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Research report

Evidence for an antihyperalgesic effect of venlafaxine in vincristine-induced neuropathy in rat

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Abstract

Venlafaxine, a new antidepressant with fewer side effects, could be of interest to reduce neuropathic pain following antineoplasic drug treatment. In the present study, we demonstrated that venlafaxine inhibits hyperalgesia in a new rat model of neuropathy induced by the antineoplasic drug vincristine, and exerts its effect preferentially via supraspinal and spinal mechanisms.

Theme: Sensory systems

Topic: Pain modulation: pharmacology

Keywords: Venlafaxine; Neuropathic pain; Antineoplasic drug; Antidepressants; Paw pressure; C reflex; Rat

Within the numerous adverse effects associated with antineoplasic drugs, painful peripheral neuropathy is frequent [19]. Tricyclic antidepressants (TCAs) together with antiepileptic drugs are still the first choice in the treatment of these pains [15,16]. However, these drugs have many side effects that limit their therapeutic use. A newer antidepressant, venlafaxine, which is devoid of TCAs adverse side effects, could have a therapeutic potential [11].

Vincristine, an antineoplasic agent widely used in cancer therapy, is neurotoxic for all treated patients and can induce peripheral neuropathy [8,20,21]. The development of a new animal model of nociceptive neuropathy using repeated injections of vincristine [4] represents an opportunity for testing the antihyperalgesic efficacy of venlafaxine. The first aim of this work was to assess the antihyperalgesic effect of venlafaxine in a model of toxic neuropathy. Secondly, we studied its effect on two differentially integrated nociceptive tests: a supraspinally integrated pain response, the vocalisation to paw pressure, and a spinal integrated test, the C-fiber evoked nociceptive reflex, to determined the preferential level of its action in the central nervous system.

These studies were conducted in accordance with IASP guidelines for animals experiments [22]. Neuropathy was induced in male Sprague–Dawley rats (Charles River, France), weighing 180–200 g (protocol adapted from Ref. [4]). Briefly, five intravenous injections of 150 μg/kg of vincristine (Oncovin®, Elly Lilly) were performed every 2 days until a cumulative dose of 750 μg/kg was reached.

For the paw pressure test, the vocalisation thresholds, expressed in grams (g), were measured with a Ugo Basil analgesimeter (Bioseb) by applying an increasing pressure to the right hind paw of unrestrained rats until a squeak was elicited (a cut-off level of 750 g was applied). Preliminary thresholds to paw pressure (the mean of two consecutive stable values which do not differ more than 10%) were determined before (control pre-neuropathic values) and 14 days after the beginning of vincristine treatment (control pre-drug values). Venlafaxine was then injected and the vocalisation thresholds were determined 15, 30, 45, 60, 90 and 120 min after this injection.

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The C-fiber evoked flexor reflex elicited in the right hind limb of anesthetized rat (0.9% halothane in 200 ml/min oxygen, Capnomac II, Datex Instruments) was recorded as described previously [6,17]. Briefly, rectangular electric pulses of 6–7 mA strength and 2 ms duration were applied every 10 s to the sural nerve receptive field by means of two stainless steel needles inserted into the skin of toes 4 and 5. The C-fiber evoked reflex response (electromyographic responses) was recorded from the ipsilateral biceps femoris muscle by utilizing another pair of stainless steel needles. Once a stable threshold C reflex response was obtained, the stimulus strength was increased by a 3-fold factor. Venlafaxine (10, 20, 40 and 80 mg/kg, s.c.) was then injected and the mean C-fiber reflex (mean of the 12 C-fiber reflex recorded during the 2-min period) was calculated every 2 min between 25 and 35 min. Validity of the C reflex response as a tool for pharmacological studies of spinal mechanisms involved in antinociception has already been demonstrated [10,17].

The data analysis was performed by a two-way analysis of variance (ANOVA) followed by a Student–Neuwmans–Keuls test for the time-course of the effect of venlafaxine on mechanical nociceptive thresholds, the significance level being 0.05. Areas under these time-course curves (AUC) were evaluated by the method of trapezoidal rule and plotted against log dose, and the ED_{50} calculated from linear fitting by utilizing standard interpolation procedures (Origin 3.5, Microcal Software). For the C reflex, results were expressed as mean percentage inhibition of the integrated C reflex responses obtained between 25 and 35 min after venlafaxine injection and plotted against log dose, the ED_{50} being calculated in the same way as for the effect of venlafaxine on mechanical test.

