Original Article

Doxepin prevents the Expression and Development of Paclitaxel-Induced Neuropathic Pain

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Abstract

Background: Peripheral neurotoxicity is a common side effect of many anticancer chemotherapy drugs, including paclitaxel. Peripheral neurotoxicity may present as changes in sensory function and mild paresthesia that, in turn, can lead to alleviation of the prescribed dose of the medication. The aim of this study was to evaluate the effectiveness of acute and chronic doxepin administration on development and expression of neuropathic pain during the treatment of cancer with paclitaxel. **Materials and Methods:** Neuropathic pain was induced in mice by paclitaxel (2 mg/kg, intraperitoneally [i.p.,] once daily from day 1 to day 5) that caused mechanical and cold allodynia. Doxepin was administrated every day from day 6 to 10 (10 and 15 mg/kg i.p.). Mechanical and cold allodynia was evaluated on day 11 of the experiment in both the test and the control group. **Results:** Daily administration of doxepin (2.5, 5, and 10 mg/kg i.p.) from day 1 to 5 significantly inhibited the development of cold and mechanical allodynia. As well doxepin administration (5 and 10 mg/kg i.p.) from the 6th day, to 10th day significantly inhibited cold and mechanical allodynia expression. To address the concerns associated with the effectiveness of chemotherapy agents on the tumor, we evaluated paclitaxel cytotoxicity effect in combination with doxepin. Our observations indicate that doxepin even at high concentrations (1 and 10 μg/ml) does not interfere with the cytotoxic effect of paclitaxel (0.05 μg/ml). **Conclusions:** These results indicate that doxepin, when administered during chemotherapy, can prevent the development and expression of paclitaxel-induced neuropathic pain.

Keywords: *Doxepin, mice, neuropathic pain, paclitaxel*

Introduction

Anticancer drugs frequently produce peripheral neuropathy, which is clinically significant and may lead to dose reduction and/or discontinuation of treatment.[1] Paclitaxel is one of the most effective chemotherapy agents, frequently used in the treatment of breast cancer.[2] However, paclitaxel produces peripheral neurotoxicity that leads to sensory abnormalities and neuropathic pain during the treatment that may persist after treatment. In some cases, neuropathic pain would diminish patients' quality of life and their daily activity.[3]

Paclitaxel stabilizes microtubules and interrupts mitochondrial function and DNA synthesis. These mechanisms may result in changes in peripheral nerves and lead to spontaneous activity of the fibers.[4]

By now, the etiology and underlying mechanisms of neuropathic pain are poorly understood and the existing treatments including anticonvulsant agents, local anesthetics, and opioids are often ineffective.[5,6]

Chronic pain can give rise to depression in these patients. Tricyclic antidepressants (TCAs) are the most common treatment methods for chronic pain treatment.[7-10]

Doxepin belongs to the TCAs and inhibits serotonin and noradrenaline (NE) reuptake.[11] Similar to other antidepressants, doxepin has shown analgesic properties.[12] Topical application of doxepin has demonstrated analgesic effects. Mika *et al*. have reported that doxepin and amitriptyline attenuated the symptoms of neuropathic pain in the mice subjected to chronic constriction injury. Chronic administration of doxepin or amitriptyline also caused allodynia and hyperalgesia in naïve mice.[13] On the other hand, Wrzosek *et al*. have

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reported that doxepin was effective in reducing thermal hyperalgesia and mechanical allodynia.[14]

Advantages of doxepin therapy include its lack of adverse interactions with prescription and nonprescription drugs and the high degree of safety seen in patients with concomitant cardiovascular and other physical disorders. Thus, doxepin appears to be an excellent choice in the long-term maintenance outpatient treatment of chronic depression.[15]

Research has also suggested an interaction between antidepressants and opioid receptors. Activation of the endogenous opioid mechanisms by serotonergic and/or noradrenergic pathways is believed to be involved in the antinociceptive effect of antidepressants.[16,17]

Chronic administration of doxepin can modify opioid receptor densities and increase endogenous opioid levels in certain brain regions.[18]

The antinociceptive effects of doxepin and the mechanisms by which it interferes with chemotherapy-induced neuropathic pain have not been well understood.

