid receptor agonist with analgesic properties. Animal models (Obara et al., 2007) and controlled patient trials (Eisenberg et al., 2005) suggest that μ-opioid receptor agonists are effective at attenuating neuropathic pain. However, side effects suppose substantial barriers to their clinical use (Andersen et al., 2004; Glare et al., 2006). There are more evidences suggesting that opioids are effective relieving neuropathic pain of primarily peripheral origin (Przewlocki and Przewlocka, 2005) than central neuropathic pain (Rowbotham et al., 2003).

Many evidences suggest that NMDA receptors play an important role in the generation of central sensitization and in the development and maintenance of chronic pain (Chizh and Headley, 2005; Eide, 2000). It is known that the NMDA receptor and its associated transduction pathway do not play a significant role in acute pain but only in the development and maintenance of chronic pain, where noxious inputs are tonically active and generate hyperexcitability in pain-transmitting neurons of the spinal cord dorsal horn (Haley et al., 1990). Strong pain stimuli activate NMDA receptors and produce hyperexcitability of dorsal root neurons. This could induce central sensitization, wind-up phenomenon, and pain memory. It has been reported that animals with paclitaxel-induced hyperalgesia has altered discharges of spinal wide dynamic range neurons and down-regulation of glutamate transporter expression in rats (Cata et al., 2006).

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On this basis, administration of NMDA receptor antagonists could be useful for the treatment of chronic pain (Subramaniam et al., 2004). In animal models of chronic pain, NMDA receptor antagonists reduce the nociceptive response and hyperalgesia (Christoph et al., 2006). Clinically-available NMDA receptor antagonists, such as ketamine or memantine (Buvanendran and Kroin, 2008; Finkel et al., 2007), have been useful on the treatment of chronic pain.

Methadone is a potent μ-receptor agonist with affinity for δ-opioid receptor. It produces analgesia in patients refractory to other μ-opioid agonists (Burgess and Pawasauskas, 2008; Shaiova, 2005) and the development of tolerance is slower for methadone than for morphine (Inturrisi, 2005). Besides that, methadone binds with low affinity to NMDA receptors and acts as a non-competitive NMDA receptor antagonist in the brain and spinal cord (Ebert et al., 1995; Mannino et al., 2006). Thus, it is possible that the final effect of methadone may be derived from a combination of actions from its opioid receptor activating and NMDA receptor blocking properties (Axelrod and Reville, 2007). This suggests that methadone could be useful in conditions of opioid "poor-responsive" pain and/or in opioid tolerant patients (Peng et al., 2008; Sartain and Mitchell, 2002).

In the present study, we used a model of neuropathic pain induced by an antineoplastic drug (Pascual et al., 2005; Polomano et al., 2001) to study and compare the analgesic effect of these three common analgesic drugs with clinical use.

2. Methods

Adult (200–250 g) male Wistar rats (Harlan Ibérica, Spain) were used in all the experiments. The animals were housed in clear plastic cages under standard laboratory conditions: controlled temperature 23 ± 1 °C, 12/12-h light/dark cycle and free access to food and water. Spontaneous behaviour was observed in the cages before starting the experimental procedures, rats showing aggressiveness or alterations of the motility were discarded (2%). Number of animals per separated experimental group was at least 8. An observer who was blind to drug treatment conducted all the behavioural assays.

2.1. Paclitaxel-induced peripheral neuropathy (Polomano et al., 2001)

After habituation to the test environments and baseline measurements of pain sensitivity (see below), animals were intraperitoneally (i.p.) injected on four alternate days (days 1, 3, 5 and 7) with paclitaxel (1 mg/kg), using an injection volume of 1 ml/kg. The final cumulative paclitaxel dose administered was 4.0 mg/kg. The vehicle was a mixture of saline and Cremophor EL 10%, a derivative of castor oil and ethylene oxide. This vehicle is used clinically for paclitaxel injections. Body weight was recorded (pre-treatment, and days 3, 5, 7, 13, 16, 18 and 22 after the first paclitaxel or vehicle administration) in the group of animals given paclitaxel ($n = 9$) and in those treated with vehicle ($n = 9$).

