The antinociceptive effect of acetaminophen in a rat model of neuropathic pain

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Received 24 March 2011; accepted 11 August 2011
Available online 22 February 2012

KEYWORDS
Acetaminophen; Antinociceptive effect; Hyperalgesia; Neuropathic pain

Abstract Acetaminophen is one of the most popular and widely used analgesics for the treatment of pain and fever but few studies have evaluated its effects on neuropathic pain. This study examined the effect of acetaminophen on thermal hyperalgesia, mechanical and cold allodynia in a rat model of neuropathic pain. Male Sprague-Dawley rats were prepared by tightly ligating the left L5 and L6 spinal nerves to produce a model of neuropathic pain. Sixty neuropathic rats were assigned randomly into six groups. Normal saline and acetaminophen (25, 50, 100, 200 and 300 mg/kg) were administered intraperitoneally to these individual groups. Thermal hyperalgesia, mechanical and cold allodynia were examined at preadministration and at 15, 30, 60, 90, 120, 180, 240 and 360 min after administering the drug. Mechanical allodynia was quantified by measuring the paw withdrawal threshold to stimuli with von Frey filaments. Cold allodynia was quantified by measuring the frequency of foot lift after applying 100% acetone. Thermal hyperalgesia was quantified by measuring the thermal withdrawal threshold. The rotarod performance was measured to detect any drug-induced adverse effects, such as drowsiness. The hepatic and renal adverse effect was also assessed by measuring the serum levels of aspartate aminotransferase, alanine aminotransferase, blood urea nitrogen and creatinine. The paw withdrawal thresholds to mechanical stimuli and the thermal withdrawal threshold were increased significantly and withdrawal frequencies to cold stimuli were reduced by acetaminophen administration in a dose-dependent manner. Acetaminophen reduces thermal hyperalgesia, mechanical and cold allodynia in a rat model of neuropathic pain, and might be useful for managing neuropathic pain.

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doi:10.1016/j.kjms.2011.11.003
Introduction

Acetaminophen is one of the most popular and widely used analgesics on the market. Despite its introduction into human medicine one century ago, the mechanism for the analgesic action of acetaminophen is not completely understood.

Several authors have reported the antinociceptive effect of acetaminophen in noninflammatory pain models in healthy animals [1–3]. Acetaminophen had a dose-dependent antinociceptive effect in formalin [4,5], substance P (SP), glutamate [6] and carrageenan [7,8] induced inflammatory pain models. In addition, acetaminophen inhibited dose-dependently the decrease in heat threshold in a rat thermal hyperalgesia model [9].

It has been reported that acetaminophen does not affect the neuropathic pain mechanism [10]. In addition, the intrathecal administration of acetaminophen did not attenuate the mechanical hyperalgesia in streptozocin-induced diabetic neuropathy [11]. In the neuropathy induced by partial ligation of the sciatic nerve, acetaminophen did not significantly reduce the response to noxious mechanical and electrical stimulation [12]. Moreover, acetaminophen is not included in the analgesics recommended in the guidelines for the treatment of neuropathic pain [10].

However, there are reports of the positive effects of acetaminophen in neuropathic pain. Acetaminophen reduces mechanical allodynia in a chemotherapy-induced neuropathic pain model [13]. Acetaminophen dose-dependently decreases the mechanical allodynia and thermal hyperalgesia, when administered locally in a neuropathic pain model induced by partial ligation of the sciatic nerve [14]. Nevertheless, the systemic antinociceptive effect of acetaminophen in a neuropathic pain model induced by the ligation of the spinal nerve has not been profiled for the thermal hyperalgesia, mechanical and cold allodynia.

Furthermore, there is evidence suggesting the central analgesic effect of acetaminophen, even though the mechanism of the analgesic action of acetaminophen is unclear. The suggested mechanism of the analgesic action of acetaminophen includes the reinforcement of the serotonergic descending inhibitory pathway [3,4,15] and interactions with the opioidergic system, endocannabinoid (CB) systems [16,17], and nitric oxide (NO) containing pathways [1,18].