This study was designed to determine the effect of acute and chronic doxepin administration and its mechanisms in alleviating symptoms of paclitaxel-induced neuropathic pain in mice.

To identify the role of the opioid system in the antinociceptive effects of doxepin in the neuropathic pain, we examined the effect of naloxone (an opioid receptor antagonist) on the antinociceptive effects of doxepin in paclitaxel-induced neuropathic pain. As well, to respond to the concerns regarding the interference of doxepin with the efficacy of chemotherapy, we evaluated paclitaxel cytotoxicity in combination with doxepin.

Materials and Methods

Animals

Male mice weighing 20–30 g were used. They were housed under a 12-h light/dark cycle and free access to food and water. The animals were randomly assigned to different treatment groups (n = 6–8 in each group). All studies were conducted in accordance with the laboratory animals care guidelines provided by the "Ethics Committee of Isfahan University of Medical Sciences" and followed the European Commission Directive (86/609/EEC) for animal experiments.

Chemicals

Paclitaxel was purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Paclitaxel was dissolved in ethanol: cremophor: saline (5:5:90, v/v/v). Doxepin was purchased from Razak Laboratory (Tehran, Iran). Naloxone was obtained from Tolid Darou (Tehran, Iran). They all dissolved in normal saline 0.9%.

General procedures for drug treatments

To induce the neuropathic pain, paclitaxel (2 mg/kg) intraperitoneally (i.p) was injected on the 1st day and the injection was repeated every day for 4 additional days with a cumulative dose of 10 mg/kg.[19] Sham group received only vehicle.

To assess the effects of doxepin on the development (chronic effect) of neuropathic pain, doxepin (2.5, 5, and 10 mg/kg i.p.) administered every day from the 1st day to the 5th day in the different groups of mice. Furthermore, doxepin was injected (2.5, 5, and 10 mg/kg) once daily from day 6 to day 10. Mechanical and cold allodynia was assessed on the 11th day after the first paclitaxel injections.[20]

To determine the effect of doxepin on the expression (acute effect) of paclitaxel-induced neuropathic pain, it was injected 30 min before pain assessment on the 11th day.

Behavioral tests of neuropathic pain

Animals were placed on top of an aluminum mesh table. They were allowed to adapt for 15 min. To assess cold allodynia, an acetone drop was applied via a needle to the plantar surface of each hind paw for three times with 30 s interval. The time spent for licking the paw was recorded. The frequency of licking was calculated and expressed as a percentage with the following formula (number of trials attending the hind-paw/ total number of trials) ×100. To assess the mechanical allodynia, von Frey filaments (Steeling, Wood Dale, IL, USA) ranging from 0.16 to 6 g were used as previously described.[17] Bending force was applied to the plantar skin of the right hind paw, and each application was held for 6 s, using the up–down method to determine the threshold sensitivity.

Evaluation of the role of opioid receptors

To identify the role of the opioid system in the antinociceptive effects of doxepin, animals were treated by naloxone (1 mg/kg i.p.) on the 11thday. Naloxone was injected 10 min before the behavioral tests in different groups of mice. In these cases, doxepin (15 mg/kg i.p.) was administrated from the 6thday to the 10th day.

In vitro cytotoxic activity

Human breast cancer cell lines, MCF-7 (estrogen receptor positive) and MDA-MB231 (estrogen receptor negative), were purchased from the National Cell Bank of Pasteur Institute of Iran. Cell lines were maintained at 37°C and 5% CO_2. The different cell populations were cultured in RPMI (Biosera, France) containing 10% fetal bovine serum (Gibco, USA).

