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Acetyl-L-carnitine prevents and reduces paclitaxel-induced painful peripheral neuropathy

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Abstract

This study examines the potential efficacy of acetyl-L-carnitine (ALC) to prevent and treat paclitaxel-induced pain. Rats received four intraperitoneal (i.p.) injections of 2 mg/kg paclitaxel on alternate days which, following a short delay induced marked mechanical hypersensitivity. Daily administration of ALC (50 mg/kg and 100 mg/kg; p.o.; concurrently with paclitaxel and for 14 days afterwards) prevented the development of paclitaxel-induced pain. This effect was long lasting, for at least 3 weeks after the last dose of ALC. In a separate experiment, daily administration of ALC (100 mg/kg; p.o.; for 10 days) to rats with established paclitaxel-induced pain produced an analgesic effect. This effect dissipated shortly after ALC treatment was withdrawn. We conclude that ALC may be useful in the prevention and treatment of chemotherapy-induced painful peripheral neuropathy.

Keywords

Chemotherapy; Paclitaxel; ALC; L-Acetylcarnitine; ALCAR; Pain

Peripheral neurotoxicity is a common and dose-related side effect of paclitaxel (Taxol®) therapy [20]. Patients describe a variety of symptoms including numbness and tingling, mechanical allodynia, cold allodynia and on-going burning pain. Such symptoms are symmetrical and bilateral, first appearing in the feet or simultaneously in the fingers and toes [9]. Unfortunately, paclitaxel-induced pain can persist for months, even years, following the cessation of paclitaxel [9,23]. There are no proven treatments to prevent or treat paclitaxel-induced pain. A recent double blind, placebo-controlled randomized clinical trial with gabapentin failed to show efficacy in these patients [25]. We have shown that ethosuximide, an anti-epileptic and T-type calcium channel blocker, is effective in rats with paclitaxel-induced pain [12], but ethosuximide has not yet been tested clinically. Thus, painful peripheral neuropathy remains a serious dose-limiting side effect of paclitaxel therapy.

Acetyl-L-carnitine (γ -trimethyl- β -acetylbutyrobetaine, ALC) is the acetyl ester of carnitine and the primary acylcarnitine in human tissues. ALC is present throughout the central and peripheral nervous systems and plays an essential role in the oxidation of free fatty acids [14]. Repeated ALC administration has been shown to promote regeneration following nerve injury, increasing regenerating axons at the transected sciatic nerve stump and significantly restoring motor function [11]. In addition, ALC significantly decreases neuronal loss in axotomized dorsal root ganglion (DRG), in a dose-related manner [24]. A few laboratory

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studies have examined the role of ALC in nociception. Repeated dosing of ALC increased acute mechanical and thermal thresholds in normal animals and reversed thermal hyperalgesia induced by kainic acid and NMDA [7,16]. Prolonged ALC dosing commencing 24 h post-chronic constriction injury (CCI) surgery inhibited the resultant mechanical allodynia at day 24 post-injury [7].

Double blind, placebo-controlled clinical trials of ALC in patients with diabetic neuropathy demonstrated reductions in pain intensity and increases in nerve conduction velocities [8,22]. HIV patients who developed a painful neuropathy whilst on nucleoside analogue reverse transcriptase inhibitor (NRTI) therapy had lower serum levels of acetyl carnitine than similar patients who did not develop a neuropathy [10]. Open label studies with ALC in patients with NRTI-induced neuropathy describe an improvement in neuropathic symptoms and an enhancement of cutaneous innervation [17,21]. In addition, a significant decrease in pain scores was seen following ALC treatment in patients with cervical and lumbosacral neuropathies of mixed etiology [18]. In the present study, we employ a rat model of paclitaxel-induced painful peripheral neuropathy to assess the potential efficacy of ALC to: (1) counteract the development of paclitaxel-induced neuropathic pain and (2) to inhibit established paclitaxel-induced neuropathic pain.

All procedures were approved by the Faculty of Medicine, Animal Care Committee of McGill University and were conducted in accordance with the guidelines for animal research by the International Association for the Study of Pain [26]. Adult male Sprague–Dawley rats (250–300 g, Frederick, Maryland breeding colony; Harlan, Indianapolis, USA) were housed in groups of three on sawdust bedding in plastic cages. Artificial lighting was provided on a fixed 12 h light/12 h dark cycle with food and water available *ad libitum*.