Fourteen days after starting the vincristine treatment, rats developed a mechanical hyperalgesia according to Ref. [4]. The decrease of the vocalization thresholds (Fig. 1) was 118.9±4.9 g. Venlafaxine induced a dose-dependent increase of the vocalization thresholds. This increase was significant at the 15th min for 10 mg/kg, between 15 and 45 min for 20 mg/kg and throughout the experiment for the highest dose used of 40 mg/kg (Fig. 1). For the two highest doses, vocalization thresholds return to pre-injury values showing a complete antihyperalgesic effect of the antidepressant. The analysis of the area under the curve confirmed the dose-dependent antinociceptive effect of the antidepressant on the paw pressure test with an ED_{50} of 16.0 mg/kg (95% confidence intervals of 10.5 and 24.3 mg/kg) was calculated (Fig. 1, inset).

Acute injection of venlafaxine at doses of 10, 20, 40 and 80 mg/kg s.c. moderately, but dose-dependently, depressed the C-fiber evoked reflex (Fig. 2). This inhibitory effect of the antidepressant on the C reflex was significant at concentrations higher than 10 mg/kg and a ceiling effect was observed with the highest dose used. A maximal inhibitory effect of 34.4% was calculated by fitting an exponential function in the log dose plot, which could be

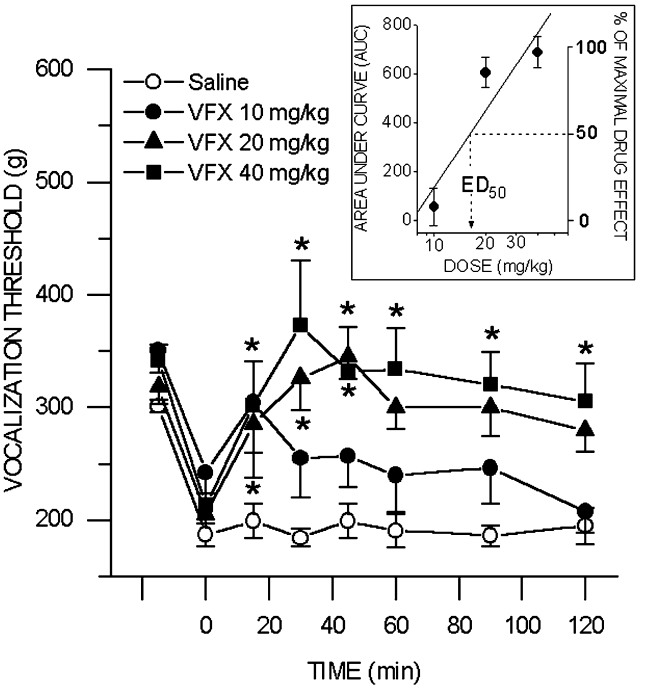

Fig. 1. Time-course of the effect of 10, 20 and 40 mg/kg, s.c. of venlafaxine (VFX) and saline on mechanical pain thresholds to paw pressure test in a vincristine model of chemical neuropathy in rats. Vocalization thresholds were determined before and after neuropathy and 15, 30, 45, 60, 90 and 120 min after venlafaxine injection (at time 0). They are expressed as mean (±S.E.M.) in grams (g). $n=8$ in each group. *$P<0.05$ versus control group (Two way ANOVA followed by a Student–Neuwmans–Keuls test). Inset: Log dose plot of the area under the curves (AUC) after venlafaxine 10, 20 and 40 mg/kg, s.c ($r=0.92$). The estimated ED_{50} is 16.0 mg/kg (95% confidence intervals of 10.5 and 24.3 mg/kg).

considered as the asymptotic maximal possible drug effect (100%) in the C reflex paradigm. Relative to the maximal drug effect, an ED_{50} of 27.2 mg/kg (95% confidence intervals of 20.4 and 35.5 mg/kg) was calculated (Fig. 2).

The main finding that emerges from this work is that venlafaxine produces a significant antihyperalgesic effect in the vincristine-induced neurotoxic pain model. This result is in line with the very recent clinical study that shows the effectiveness of venlafaxine in neuropathic pain following treatment of breast cancer [18] which validates the pharmacological interest of the new rat model of neuropathic pain we used. In fact, after vincristine treatment, rat developed hyperalgesia to painful mechanical stimulation (reduction of 35.4±1.4% of the vocalization threshold to paw pressure), which is in agreement with earlier studies [1,4,13] demonstrating that this antineoplasic drug can induce mechanical (paw pressure test) and thermal hyperalgesia (plantar test), as well as tactile allodynia to Von Frey hair application.