2.2. Behavioural assays

2.2.1. Heat hyperalgesia and mechanical allodynia

The plantar surface of hindpaws (sciatic nerve territory) was tested for heat hyperalgesia and tactile allodynia to evaluate the effect of the administration of the different treatments. Tests were carried out on the same day.

2.2.2. Hind paw heat hyperalgesia

Heat hyperalgesia of the hind paw was tested using methods described by Bennett and Hargreaves (1990). Heat hyperalgesia of the hindpaw was tested using plantar test. It was examined by measuring the latency (withdrawal time) of the hindpaws from a focused beam of radiant heat applied to the plantar surface using a plantar test apparatus (Ugo Basile). Briefly, the rat was placed within a plastic compartment on a glass floor; a light source beneath the floor was aimed at the mid plantar surface of the hindpaw. The withdrawal reflex interrupts the light and automatically turns off the light and a timer. The intensity of the light was adjusted at the start of the experiment such that the control average baseline latencies were about 8 s and a cut-off latency of 30 s was imposed. The withdrawal latency of each paw was measured during three trials at 2 min intervals and the mean of the three readings was used for data analysis.

2.2.3. Tactile allodynia

Tactile allodynia was assessed, as previously described (Fox et al., 2001), by measuring the withdrawal threshold to calibrated von Frey hairs with intensities ranging from 0.9 to 40 g. Filaments exerting a force above 40 g were not used as they lifted the paws. On the day of the experiment animals were placed in a Perspex chamber with a mesh metal floor and allowed to acclimatize for 15 min. Starting with the lowest filament force, von Frey hairs were applied perpendicular to the mid plantar surface of both hindpaws, with sufficient force to cause slight bending against the paw, and held until a response was achieved, the mechanical stimulation was maintained for 2 s (maximum), this was repeated five times at an interval of 1–2 s. When the paw was sharply withdrawn, or when there was flinching upon removal of the hair, a positive response was noted; when at least 3 out of 5 responses were positive (60%), this value was accepted as tactile threshold. If less than 3 positive responses were noted to any hair trial, the process was repeated with the next higher force hair.

2.3. Treated groups

Mechanical and thermal withdrawal thresholds were tested consecutively on the following groups of animals ($n = 9$):

1. A group injected with the paclitaxel vehicle. Tests were carried out following the same schedule their corresponding treated groups.
2. Paclitaxel treated animals: mechanical and thermal withdrawal thresholds were tested before the administration of paclitaxel (day B) and on days: 3, 5, 7, 18, 21 and 22 after starting the administration of paclitaxel. When an injection of paclitaxel had to be given on the same day as behavioural testing, rats were injected after the measurements had been taken.

After these procedures rats were randomly allocated to receive one of the following treatments:

1. Saline
2. Morphine (1, 2.5, 5 or 10 mg/kg i.p.)
3. Ketamine (12.5, 25 or 50 mg/kg i.p.)
4. Methadone (2.5 or 5 mg/kg i.p.)
5. Naloxone (1 mg/kgi.p.) + Morphine (5 mg/kg i.p.) or Methadone (5 mg/kg i.p.)
6. Ketamine (12.5 mg/kg i.p.) + Morphine (1 mg/kg i.p.)

Tests were carried out 25 min after intraperitoneal injection. Naloxone was administered 10 min before morphine or methadone injection. Mechanical and thermal withdrawal thresholds were tested in both paws in each group in this sequence. To avoid an overlap, drugs were administered in an interval of 15 min per group of three animals. The doses of ketamine used were obtained from previous works (Pelissier et al. 2003; De Vry et al., 2004).

2.4. Drugs

Methadone (kindly supplied by Laboratorios Dr. Esteve, S.A.), morphine, ketamine and Cremophor EL (Sigma-Aldrich, Spain), paclitaxel (kindly supplied by Bristol-Myers Squibb). Morphine, methadone, naloxone and ketamine were dissolved in saline.