In vitro cytotoxicity assay was initiated by separately plating (180 µl) the two cell line (5 × 10^4 cells/ml of media) in 96-well microplates and incubation for

24 h (37°C, air humidified 5% CO2). After 24 h blue insoluble formazan crystals. The metabolic activity in each well was determined by a rapid colorimetric assay using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). Plates were read using an enzyme-linked immunosorbent assay plate reader at 540 nm. The cell viability was determined by the formula 1 (below) and was compared with untreated control.[21] doxepin and paclitaxel were added together to the 96-well microplate to obtain 1 and 10 µg/ml and 0.05 µg/ml concentrations, respectively. They also were added to the plates separately with the mentioned doses. Wells containing 180 µl of the cell suspension and 20 µl of DMSO (1%) were considered as negative control while the blank wells contained only 200 µl of the RPMI medium. The micro-plates were further incubated for 48 h. Each well was then treated with 20 µl of MTT solution for 3 h. Afterward, the media in each well was replaced with 200 µl DMSO to dissolve the Formula 1:

$$\%\text{survival} = \frac{\text{Mean of the well absorbance} - \text{Mean of the blank absorbance}}{\text{Mean of the negative control absorbance} - \text{Mean of the blank absorbance}} \times 100$$

Data analysis

Data are presented as mean ± standard deviation. Data were compared by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference *post hoc* test for multiple comparisons. Time-course analysis of behavioral data was compared by repeated-measures ANOVA for each experimental group. Two-way ANOVA was used to compare the antagonistic effect of naloxone.

Results

Time-course of paclitaxel-induced cold and mechanical allodynia

As shown in Figures 1a and 2a, mice which were treated with paclitaxel had become much more sensitive to acetone application as demonstrated by paw withdrawal frequency ($F_{4,29}$ = 12.67) and duration of paw licking ($F_{4,29}$ = 8.071) in comparison with the control group ($P < 0.001$). Paclitaxel injection led to a significant decrease of paw withdrawal threshold in comparison with the control group as shown in Figure 3 ($F_{4,29}$ = 4.521) ($P < 0.01$).

Effect of doxepin on the expression of cold allodynia

Acute administration of doxepin (10 and 15 mg/kg i.p.) 30 min before acetone test on the 11thday significantly blocked paw withdrawal frequency ($F_{4,29}$ = 12.67) and duration of paw licking ($F_{4,29}$ = 8.071) [Figures 1a and 2a] ($P < 0.001$).