Therefore, this study examined the effect of the intraperitoneal administration of acetaminophen on thermal hyperalgesia, mechanical and cold allodynia in a rat model of neuropathic pain to determine if acetaminophen is a new treatment modality for neuropathic pain diseases.

Materials and methods

Animals

All experimental procedures were approved by our institutional animal investigation committee. Male Sprague-Dawley rats weighing 100–150 g each were used. Food and water were provided ad libitum. The rats were housed in groups of three to four in plastic cages with soft bedding and maintained on a 12:12-h light-dark cycle. The experimental rats were allowed at least 5 days to adjust to their environment before conducting the experiment.

Spinal nerve ligation

The method reported by Kim and Chung [19] was used to produce a neuropathic pain model by ligating the left L5 and L6 spinal nerves. The surgical procedures were performed under general anesthesia delivered through an open mask system. Anesthesia was induced by 4% Isoflurane and maintained with 1–2% in a 1:1 mixture of nitric oxide and oxygen at a flow rate of 2 L/min. After surgery, the rats were allowed to recover for 7 days before starting the behavioral tests. Those animals that showed a foot withdrawal response to the von Frey filaments (Semmes Weinstein Von Frey Aesthesiometers, Stoelting CO, IL, USA) with an applied bending force of 4 g or less were considered to be neuropathic [20–22], and they were used in the tests. The rats that exhibited motor deficiency (such as paw dragging or limping) or those that failed to exhibit subsequent mechanical allodynia were excluded.

Drug administration

Sixty neuropathic rats were divided randomly into six groups (n = 10 in each group) before the intraperitoneal administration of the drugs. The intraperitoneal injections were performed without anesthesia. The control group received 0.9% normal saline 10 mL/kg (the NS group). There were five experimental groups, PAR 25, PAR 50, PAR 100, PAR 200, and PAR 300 groups, which were given 25, 50, 100, 200, and 300 mg/kg of acetaminophen in a volume of 10 mg/mL (Acetaminophen, Perfalgan®, BMS Pharmaceutical Korea Limited. Seoul, Korea), respectively.

Behavioral tests

To avoid circadian rhythm errors, all behavioral tests were conducted at fixed times (1 PM to 7 PM) by the same person who was unaware of which solution had been administered and which dose was used. After the intraperitoneal injection, the rats were placed on a metal mesh covered with a plastic dome (8 × 8 × 18 cm) to assess the mechanical and cold allodynia. Thermal hyperalgesia, mechanical and cold allodynia were assessed before the intraperitoneal injection and also at 15, 30, 60, 90, 120, 180, 240, and 360 min after the injection.

The thresholds for mechanical allodynia were measured using a series of von Frey filaments (from 0.07 to 15.0 g). The third metatarsal bone area of the left hindpaw was stimulated with von Frey filaments at 3–4 s intervals using the up-down method [20]. The minimal pressure (g) that initiated a response was recorded. The threshold was recorded as 15.0 g if the strongest hair did not elicit a response. Avoidance responses, such as lifting, shaking or licking the paw and running away, were considered as positive responses.

Cold allodynia was measured as the number of foot withdrawal responses after applying cold stimuli to the plantar surface of the paw [23]. The tests were repeated
five times at intervals of approximately 3–5 min between each test. The response frequency to acetone is expressed as the percentage response frequency [(number of paw withdrawals/number of trials) × 100].

Thermal hyperalgesia was measured using an increasing-temperature hot plate (IITC life Science Inc. Victory Blvd Woodland Hills, CA, USA) [24]. The thermal withdrawal threshold, i.e., the lowest temperature to evoke nocifensive behavior, was measured. The rat was placed into the observation chamber on the plate with a starting temperature of approximately 30°C. The plate was then heated up at a rate of 12°C/min until the animal showed nocifensive behavior. The typical response was hindpaw licking, while shaking and lifting of the paw or jumping was observed on rare occasion. The cutoff temperature was set to 50°C.