2.5. Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). In order to facilitate the comparison between the effects observed using different tests, data are expressed as percentage of the control value in non-treated animals. Statistical analyses for significant differences between groups were performed using Student's *t*-test. Paired *t*-test was used to compare values obtained from the paws of the same animal. Statistical analyses for significant differences between multiple groups were performed by analysis of variance ANOVA followed, when appropriate, by Newman-Keuls test or Bonferroni test. $P<0.05$ was considered as statistically significant.

This investigation was conducted following a protocol that was approved by the ethical committee of Rey Juan Carlos University and in agreement with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

3. Results

The increase of body weight evaluated in the group of animals treated with paclitaxel 1 mg/kg ($n=9$, 23.76%) did not differ significantly from the increase recorded in rats treated with the vehicle ($n=9$, 30.55%). None of the paclitaxel treated rats showed any obvious changes in spontaneous behaviour when observed in their home cages.

3.1. Thermal hyperalgesia and tactile allodynia

No difference between data recorded from the left or right hindpaws was found, and therefore data presented from Fig. 1 onwards is calculated using the mean value obtained from both hindpaws. Control data indicated that neither heat latency nor mechanical threshold responses differed when compared after vehicle paclitaxel administration and day B (Fig. 1). The thermal latency response was increased by 2.78 ± 3.4% ($n=9$) at day 21 after vehicle treatment compared to the pre-treatment period (day B). The von Frey threshold was increased by 5.93 ± 8.8% ($n=9$) at day 21 after vehicle treatment compared to the pre-treatment period (day B).

As expected, paclitaxel significantly reduced the thermal latency and mechanical threshold when compared to the vehicle-treated group ($p<0.001$, Fig. 1). When the reduction of the thermal latency (hyperalgesia) was evaluated, the difference between pre-treatment control values (day B) and those recorded on days 18–21 reached statistical significance (Fig. 1-A). Tactile threshold (allodynia, Fig. 1-B) was significantly reduced after paclitaxel treatment from 5 to 21 days when compared to day B ($p<0.001$).

The mean of pre- (day B) and post-paclitaxel (day 20) threshold for mechanical test was 18.4 ± 2.6 g and 8.09 ± 1.1 g respectively. The mean of pre and post-paclitaxel was 7.9 ± 0.3 s and 5.97 ± 0.25 s respectively.

3.2. Effect of i.p. administration of morphine on mechanical and thermal withdrawal thresholds

After repeated i.p. administration of paclitaxel, administration of morphine (2.5, 5 and 10 mg/kg) at day 22 was able to abolish the reduction in the mechanical and thermal withdrawal thresholds (Fig. 2) in a dose dependent manner. The dose of 1 mg/kg was not able to produce any effect on hyperalgesia and allodynia induced by paclitaxel.

The effect of 2.5 mg/kg of morphine was not significantly different from basal values recorded before paclitaxel administration on latency response time to heat stimulation (93.73 ± 2.8% vs 100% day B) and tactile threshold to mechanical stimulation (93.92 ± 9.5% vs 100% day B) (Fig. 2). At the doses of 5 and 10 mg/kg the opioid agonist increased the withdrawal threshold responses to heat and to tactile stimulation when compared with values obtained before paclitaxel treatment (day B). The higher doses of morphine (5 and 10 mg/kg) increased twice the tactile withdrawal threshold. These doses of morphine produce an analgesic effect on thermal hyperalgesia.