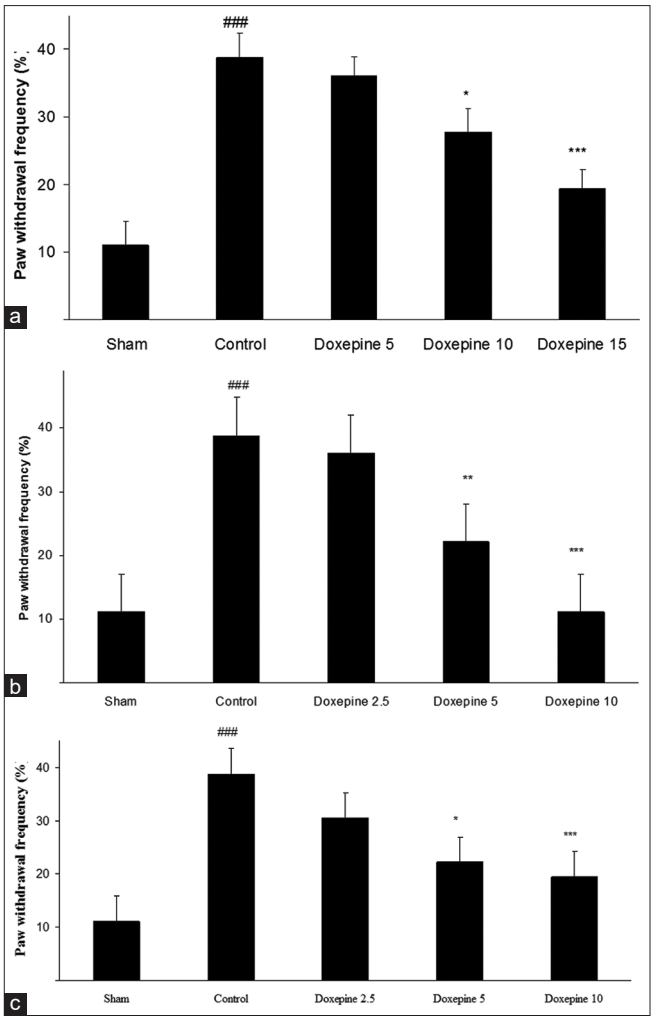

Figure 1: Paw withdrawal frequency. (a) Effect of acute treatment with doxepin (5, 10, and 15 mg/kg intraperitoneally) on the paw withdrawal frequency. (b), Effect of chronic treatment with doxepin (2.5, 5, and 10 mg/kg intra peritoneally) from 1st day to the 6th day. (c) Effect of chronic treatment with doxepin (2.5, 5, and 10 mg/kg intraperitoneally) from 6th day to the 10th day. The results are expressed as mean ± standard error of mean, ###*P* < 0.001 versus sham group, **P* < 0.05, ****P* < 0.01 and ******P* < 0.001 versus control (mice were treated with paclitaxel 2 mg/kg intra peritoneally) group, *n* = 6 in all groups

Effect of doxepin on the expression of mechanical allodynia

As shown in Figure 3a, a statistically significant difference was observed between the animals treated with acute administration of doxepin (10 and 15 mg/kg i.p.) on day 11 and the control group ($F_{4,29}$ = 4.521) ($P < 0.01$).

Effect of doxepin on the development of cold allodynia

Chronic treatment with doxepin (5 and 10 mg/kg) from the 1st day to the 6th day exerted significant antinociceptive effects against paw withdrawal frequency ($F_{4,29}$ = 15.44) and duration of paw licking ($F_{4,29}$ = 14.423) in comparison to the control group [Figures 1b and 2b] ($P < 0.001$). As well, administration of doxepin (5 and 10 mg/kg i. p.) from the day 6 to the day 10 reduced paw withdrawal