The changes in locomotor function in the neuropathic rats were evaluated by rotarod tests (Acceler rota-rod for rats 7750; Ugo Basile, Comerio-Varese, Italy). This apparatus consisted of a base platform and a rotating rod, 7 cm in diameter, with a nonskid surface. The rod, 50 cm in length, was divided into four equal sections by five discs and four rats could be tested simultaneously. A V-shaped counter-trip plate was positioned under each drum section, 26 cm below the rod on the base platform. The neuropathic rats were acclimatized to the revolving drums, and habituated to handling in order to ameliorate any stress during the tests. The rats were given three training trials on the revolving drums (10–15 rpm) for 2 days before the actual day of the test. The rats were placed on the drum rotating at 10–15 rpm. The performance time on the rod measured until the rat fell from the drum onto the counter-trip plate or reached a cutoff or a maximum of 150 s. The rats that could remain on the revolving drum for a minimum of 150 s were selected for drug testing. The mean of three training runs served as the control performance time. The rotarod performance time was measured at 15, 30, 60, 90, 120, 180, 240, and 360 min after an intraperitoneal injection. Each test was performed three times at 5-min intervals, and the mean values were compared. The tactile, cold and heat testing all took place at the same time (i.e., each test was repeated in order at each time interval) and only rotarod testing was performed separately at each time interval due to a lack of time.

Serum analysis

Approximately 1 mL of blood was collected from the tail vein to assess the hepatic and renal adverse effects before and 24 h after the acetaminophen or normal saline injection. The plasma was separated by centrifuging the blood for 15 min at 3000 rpm and stored at −70°C until the analysis. The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine (CREA) were measured using Hitachi 7180 Automatic Clinical Analyzer (Hitachi high-Technologies, Japan).

Statistical analysis

The results are expressed as the mean ± SEM. Statistical analysis was performed with SPSS (version 15.0; SPSS, Chicago, IL, USA). The percentage withdrawal frequency, withdrawal threshold and rotarod performance time were assessed using repeated measure of analysis of the variance (ANOVA), followed by a post hoc Dunnet’s tests for multiple comparisons. For the dose-response curves, the withdrawal data was converted to a percentage of the maximum possible effect (%MPE) using the formula: %MPE for mechanical allodynia and thermal hyperalgesia = (post drug threshold-baseline threshold)/(cutoff threshold-baseline threshold) × 100; %MPE for cold allodynia = (baseline withdrawal frequency-post drug withdrawal frequency)/(baseline withdrawal frequency) × 100. The dose-response data was analyzed by one-way ANOVA. The serum AST, ALT, BUN and CREA levels were analyzed by ANOVA one-way variance, followed by a post hoc Dunnet’s tests for multiple comparisons and a paired t test for the levels before and after drug administration. p < 0.05 was considered significant.

Results

Mechanical allodynia

After spinal nerve ligation, all the rats developed mechanical allodynia with a paw withdrawal threshold <2.0 g. The withdrawal threshold in the control and PAR 25 groups was not significantly different from that of the preadministration. Compared to the preadministration values, the withdrawal threshold in the PAR 50, PAR 100, PAR 200 and PAR 300 groups significantly increased from 30 to 180 min, 30 to 240 min, 15 to 240 min and 15 to 240 min, respectively. The withdrawal threshold in the PAR 25 and PAR 50 groups was not significantly different from that of the control group. Compared to the control group, the withdrawal threshold in the PAR 100, PAR 200 and PAR 300 groups significantly increased from 90 to 180 min, 15 to 180 min and 15 to 240 min, respectively (Fig. 1A). The %MPE for mechanical allodynia was 3.9 ± 1.9, 22.8 ± 7.5, 27.2 ± 4.0, 31.8 ± 8.6, and 37.5 ± 8.3% at 25, 50, 100, 200, and 300 mg/kg, respectively. The administration of acetaminophen attenuated mechanical allodynia in a dose dependent manner (Fig. 2).