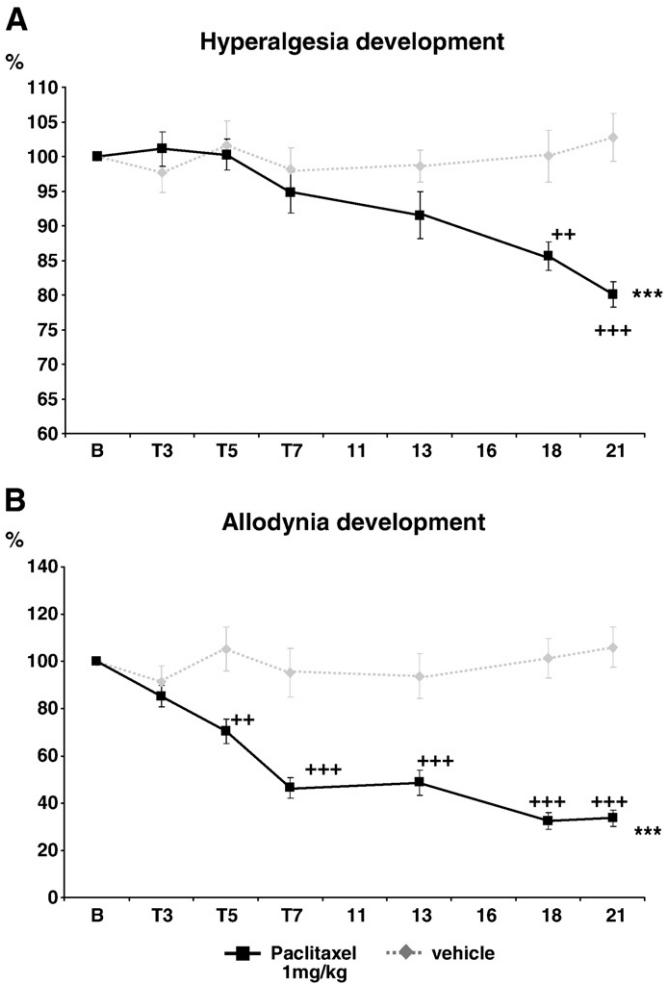

Fig. 1. Development of hyperalgesia (A, plantar test) and allodynia (B, von Frey hairs) after paclitaxel and paclitaxel vehicle control treatments. Data is expressed as the percentage of the mean pre-paclitaxel control response ± SEM against the pre-treatment period (B) and at successive treatment days T(n). + Statistically significant differences vs day B (one-way ANOVA plus post hoc Newman-Keuls test). * Statistically significant differences between paclitaxel treatment vs vehicle treatment ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, two-way ANOVA). $n=9$ (vehicle-treated animals), $n=9$ (paclitaxel treated animals).

In order to confirm the involvement of opioid receptor, the effect of morphine (5 mg/kg) was tested after treatment with 1 mg/kg of the μ-opioid receptor naloxone. In this group of rats the effect of morphine was antagonized in both tests (Fig. 2).

3.3. Effect of i.p. administration of ketamine on mechanical and thermal withdrawal thresholds

The administration of ketamine (12.5, 25 and 50 mg/kg) at day 22 was also able to abolish the reduction in the mechanical and thermal withdrawal thresholds only at highest dose (Fig. 3) and returned it to basal values. At the dose of 12.5 mg/kg the NMDA antagonist did not produced any effect in the withdrawal threshold responses to heat or the mechanical threshold to tactile stimulation. The dose of 25 mg/kg only increased the tactile threshold (compare vs day 21) and had no effect on heat hyperalgesia.

When subanalgesic doses of morphine (1 mg/kg) and ketamine (12.5 mg/kg) were used, an additive effect on heat hyperalgesia was