In this study, mechanical hypersensitivity was measured because this model displays only a mild and transient heat-hyperalgesia, as previously reported [12]. Following habituation to the behavioural testing environment, mechanical behavioural testing was performed as previously described [12]. Briefly, von Frey filaments with bending forces of 4, 8 and 15 g were applied to the mid-plantar area of each hind paw, in ascending order of force. Each application of a von Frey filament to the hind paw was held for 5 s and each hind paw was stimulated five times with each of the three von Frey filaments. Total withdrawal responses of the rat to each von Frey filament were counted (out of 10) and expressed as an overall percentage response. Three baseline measurements of mechanical sensitivity were taken prior to paclitaxel administration. Paclitaxel, 2 mg/kg per ml: Taxol® (Bristol-Myers-Squibb: 6 mg/ml paclitaxel in Cremophor EL and dehydrated ethanol) was diluted with saline. All rats received four intraperitoneal (i.p.) injections of paclitaxel on alternate days (days 0, 2, 4 and 6) as previously described [12]. Acetyl-L-carnitine hydrochloride (Sigma–Aldrich, Oakville, Ont., Canada) dissolved in distilled water was administered via oral gavage (p.o.) in either a prophylactic or treatment dosing paradigm to paclitaxel-treated rats. For the duration of the experiments, the experimenter was blind to the drug treatment.

In the prophylactic paradigm, rats received p.o. doses of either 50 mg/kg ALC ($n = 10$), 100 mg/kg ALC ($n = 10$) or vehicle (distilled water, $n = 10$) for 21 consecutive days (day 1 through to day 20). Mechanical sensitivity was assessed on days 7, 12, 16, 21, 23, 26, 29, 34, 37 and 41 following the initiation of paclitaxel treatment (see Fig. 1). In the treatment paradigm, rats received p.o. doses of either 100 mg/kg ALC ($n = 13$) or vehicle (distilled water, $n = 14$) for 10 consecutive days (day 17 through to day 26), once the paclitaxel-induced pain was clearly evident (day 16 post-paclitaxel initiation). Mechanical sensitivity was assessed on days 19, 21, 23, 25, 27, 30, 33 and 36 following the initiation of paclitaxel treatment (see Fig. 2).

Fig. 1 shows the effects of prophylactic dosing of ALC on responses to von Frey stimulation from normal baseline levels (pre-paclitaxel) to day 41 post-paclitaxel initiation. The vehicle group displayed the expected marked and long-lasting mechanical hypersensitivity. Mechanical hypersensitivity was evident, after a short delay period, at day 16 and peaked at day 26–29 post-paclitaxel initiation; this is the same time-course that we have previously demonstrated with this model [12]. Responses of the vehicle-treated group to 4 g and 8/15 g von Frey filaments were significantly different from pre-paclitaxel readings beginning on day 23 and day 16, respectively, and for the rest of the experiment (Fig. 1A–C, $p < 0.01$ one-way repeated measures ANOVA with Dunnett's post hoc tests).

Prophylactic dosing with either 50 or 100 mg/kg ALC prevented the development of mechanical hypersensitivity (Fig. 1A–C). The effects of prophylactic ALC were prolonged, with 100 mg/kg ALC still preventing paclitaxel-induced mechanical hypersensitivity at day 41 post-paclitaxel initiation (i.e. 21 days after the last dose of ALC). Responses to von Frey 4 g were significantly inhibited in the ALC-treated groups at days 26, 29 and 37 compared to the vehicle treated group ($p < 0.05$, one-tailed unpaired t -tests with Bonferroni correction). Responses to von Freys 8 and 15 g were significantly inhibited in ALC-treated groups from day 16 and day 21, respectively, and for the duration of the experiment when compared to the concurrent vehicle-treated group ($p < 0.05$, one-tailed unpaired t -tests with Bonferroni correction). Thus, from the point of emergence and throughout the peak of paclitaxel-induced pain seen in the vehicle-treated group, ALC treatment prevented behavioural changes.