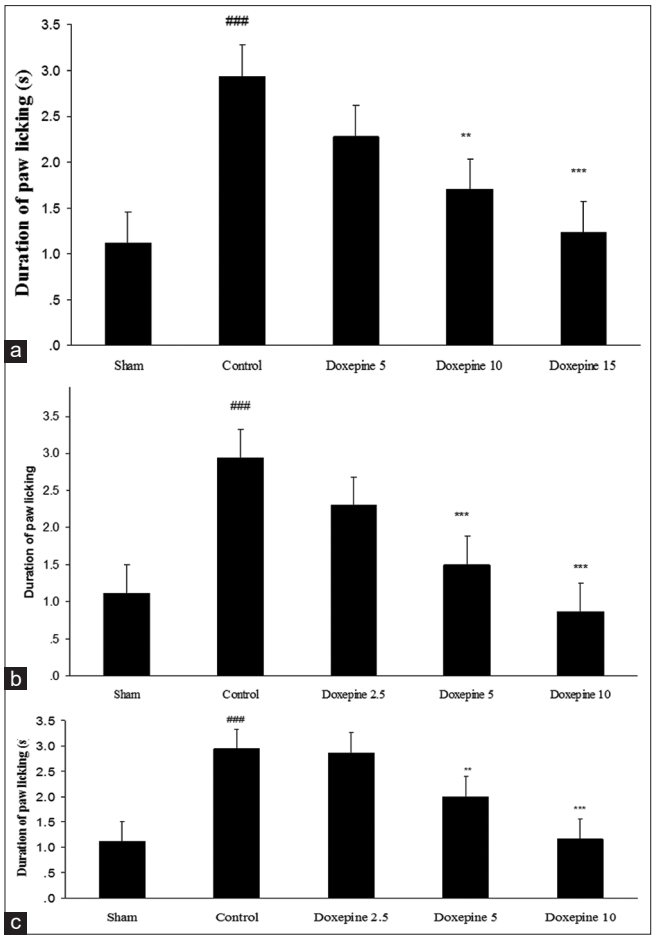

Figure 2: Duration of paw licking. (a) Effect of acute treatment with doxepin (5, 10, and 15 mg/kg intraperitoneally) on the duration of paw licking. (b) Effect of chronic treatment with doxepin (2.5, 5, and 10 mg/kg intraperitoneally) from 1st day to the 6th day. (c) Effect of chronic treatment with doxepin (2.5, 5, and 10 mg/kg intraperitoneally) from 6th day to the 10th day. The results are expressed as mean ± standard error of mean, ###*P* < 0.001 versus sham group, ***P* < 0.01 and ****P* < 0.001 versus control (mice were treated with paclitaxel 2 mg/kg intraperitoneally) group, *n* = 6 in all groups

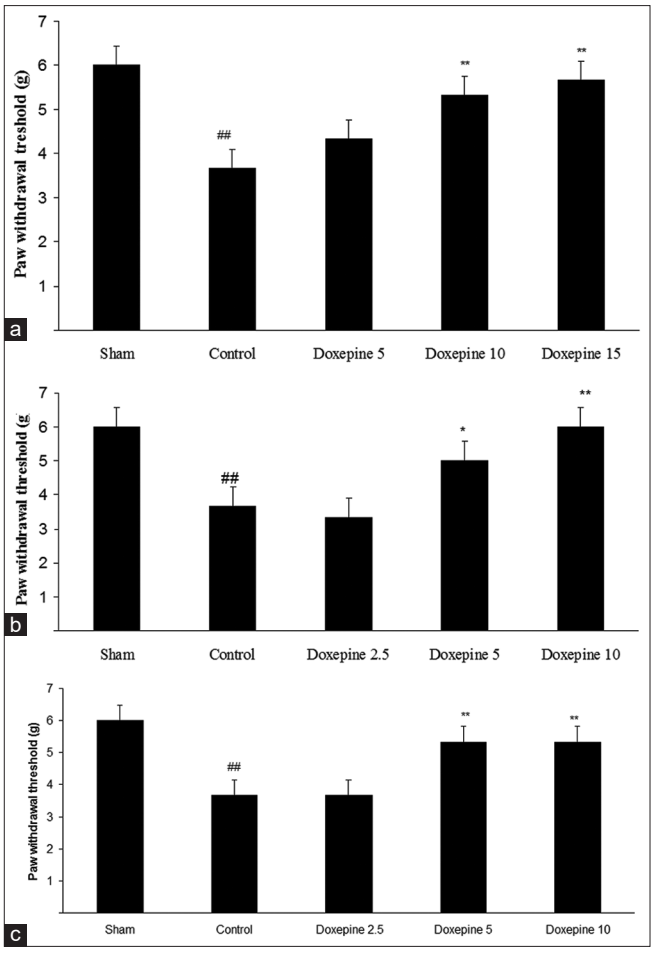

Figure 3: Mechanical allodynia, (a), Effect of acute treatment with doxepin (5, 10, and 15 mg/kg intraperitoneally) on the paw withdrawal threshold. (b) Effect of chronic treatment with doxepin (2.5, 5, and 10 mg/kg intraperitoneally) from 1st day to the 6th day. (c) Effect of chronic treatment with doxepin (2.5, 5, and 10 mg/kg intraperitoneally) from 6th day to the 10th day. The results are expressed as mean ± standard error of mean, ##*P* < 0.01 versus sham group, **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 versus control (mice were treated with paclitaxel 2 mg/kg intraperitoneally) group, *n* = 6 in all groups

frequency ($F_{4,29}$ = 6.303) and duration of paw licking ($F_{4,29}$ = 6.785) [Figures 1c and 2c] ($P < 0.001$).