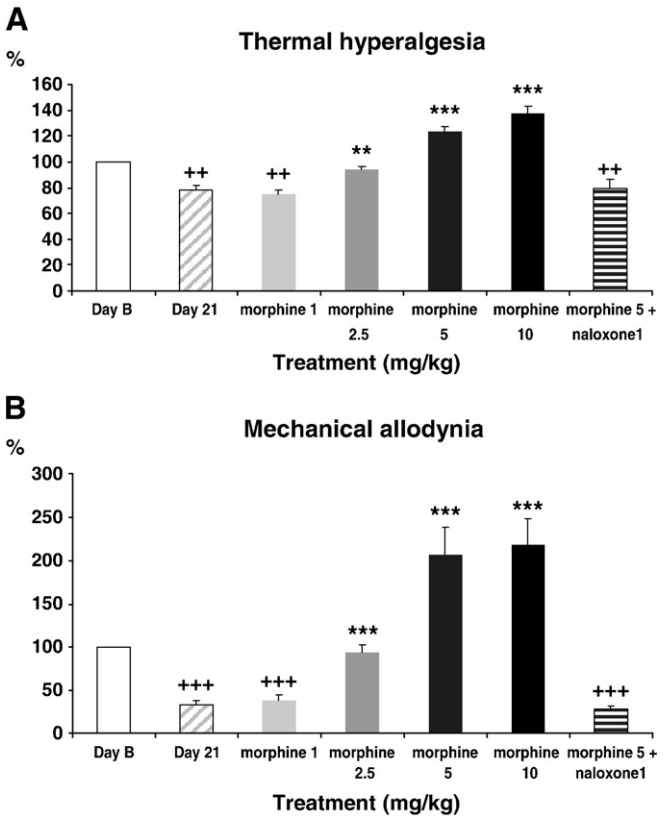

Fig. 2. Effect of morphine on paw hyperalgesia (A) and allodynia (B). Bars show the percentage of the mean ± SEM recorded on day B (before paclitaxel treatment), on day 21 (before drug administration) and on day 22 (drug administration). + Statistical difference vs day B, * statistical difference vs day 21 (**$p<0.01$, ***$p<0.001$, one-way ANOVA plus post hoc Bonferroni test). $n=9$ (for each group).

observed (Fig. 3). Using this combination, an antinociceptive effect was reached without any change on tactile allodynia.

3.4. Effect of i.p. administration of methadone on mechanical and thermal withdrawal thresholds

The administration of methadone (2.5 and 5 mg/kg) at day 22 was also able increase the thermal and mechanical withdrawal thresholds (Fig. 4). The highest dose produced an antinociceptive effect in the withdrawal threshold responses to heat and mechanical threshold to tactile stimulation. The dose of 2.5 mg/kg only increased significantly the heat threshold (compare vs day 21) and had no effect on allodynia.

To confirm the involvement of opioid receptor, the effect of methadone (5 mg/kg) was tested after treatment with 1 mg/kg of naloxone. The analgesic effect of methadone was completely antagonized by naloxone in both tests (Fig. 4).

4. Discussion

Paclitaxel is one of the most effective and frequently used chemotherapeutics for the treatment of solid tumours. However, paclitaxel produces peripheral neurotoxicity with patients reporting sensory abnormalities and neuropathic pain during and often persisting after paclitaxel therapy (Cella et al., 2003; Dougherty et al., 2004). The mechanisms underlying this dose-limiting side effect are currently unknown and there are no validated drugs for its prevention or control.

Animal models have been established to investigate mechanisms underlying the development of anti-tumoral drug-induced neuropathies and to search for therapies that can improve the management of these patients (Nozaki-Taguchi et al., 2001; Polomano et al., 2001). Investigations into this serious clinical problem have progressed with the development of rat models of chemotherapy-induced pain using different systemic dosing schedules of paclitaxel. Present data were obtained using the paclitaxel model (Polomano et al., 2001). In previous studies, our group reported the analgesic effect of a synthetic cannabinoid, WIN 55, 212-2 on this model (Pascual et al., 2005) but the use of cannabinoids is not regularly established in all the countries and the study of other therapeutic approaches is interesting.

In this paper we examined if three common analgesic drugs with clinical use would have an antinociceptive effect on neuropathic pain induced by paclitaxel. These analgesic drugs were selected because morphine is the reference opioid compound, ketamine is a NMDA antagonist used in several pain states and methadone shares both mechanisms of action. We wanted to know if a dual approach may be useful and could permit to reduce the opioid doses.