Cold allodynia

Before drug administration, all the rats showed a withdrawal frequency from 80% to 100% when acetone was applied, without any difference among the groups. Compared to the preadministration values, the administration of acetaminophen reduced the withdrawal frequency to acetone application from 90 to 120 min for the PAR 25 group, from 15 to 180 min for PAR 50, and from 15 to 240 min for the PAR 100, PAR 200, and PAR 300 groups. Compared to the control group, the administration of acetaminophen reduced the withdrawal frequency to acetone application in a dose dependent manner: 120 min for the PAR 25 group, from 30 to 180 min for PAR 50 and PAR 100 groups, and from 15 to 240 min for the PAR 200 and PAR 300 groups (Fig. 1B). The %MPE for cold allodynia was 24.5 ± 4.0, 47.0 ± 3.0, 53.5 ± 3.9, 68.0 ± 6.8 and 88.0 ± 6.1% at 25, 50, 100, 200 and 300 mg/kg, respectively.
The administration of acetaminophen attenuated cold alldynia in a dose dependent manner (Fig. 2).

Thermal hyperalgesia

The withdrawal temperature of all neuropathic rats was 42.8 ± 0.5°C before drug administration. The withdrawal temperature was increased by the administration of acetaminophen reaching a maximum in 120 min after administering drug and then returning gradually to the preadministration level. Compared to the control group, acetaminophen significantly increased the withdrawal temperature from 90 to 180 min for the PAR 25 group, from 15 to 180 min for PAR 50, PAR 100 and PAR 200 groups, and from 15 to 240 min for the PAR 300 group (Fig. 1C). The %MPE for thermal hyperalgesia was 25.8 ± 3.8, 40.1 ± 2.6, 47.8 ± 3.5, 52.8 ± 3.9 and 70.7 ± 3.2% at 25, 50, 100, 200 and 300 mg/kg, respectively. The administration of acetaminophen attenuated thermal hyperalgesia in a dose dependent manner (Fig. 2).

Rotarod performance

The rotarod performance time was not reduced by the administration of acetaminophen (Fig. 3).

Effect of acetaminophen administration on hepatic and renal function

The serum AST and BUN levels were significantly higher in the rats injected with 300 mg/kg acetaminophen than the rats in the control and other acetaminophen groups (PAR...
The serum AST, ALT, BUN and CREA levels were not increased in the 24 h period after the administration of acetaminophen, except for 300 mg/kg (Fig. 4).

Discussion

This study demonstrated that intraperitoneally administered acetaminophen had a dose-dependent antiallodynic and antihyperalgesic effect on a rat model of neuropathic pain that did not produce sedation. This suggests that acetaminophen may be an effective therapy for neuropathic pain.

Although acetaminophen is one of the most popular and widely used analgesics, the mechanism of the analgesic action of acetaminophen is unclear. However, there is evidence suggesting the central analgesic effect of acetaminophen. The mechanism of the analgesic action of acetaminophen is an increase in the central nervous system levels of serotonin released from the brainstem serotonergic neurons, suggesting central mediation [3,4,15]. The other antinociceptive mechanism of acetaminophen suggested is that acetaminophen could act either directly as an opioid or CB receptor ligand or indirectly by increasing the endogenous opioid or CB receptor ligands [16,17]. The metabolism of acetaminophen in the brain and in dorsal root ganglia leads to the formation of N-(4-hydroxyphenyl)-arachidonylamine (AM404), which inhibits the cellular reuptake of CB [16]. In addition, the suggested mechanisms of the analgesic action include the inhibition of prostaglandin synthesis [25–27], and an interaction with the NO containing pathways [1,18]. Therefore, the systemic administration of acetaminophen was used to evaluate the antinociceptive effect on a rat model of neuropathic pain in the present study.