Effect of doxepin on the development of mechanical allodynia

Chronic administration of doxepin (5 and 10 mg/kg i.p.) from the 1st day to the 6th day ($F_{4,29}$ = 10.515) and also from 6th day ($F_{4,29}$ = 6.776), after the establishment of neuropathic pain to the 10th day, noticeably reduced paw withdrawal threshold compared to the control group [Figure 3b and c] ($P < 0.01$).

Effects of naloxone on doxepin-induced effects

Naloxone (1 mg/kg i.p.) did not induce any significant changes in the nociceptive thresholds.

Figure 4a-c show the effect of naloxone (1 mg/kg i.p.) on the antinociceptive effect of doxepin injection (15 mg/kg i.p.). Naloxone could not change anti allodynic effects of doxepin on the frequency of paw withdrawal ($F_{3,21}$ = 16.356) [Figure 4a], duration of paw licking ($F_{3,21}$ = 5.954) [Figure 4b] and mechanical allodynia ($F_{3,21}$ = 5.213) [Figure 4c].

Cytotoxicity effect of doxepin

The cytotoxicity effect of doxepin on MCF-7 and MDA cell lines was evaluated through microculture tetrazolium assay (MTT). Results of the cytotoxicity evaluation against MCF-7 and MDA cell lines are shown in Figure 5. Doxepin (1 and 10 µg/ml) exhibited no significant activity against MCF-7 and MDA cell lines. On the contrary paclitaxel (0.05 µg/ml) exhibited significant activity against both cell lines. As well, doxepin did not inhibit the cytotoxic effect of paclitaxel [Figure 5a and b].

Discussion

Chemotherapy-induced peripheral neuropathy is a relatively common and serious consequence of cancer