It is well known that treatment with paclitaxel induces a significant reduction in the latency to heat stimuli and in the mechanical threshold response. Although it is accepted that paclitaxel provokes peripheral neuropathy, there is no general agreement on the symptoms and intensity that are recorded, because they depend on the strain and, probably, on small differences in each laboratory (Polomano et al., 2001). As expected (Polomano et al., 2001; Pascual et al., 2005) the general health and survival rate was good and no difference was found in weight gain in rats treated with either vehicle or paclitaxel.

There is a controversy in relation to the analgesic efficacy of opioids in neuropathic pain. In this model, morphine was able to abolish the tactile allodynia and the thermal hyperalgesia induced by

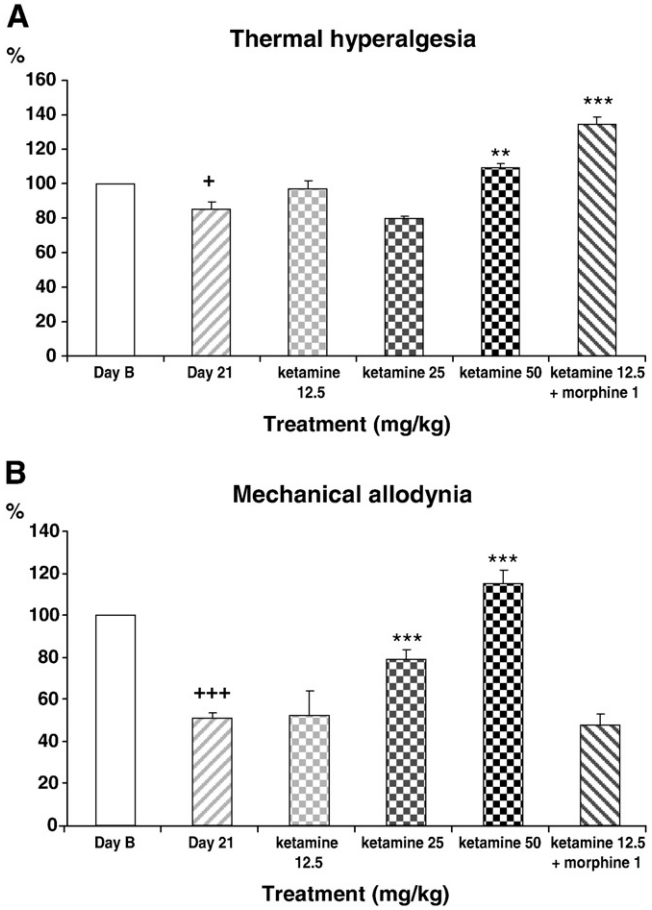

Fig. 3. Effect of ketamine on paw hyperalgesia (A) and allodynia (B). Bars show the percentage of the mean ± SEM recorded on day B (before paclitaxel treatment), on day 21 (before drug administration) and on day 22 (drug administration). + Statistical difference vs day B, * statistical difference vs day 21 (*$p<0.05$, **$p<0.01$, ***$p<0.001$, one-way ANOVA plus post hoc Bonferroni test). $n=9$ (for each group).