Acetaminophen administered orally (200, 400 and 800 mg/kg), intravenously (50, 100, 200 and 300 mg/kg) and intrathecally (100 and 200 µg/rat) induced a significant dose-dependent antinociceptive effect in the paw-pressure test [2]. The intraperitoneal administration of acetaminophen (400 mg/kg) also had an antinociceptive effect in the hot-plate test [1]. In addition, acetaminophen had a significant antinociceptive effect in an inflammatory pain model in rats [4–8]. Pini et al. [4] reported that the intraperitoneal administration of acetaminophen had a significant antinociceptive effect in the formalin test at a dose of 300 mg/kg. In another study, the oral administration of acetaminophen (200 and 300 mg/kg) dose-dependently inhibited the nociceptive behavior in both phases of the formalin test and reduced the intrathecal SP and glutamate-induced nociceptive behavior [6]. However, the antinociceptive effect of acetaminophen in neuropathy is controversial. Lynch et al. [13] reported that the oral administration of acetaminophen attenuated the mechanical allodynia in a rat chemotherapy-induced neuropathic pain model but neither cold allodynia nor thermal hyperalgesia was assessed in that study. The local, not systemic administration of acetaminophen decreased the mechanical allodynia and thermal hyperalgesia in the neuropathic pain model by partial ligation of the sciatic nerve [14]. Unlike the present study, their study evaluated the local peripheral effect of acetaminophen but did not examine cold allodynia.

In contrast, there are some reports showing that acetaminophen lacks the antinociceptive activity in experiments performed in neuropathy [12,28] and does not affect the neuropathic pain mechanisms [10]. The intrathecal administration of acetaminophen did not increase the paw withdrawal threshold to mechanical stimulation at a dose of 1–7 mg in streptozotocin-induced diabetic neuropathic pain model in rats [11]. Curros-Criado et al. [12] reported...
that the intrathecal administration of acetaminophen did not reduce the response to noxious mechanical and electrical stimulation in the neuropathic pain model by partial ligation of the sciatic nerve. These results are inconsistent with those of the present study. Kim et al. [29] compared three animal models of neuropathic pain and reported that mechanical allodynia in the spinal nerve ligation neuropathic pain model was greater than in the partial sciatic nerve ligation neuropathic pain model but reduced more after the sympathectomy. In addition, the number and types of injured fibers in the partial sciatic nerve ligation neuropathic pain model are difficult to control [19]. Therefore, the number and types of sciatic nerve axons ligated also differ from experiment to experiment due to the inability to damage precisely the same part of the nerve in each animal. On the other hand, the present surgical procedure tightly ligates the same spinal nerves in each animal. Therefore, the only potential variability between experimental subjects would be the differences between individual rats in the proportion of the sciatic nerve contributed by its three spinal segments, which is normal biological (not experimental) variability. The streptozotocin-induced diabetic neuropathic pain model was also different from the spinal nerve ligation neuropathic pain model, which produces both mechanical allodynia and thermal hyperalgesia ipsilaterally to the lesion. The streptozotocin-induced diabetic neuropathic pain model in rats produces long-lasting mechanical allodynia, but the thermal hyperalgesia is variable [11,30,31]. In addition, diabetic animals are chronically ill, with a reduced growth rate, polyuria, diarrhea and show markedly reduced motor activity. Therefore, there is some doubt as to whether the changes in the nocifensive reflex activity, which is used as a measure of allodynia, are genuinely indicative of peripheral neuropathy or can be attributed to the poor health of the animals. Moreover, the mechanical hyperalgesia is largely resistant to a range of pharmacological tools [30]. The profound ill-health of the animals, together with the poor activity of a range of potential analgesic drugs, may raise these questions. Therefore, these differences in pain models might be related to the discrepancy of the results.