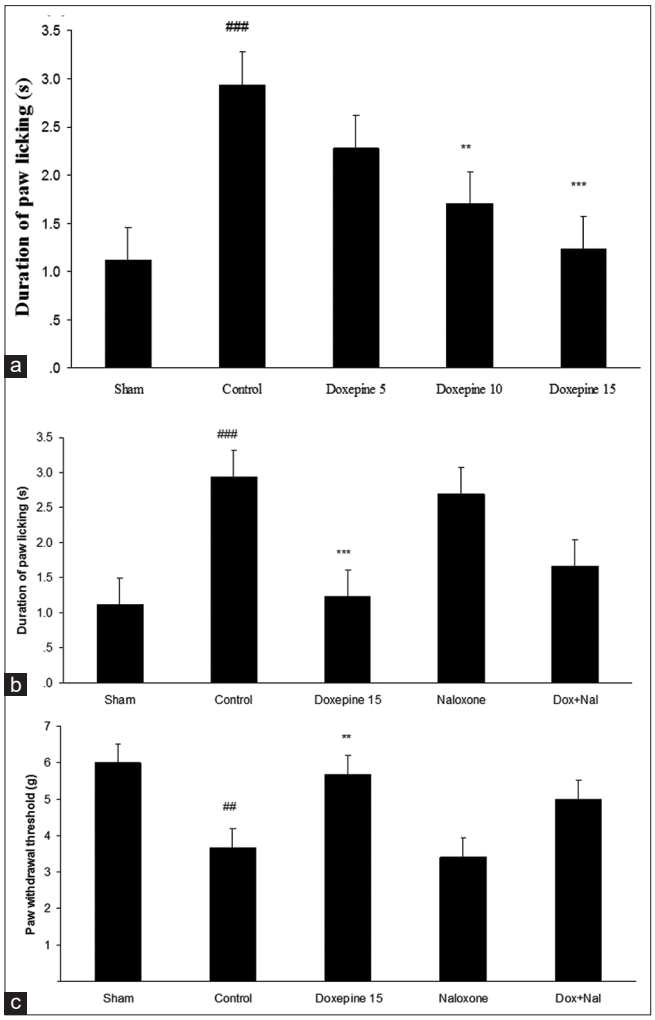

Figure 4: (a) The effect of naloxone (1 mg/kg intraperitoneally) on the antiallodynic effect of acute treatment with doxepin (15 mg/kg intraperitoneally) on the paw withdrawal frequency. (b) (1 mg/kg intraperitoneally) on the antiallodynic effect of acute treatment with doxepin (15 mg/kg intraperitoneally) on the duration of paw licking. (c) (1 mg/kg intraperitoneally) on the anti allodynic effect of acute treatment with doxepin (15 mg/kg intraperitoneally) on the paw withdrawal threshold. The results are expressed as mean ± standard error of mean, $n = 6$ in all groups. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus sham group, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control (mice were treated with paclitaxel 2 mg/kg intraperitoneally) group

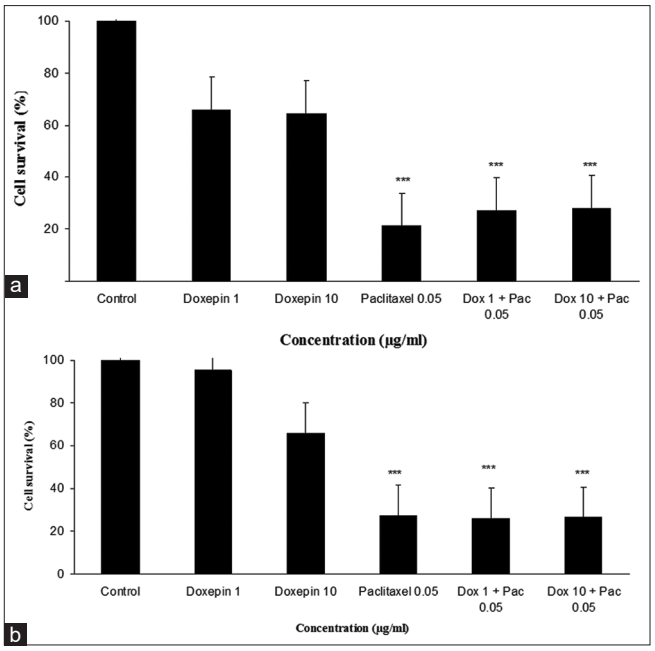

Figure 5: Cytotoxicity effect of doxepin, (a) Evaluation of the cytotoxicity of doxepin (1 and 10 µg/ml) alone and in combination with paclitaxel (0.05 µg/ml) on MDA cell lines. (b) Evaluation of the cytotoxicity of doxepin (1 and 10 µg/ml) alone and in combination with paclitaxel (0.05 µg/ml) on MCF-7 cell lines, $^{***}P < 0.001$ versus control (vehicle treated cells) group, Dox 1+ Pac 0.05 (cells were treated with doxepin 1 µg/ml in combination with paclitaxel 0.05 µg/ml), Dox 10+ Pac 0.05 (cells were treated with doxepin 10 µg/ml in combination with paclitaxel 0.05 µg/ml)

treatment. Chemotherapy-induced peripheral neuropathy is often the main reason for reduction in dose of the medication and/or discontinuation of the therapy, hence it may limit the application of lifesaving agents. This study suggests that systemic administration of doxepin inhibits paclitaxel-induced neuropathic pain.

Considering our results, acute doxepin (10 and 15 mg/kg i.p.) administration inhibited the expression of paclitaxel-induced mechanical and cold allodynia in mice. The repeated co-administration of doxepin (5 and 10 mg/kg i.p.) and paclitaxel prevented the development of both kinds of allodynia. When doxepin (5 and 10 mg/kg i.p.) administration was initiated from the 6th day, once the model had been fully established, and the administration continued for 4 additional days, mechanical and cold allodynia significantly blocked. Results from doxepin cytotoxicity evaluation indicated no cytotoxic effect from doxepin itself and demonstrated that doxepin has no interference with the cytotoxicity of paclitaxel.