When comparing to pre-paclitaxel baseline levels, a few statistically significant post-paclitaxel changes were seen in the ALC-treated groups. For the 50 mg/kg ALC-treated group, significant differences were seen at day 29 in von Frey 4 g responses, at days 37 and 41 in von Frey 8 g responses, and at day 41 in von Frey 15 g responses (Fig. 1A–C, $p < 0.05$, oneway repeated measures ANOVA with Dunnett's post hoc test). For the 100 mg/kg ALC-treated group, significant differences from pre-paclitaxel baseline levels were seen at days 12, 26 and 41 in von Frey 4 g responses, at day 23 in von Frey 8 g responses, and at days 23 and 37 in von Frey 15 g responses (Fig. 1A–C, $p < 0.05$, one-way repeated measures ANOVA with Dunnett's post hoc test). Although these fluctuations from the pre-paclitaxel baseline are statistically significant, they did not consistently occur within the time course of the experiment. We think it is likely that these changes reflect variability in behaviour that are not related to pain, but we can not exclude the possibility that ALC's inhibitory effect is slightly less complete.

As there was little difference between the prophylactic effects of the two doses of ALC examined, we used the higher dose to test the effect of ALC on established paclitaxel-induced pain (Fig. 2). At day 16 post-paclitaxel initiation, significant mechanical hypersensitivity is evident in the responses to all von Frey filaments compared to pre-paclitaxel responses ($p < 0.0001$, one-tailed paired t -tests). ALC significantly inhibited paclitaxel-induced mechanical hypersensitivity compared to the concurrent vehicle-treated group (Fig. 2A–C). Both von Frey 4 g responses and von Frey 15 g responses were significantly inhibited (by 42–55% and 32–47%, respectively) at day 21, 23, 25 and 27 (Fig. 2A–C, $p < 0.05$, one-tailed unpaired t -tests with Bonferroni correction). Von Frey 8 g responses were significantly inhibited by ALC at day 19, 25 and 27 by 38–42% (Fig. 2B, $p < 0.01$, one-tailed unpaired t -tests with Bonferroni correction). However, when comparing to pre-paclitaxel baseline levels, significant mechanical hypersensitivity was still evident in the ALC-treated group, from day 16 onwards for all von Frey responses ($p < 0.01$, one-way repeated measures ANOVA with Dunnett's post hoc test). Thus, ALC inhibited established paclitaxel-induced pain but was unable to reverse it. In contrast, to the prophylactic effects of ALC, the inhibitory effects of ALC on established paclitaxel-induced hypersensitivity were short-lived; responses were still attenuated a day following the last dose of ALC (day 27), but not 4 days post-ALC (day 30).

We did not observe any adverse effects of i.p. paclitaxel or oral ALC administration. There were no signs of ill health such as alopecia, diarrhoea or weight loss due to the chemotherapy. All rats gained weight normally throughout the duration of the experiments and no sedation or ataxia was observed with ALC administration.

We have shown that prophylactic administration of oral ALC can prevent the development of paclitaxel-induced painful peripheral neuropathy. ALC prevented the emergence of significant paclitaxel-induced pain up to day 41 post-paclitaxel initiation, i.e. 3 weeks following the last dose of ALC. The experiment was terminated at this point. We have previously shown that significant mechanical hypersensitivity can still be observed with this model in responses to von Freys 8 and 15 g, but not von Frey 4 g, at day 93 post-paclitaxel initiation [12]. Here we have again shown that paclitaxel-induced pain peaks (day 26–29) and that it is declining by day 41. The mechanical hypersensitivity at day 93, although significantly different from pre-paclitaxel baseline, is approximately half the magnitude it was at its peak [12]. As the pain induced by paclitaxel is so long-lasting, we cannot eliminate the possibility that some degree of pain could develop in the ALC-treated groups after day 41. However, we think that if we had continued the experiment beyond day 41, it is likely that any mechanical hypersensitivity that may have developed would be slight due to the natural decline of pain behaviour after this point.