Concerning treatment of pain in chemotherapy, tricyclic antidepressants are the first choice [16] but they induce side effects that limit their therapeutic potential. Venlafaxine is a newer antidepressant that has lower side effects

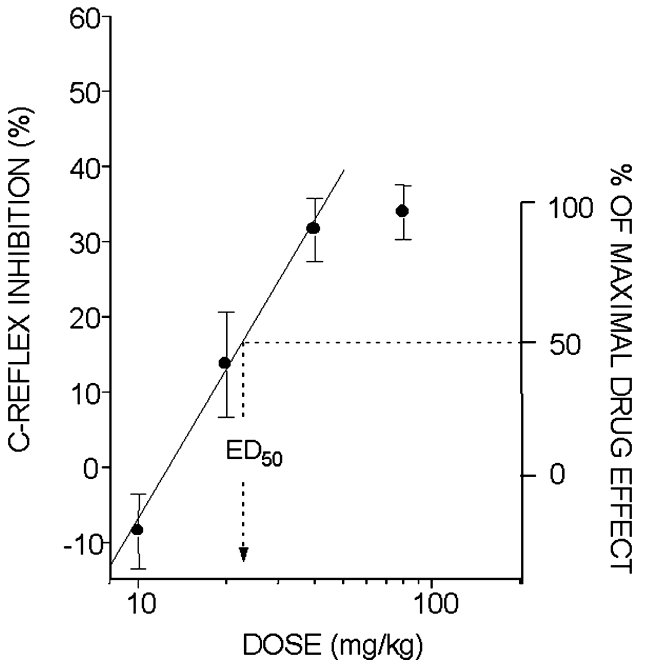

Fig. 2. Log dose plot of the effect of venlafaxine (10, 20, 40 and 80 mg/kg, s.c.) on the C-fiber evoked nociceptive reflex in a vincristine model of chemical neuropathy in rats. Values correspond to percentage inhibition of the C reflex response and are expressed as mean (±S.E.M.). $n=8$ in each group. For linear regression analysis the point corresponding to the dose of 80 mg/kg was discarded ($r=0.97$). The estimated ED_{50} is 27.2 mg/kg (95% confidence interval of 20.4 and 35.5 mg/kg).

[11] and thus can have a clinical relevance for the treatment of painful neuropathy. The present results show that this antidepressant can relieve mechanical hyperalgesia in a rat model of chemical neuropathic pain. Its effect is characterized by an inhibition of the decrease of vocalization thresholds to the paw pressure test, hence an inhibition of vincristine-induced hyperalgesia. It has been already demonstrated that antidepressants can be effective in this mechanical pain test in other animal models of painful neuropathy, such as mononeuropathic rats [2] or diabetic rats [5]. Venlafaxine has also shown antinociceptive effects in mononeuropathic rats by relieving thermal hyperalgesia in the plantar test [9].

In the present study, we explored the relative contribution of spinal versus supra-spinal mechanisms by comparing the effect of systemic venlafaxine on two tests integrated at different levels on the neuroaxis. Venlafaxine produced a moderate inhibitory effect on the C-fiber mediated reflex evoked in vincristine-induced neuropathic rats, which seems apparently inconsistent with the potent antinociceptive effect exerted in the paw pressure testing. However, the venlafaxine ED_{50} for paw pressure testing and for C reflex inhibition are not significantly different when compared each other. In addition to the different kind of nociceptive stimulus considered in this comparison (electrical versus mechanical), the main factor for this apparent differential effectiveness of venlafaxine could be related to a definition of a strong inhibitory effect. Thus, 100% inhibition of C reflex nociceptive response means ‘no pain’ (at least for that mediated by C-fibers), while 100% inhibition of mechanical hyperalgesia does not imply ‘no pain’ (at least for mechanical pain) but only suppression of mechanical hyperalgesia. Further, the inhibitory effect induced by the high dose of venlafaxine (80 mg/kg) on the integrated C reflex of neuropathic rats (about 35% inhibition) is not less than that produced by high doses of other antidepressants such as desipramine [7] and clomipramine [14] on normal rats. Thus, both supra-spinal and spinal mechanisms may play a role in the antihyperalgesic effect of venlafaxine. In fact, a spinal mechanism must be involved to reduce the C-fiber reflex. This spinal action could be directly exerted via activation of opioid or adrenergic receptors, but venlafaxine does not strongly bind to these receptors [12]. Its action could be consecutive to the activation of descending inhibitory pathways conveyed by the dorsolateral funiculus, as shown for clomipramine [3]. Moreover, Mestre et al. [10] reported that clomipramine depress the C-fiber-evoked spinal reflex by acting at a supra-spinal modulatory site. It could be possible that venlafaxine act in the same way even if a direct spinal effect cannot be ruled out.

In conclusion, the present results indicate a therapeutic potential of venlafaxine, a better tolerated antidepressant, for chemotherapy-induced painful neuropathy. The antinociceptive effect of venlafaxine in the vincristine rat model seems to involve both supra-spinal and spinal mechanisms, which need further works to be elucidated. Further clinical and experimental studies are required to determine the potential benefit of venlafaxine for the treatment of antineoplasic drugs-induced peripheral neuropathy.

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