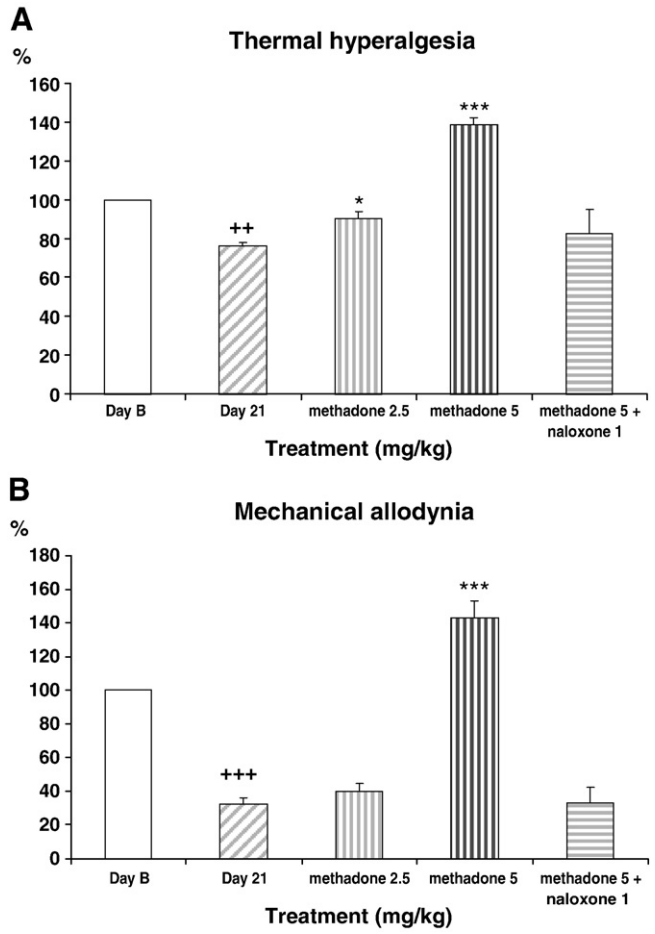

Fig. 4. Effect of methadone on paw hyperalgesia (A) and allodynia (B). Bars show the percentage of the mean ± SEM recorded on day B (before paclitaxel treatment), on day 21 (before drug administration) and on day 22 (drug administration). + Statistical difference vs day B, * statistical difference vs day 21 ($p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, one-way ANOVA plus post hoc Bonferroni test). $n=9$ (for each group).

paclitaxel. We observed a dose dependent effect. Similar results have been published with other models of neuropathic pain (Bulka et al., 2002; Erichsen et al., 2005; Ossipov et al., 1999b for review). Nevertheless, in the study of Flatters and Bennett (2004) morphine 4 mg/kg was ineffective and 8 mg/kg only elicited up to a 50% reversal of mechanical allodynia/hyperalgesia. This discrepancy could be due to the differences on the protocol and on the rat strain.

The lower dose of ketamine produced no significant reversal of the allodynia/hyperalgesia, suggesting that NMDA receptor activation is not the main source of the paclitaxel induced pain. This result is in agreement with the effect of ketamine in chronic constriction model of neuropathic pain (De Vry et al., 2004). Flatters and Bennett (2004) reported similar results with other NMDA antagonist, MK801 on paclitaxel model. The analgesic effect was only obtained at high doses which overlap with motor side effects (De Vry et al., 2004).

Flatters and Bennett (2004) found that MK-801 (NMDA antagonist) did not produce any significant reversal of paclitaxel-induced pain. Other authors report a complete reversal of mechanical allodynia induced by chronic constriction injury at the same dose of MK-801. These data suggest that chemoteraphy induced neuropathies are fundamentally different from other neuropathies, with NMDA receptor activation and the ensuing central sensitisation not playing a key role in the generation of behavioural abnormalities. In this paper, Flatters and Bennett found small changes in axonal mitochondria of sensory nerves that might contribute to paclitaxel-induced pain and there is no axonal degeneration. Nevertheless in the CCI model, nerve fibers are clearly disrupted. This could explain the differences in effectiveness of these drugs in different models.

Some differences in the effect of ketamine at dose of 25 mg/kg can be observed when comparing the effect on allodynia and hyperalgesia. It suggests that mechanical allodynia is more sensitive to the effect of ketamine, as antiallodynic effect is achieved with this dose. This could be due to the effect of paclitaxel on the mechanical threshold is more pronounced than on the thermal threshold and the mechanisms that produce mechanical allodynia and thermal hyperalgesia could be different; for this reason ketamine could not act in the same way.

This data would be consistent with other studies that use antineoplastic drugs to produce neuropathies. Cisplatin produces mechanical allodynia but not hyperalgesia (Vera et al., 2007) and the oxaliplatin produces cold-hyperalgesia but mechanical allodynia (Sakurai et al., 2009).