In addition, the discrepancy of the results might be related to the difference in animals (Male Sprague-Dawley rats, initially weighing 200–230 g vs. Male Wistar rats weighing 235–350 g vs. Male Sprague-Dawley rats weighing 100–150 g). Different strains of rats show different neuropathic pain behaviors [32,33]. Compared to Sprague-Dawley rats, Wistar rats showed generally low frequencies of foot withdrawals to repeated mechanical stimuli with the von Frey filament in the spinal nerve ligation neuropathic pain model [32]. The age and size of the rats also affects the development of allodynia in the neuropathic pain model [34–36]. Young rats displayed much more vigorous behavioral signs of mechanical allodynia and ongoing pain after ligation of spinal nerve than the old rats [35]. Finally, the difference in the route of drug administration (intrathecal vs. intraperitoneal), or the types of nociceptive stimulation used in these studies might also explain the discrepancy of the results. Curros-Criado et al. [12] reported that the highest dose (960 μmol/kg) of acetaminophen administered intravenously decreased the response to electrical stimulation, but the intrathecal administration of acetaminophen did not reduce the response to electrical stimulation. The intrathecal and intravenous administration of acetaminophen did not reduce the response to noxious mechanical in their study.

Acetaminophen was reported to induce drowsiness [16]. In animal behavioral tests, drowsiness after drug administration may reduce the response to stimulation and mimic the antiallodynic and antihyperalgesic effects. A rotarod test was performed to examine the effect of acetaminophen on the motor coordination to exclude drowsiness-induced antiallodynic and antihyperalgesic effect. The experimental rats did not show a significant decrease in rotarod performance. Therefore, the antiallodynic and antihyperalgesic effects produced by 25–300 mg/kg acetaminophen were not caused by drug-induced drowsiness. Acetaminophen also has an effect on the hepatic and renal function. Therefore, the serum AST, ALT, BUN and CREA levels were measured before and 24 h after the acetaminophen injection. Twenty-four hours was chosen as a time point because preliminary experimental results showed the highest AST, ALT, BUN and CREA levels at 24 h.
after the acetaminophen injection. The serum AST, ALT, BUN and CREA levels were not increased at 24 h after the administration of acetaminophen. The acetaminophen doses administered in the present study were within the range used by others in comparable studies [1,5,7]. The 400 mg/kg dose of acetaminophen administered intraperitoneally is a subtoxic level in rats and the motor activities also remained unchanged by such doses [1]. Only the highest dosed (300 mg/kg) rats showed an elevation of the serum AST, ALT, BUN and CREA levels.

Acetaminophen is not recommended in the guidelines for the treatment of the neuropathic pain. The analgesic effects of acetaminophen, alone or combination with other analgesics, on neuropathic pain have been rarely reported in published clinical trials [37,38]. Gulcu et al. [37] suggested that an acetaminophen infusion in a dose of 4 g/day might be effective for neuropathic pain. The combination of oxycodone 5 mg and acetaminophen 325 mg at up to 8 hour intervals improved the level of pain in patients with neuropathic pain [38], and tramadol 37.5 mg/acetaminophen 325 mg combination tablets with a maximal dose of four tablets per day were effective in the treatment of chronic lower back pain [39]. Based on these published clinical trials and the current animal experiments, acetaminophen, alone or combination with other drugs may suitable for treating neuropathic pain but further studies will be needed in a clinical setting.

In conclusion, intraperitoneally administered acetaminophen had a dose-dependent antiallodynic and anti-hyperalgesic effect in this neuropathic rat model. In addition, the administration of acetaminophen at 200 mg/kg or below does not increase the serum AST, ALT, BUN and CREA levels. This study shows that acetaminophen may be a treatment modality for a broad range of neuropathic pain diseases that are accompanied by allodynia or hyperalgesia. However, proper human clinical trials will be needed to confirm similar responses in the human population.

References