This is of note that none of the adverse effects commonly associated with administration of doxepin, including dizziness, drowsiness, or ataxia were not observed. This result is consistent with previous studies that suggested doxepin does not decrease motor performance when consumed at low doses (i.e. <30 mg/kg).[13,15]

Since pain is a complex neurobiological phenomenon with a diversity of neurochemical factors contributing to both peripheral and central pain-signaling mechanisms, it is likely that various mechanisms are involved in the antinociceptive effect of doxepin.

The primary focus, with regard to the mechanism of action of doxepin, was its ability to inhibit biogenic amine reuptake.[22] Doxepin inhibits the reuptake of serotonin (5-HT) and to a lesser extent, NE.

Given that both 5-HT and NE are involved in the nociception, it is probable that antidepressants modulating these neurotransmitters exhibit antinociceptive effects, although studies suggest that antidepressants that target both 5-HT and NE neurotransmission are more effective than serotonergic agents in relieving various types of chronic pain.

Investigations also propose an interaction between antidepressants and opioid receptors. Activation of the endogenous opioid mechanisms by serotonergic and/or noradrenergic pathways is believed to be involved in the antinociceptive effect of antidepressants.[16,17]

Chronic antidepressant administration is capable to modify opioid receptor densities and increase endogenous opioid levels in certain brain regions.[18] It appears that antidepressants may interact both directly and indirectly with endogenous opioid systems to establish analgesia.[23]

For example, a study evaluating venlafaxine and mirtazapine found that both the agents significantly potentiated antinociceptive effects through interactions with multiple opioid receptors. Other investigations have demonstrated inhibitory effects of naloxone, an opioid antagonist on the analgesic effects of antidepressants.[8] Additional data suggest that dual-acting antidepressants (e.g., TCAs) may produce analgesic effects as a result of both direct and indirect interactions with opioid systems.[24]

The present study investigated if opioid-related mechanisms contribute to the antinociceptive effects of doxepin in paclitaxel-induced neuropathic pain. Our results showed that naloxone as an opioid antagonist could not reverse the antinociceptive effects of doxepin.

These results demonstrate that doxepin, as a TCAs, differs from other TCA drugs in the antinociception mechanism by which it applies its analgesic effect.

Interestingly, TCAs exhibit diverse pharmacological properties and this may account for a variety of specific pharmacological profiles between agents.

The differential involvement of myelinated and unmyelinated peripheral nerve fibers in the pathogenesis of each sign of pain has been already reported. Mechanical allodynia is evoked by inputs from myelinated fibers (mainly Aβ-fibers, but also Aβ-fibers), whereas heat hyperalgesia is evoked mainly by unmyelinated C-fibers, and both Aδ-and C-fibers are involved in cold allodynia.[25]

It is well known that voltage-gated Na + channels play a critical role in neuronal function and in the development of chemotherapy-induced peripheral neurotoxicity.[26] Doxepin inhibits sodium channels which may participate in its antinociception effects.[27] The effect of doxepin cream has been approved in dermatitis induced by radiotherapy.[28] However, further studies are needed to validate this theory.

Conclusions

This study indicates that doxepin, when administered during or upon completion of chemotherapy, can prevent the expression and development of neuropathic pain. This effect is not inhibited by naloxone which, interestingly, distinguishes doxepin from other TCAs. Further investigations are required to clarify the possible mechanisms of doxepin in the attenuation of neuropathic pain.

In addition, we demonstrated that doxepin does not interfere with the cytotoxic effect of paclitaxel. These results suggest a potential application for doxepin in the management of neuropathic pain during chemotherapy with paclitaxel. However, further preclinical and clinical studies are warranted to validate this effect and establish the effective doses of doxepin in human.

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Conflict of interest

The authors declare no conflicts of interest.

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