Other studies have examined prophylactic therapies in models of paclitaxel-induced neuropathy that employed different dosing schedules and larger cumulative doses of paclitaxel than those used here. These models of paclitaxel-induced neuropathy result in a loss of sensory function, demonstrated by thermal hypoalgesia, as opposed to the paclitaxel-induced pain syndrome produced by the model we have employed. The higher doses of paclitaxel used in these models are often associated with degeneration, e.g. [6]. In comparison, the model used in this study shows no signs of degeneration [13], suggesting that paclitaxel-induced pain could be an early indicator of peripheral neurotoxicity. Pisano et al. [19] examined the effects of ALC on sensory nerve conduction velocities in paclitaxel-treated rats. Paclitaxel was given in a higher cumulative dose (25 mg/kg) than used in the present study (8 mg/kg) for a total 15 days prior to and during an i.v. paclitaxel dosing schedule. ALC partially reversed the paclitaxel-induced decrease in sensory nerve conduction velocities, but had no effect on the anti-tumour properties of paclitaxel [19]. Nerve growth factor (NGF) [1], glutamate [4], leukaemia inhibitory factor (LIF) [3] and prosaptide [5] have been shown to prevent paclitaxel-induced thermal hypoalgesia when given prophylactically.

ALC was not as effective in the treatment of paclitaxel-induced pain as it was in the prevention of the development of the pain syndrome. However, a significant attenuation of established paclitaxel-induced mechanical hypersensitivity was observed following repeated dosing. The analgesic effect dissipated shortly after ALC discontinuation. A recent open label study found that 8 weeks of ALC treatment lead to an overall improvement in the clinical neuropathy grade of patients with established paclitaxel- and cisplatin-induced neuropathies [2].

A few studies have examined the potential mechanism underlying the anti-nociceptive effect of ALC. Muscarinic receptors appear to play a role in acute ALC analgesia, as selective M₁ receptor antagonists and antisense knockdown of the receptor prevented the ALC enhancement of acute thermal thresholds [16]. Phospholipase C (PLC) inhibitors and LiCl (an inhibitor of phosphatidylinositol synthesis) antagonized ALC-induced antinociception, suggesting the involvement of the PLC-IP₃ pathway [15]. The ALC-induced increase in nociceptive thresholds of normal and nerve-injured rats was accompanied by increases in mGlu2/3 immunoreactivity in the lumbar spinal cord [7]. Such mGlu receptor up-regulation appears to be functionally relevant as the selective mGlu2/3 receptor antagonist, LY341495, attenuated the anti-nociceptive effects of ALC in normal and CCI rats [7]. It cannot be assumed that the

anti-nociceptive effects of ALC occur via the same mechanisms under normal conditions and post-injury conditions. Thus, further studies are required to identify if the receptor systems described above are also involved in the actions of ALC on chemotherapy-induced pain.

Chemotherapy-induced painful peripheral neuropathy remains a serious clinical problem. Our data show that oral ALC can effectively prevent the development of paclitaxel-induced pain and can also attenuate established paclitaxel-induced pain. ALC is well tolerated in humans [8,22] and there is evidence that it does not affect the anti-tumour properties of chemotherapeutics [19]. Therefore, we hypothesize that ALC therapy initiated at the same time as chemotherapy may reduce the number of patients who develop painful peripheral neuropathy.

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Fig. 1.

Effect of prophylactic ALC on the development of paclitaxel-induced pain. Oral ALC or vehicle was administered for 21 consecutive days (day 1 through day 20). Graphs show the mean $\pm$ S.E.M. of the response frequency to mechanical stimulation by: (A) von Frey 4 g; (B) von Frey 8 g and (C) von Frey 15 g before paclitaxel (pre-paclitaxel) and up to day 41 post-paclitaxel initiation. $*p < 0.05$, one-tailed unpaired *t*-tests with Bonferroni correction comparing 50 mg/kg ALC treatment or 100 mg/kg ALC treatment to vehicle treatment, all groups $n = 10$.

Fig. 2.

Effect of ALC on established paclitaxel-induced pain. Oral ALC or vehicle was administered for 10 consecutive days (day 17 through day 26), once the paclitaxel-induced pain was clearly evident (day 16 post-paclitaxel initiation). Graphs show the mean $\pm$ S.E.M. of the response frequency to mechanical stimulation by: (A) von Frey 4 g; (B) von Frey 8 g and (C) von Frey 15 g before paclitaxel (pre-paclitaxel) and up to day 36 post-paclitaxel initiation. $*p < 0.05$, one-tailed unpaired t -tests with Bonferroni correction comparing ALC treatment ($n = 13$) to vehicle treatment ($n = 14$).