The most interesting data was the additive effect of subanalgesic doses of morphine and ketamine, although this kind of interaction can only be established by isobolographic analysis. These results would be in agreement, although with the hypothesis on the possible interactions between the NMDA system and the opioid system, where the opiates decrease the release of excitatory amino acids and NMDA receptor antagonist block the central hiperexcitability (Martinez et al., 2002), Ketamine, one of a few clinically-available NMDA receptor antagonists, is known to improve the analgesic efficacy of opioids in humans and rodents (Holtman et al., 2008). Previous results demonstrated that blocking the NMDA receptor enhances the analgesic effect of morphine in formalin test (Hama et al., 2006; Sevostianova et al., 2005), chronic constriction injury (Pelissier et al., 2003) and clinical analgesia (Kollender et al., 2008). Morphine-NMDA receptor antagonist combinations have been studied extensively, several studies reported the potentiation of morphine but some studies reported negative findings with the same drug combination. It could be due that the majority of the rodent studies use a tail flick or tail withdrawal procedure, in which the responses might have a larger spinal component. The studies with the hot plate procedure, morphine potentiation are usually reported. Nevertheless, Pelissier et al. (2003) reported the supradditive analgesic effect of combination of opioids and ketamine in mononeuropathic rats.

Therefore the potentiation of morphine by MNDA antagonist could be depend on the antinociceptive assay used, pain model and other factors which might affect to analgesic effect of morphine (Fischer et al., 2005).

Another data in favour of the mixture of two drugs is that NMDA antagonits attenuate the development of tolerance to antinociceptive effects of opioids (Bilsky et al., 1996; Holtman et al., 2008). It has also been reported that μ receptor activation by opioids leads to a sustained increase in glutamate synaptic effectiveness at the level of NMDA receptors. Opioids when used alone in large doses for a prolonged period induce tolerance, which may lead to increased postoperative pain (Subramaniam et al., 2004). Ketamine, by blocking these NMDA receptors, can prevent the development of tolerance and hyperalgesia (Haugan et al., 2008).

The differences found between hyperalgesia and allodynia could be due of the different fibres involved conducting pain sensation. Tactile allodynia has been suggested to be mediated through large diameter, Aβ-afferent fibers, whereas thermal hyperalgesia is likely to be mediated through small diameter, unmyelinated high threshold C-fibers (Ossipov et al., 1999a; Shir and Seltzer, 1998). Morphine has been shown to block small (C and Aδ) but not large diameter (Aβ) fibre evoked responses into the dorsal horn (Tian et al., 2005). Therefore, thermal hyperalgesia could be more sensitive to morphine administration than allodynia. One possibility could be that high doses of morphine block all the fibers but low doses selectively block C-fibers. Regarding NMDA receptors, application of glutamate can increase the activities of C and Aδ afferent fibers (Du et al., 2001). For this reason, the co-administration with ketamine could help to reach only the antihiperalgesic but not antiallodynic effect blocking the activity of C and Aδ afferent fibers.

Treatment with methadone produced an antinociceptive effect in both tests. Methadone showed antiallodynic effect only at larger doses. The antinociceptive effect was totally antagonized by naloxone. These data suggest that the analgesic effect of methadone is mediated by opioid receptor activation. Similar results were reported in the spinal nerve ligation model of neuropathic pain (Lemberg et al., 2006).

Our results confirm that: the activation of opioids receptor produced analgesia on this model of neuropathic pain; the blockade of NMDA receptors produce antinociception but at high doses with motor impairments; low doses of NMDA antagonist enhancing the analgesic effect of morphine.

In conclusion, considering the clinical utility of NMDA antagonists is compromised by its small therapeutic window (Persson et al., 2002) our findings suggest that this problem could be solved using small doses of NMDA antagonist to enhance opioid analgesic effect reducing side effects of both drugs. The modulation of opioid-induced analgesia by NMDA receptor antagonist is of clinical interest. If low doses of NMDA receptor antagonist can enhance the effects of opioid, either by potentiating their effects or by prolonging their duration of action without enhancing undesirable side effects, combinations might be useful for the treatment of neuropathic pain. These data should be taken with caution because the motor function of these analgesic doses has not been tested.

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