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Original Article

Sildenafil Attenuates Vincristine-induced Early Onset Tactile Allodynia in Rats

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Abstract

Cancer chemotherapies are often associated with painful neuropathies due to diverse causes. A deficit in nitric oxide is postulated to be involved in the pathogenesis. The purpose of the study was to evaluate the effect of sildenafil, a PDE-5 inhibitor, on nociception in neuropathy due to vincristine administration in rats.

Neuropathy was produced in male albino rats by vincristine administration. Animals received either of the following treatments on days 10-14: saline, gabapentin, sildenafil and sildenafil+L-NAME. The animals were assessed using 3 models of nociception viz., tactile allodynia using a monofilament, thermal allodynia by hot-plate method and tail flick test.

Significant tactile allodynia developed by day 5. There was no significant change in allodynia in the hot-plate test or tail flick test. Sildenafil (0.5, 1 and 2 mg/kg) showed a dose response effect in attenuating the vincristine-induced allodynia. Sildenafil (1 and 2 mg/kg) and gabapentin (50 mg/kg) attenuated tactile allodynia significantly ($p<0.05$) on all days whereas had no effect in thermal allodynia by hot-plate method and tail-flick test. Addition of L-NAME reversed sildenafil's attenuation of tactile allodynia.

The present study demonstrates the involvement of nitric-oxide in the pathogenesis of vincristine-induced early onset tactile allodynia which can be reversed by sildenafil.

Introduction

Neuropathic pain is a complex condition that may develop when nerve fibres are damaged or dysfunctional. A wide variety of insults including metabolic disorders like diabetes mellitus (DM), traumatic nerve injury, and neurotoxic drugs like vincristine result in peripheral neuropathy associated with loss of sensation and numbness in the feet, hands, and legs accompanied by painful tingling or burning sensation (1, 2). These neuropathies are characterized by hyperexcitability of the nociceptors and changes in central pathways that modulate

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sensory transmission (3-5).

Chemotherapy induced peripheral neuropathy (CIPN) has a high degree of similarity in pattern and spectrum of clinical manifestations caused by various chemotherapeutic agents (e.g. vincristine, platins, thalidomide), which includes length dependent, symmetrical glove and stocking distribution with predominantly sensory symptoms. The pathophysiology is poorly understood but interference with tubulin function and direct damage to sensory nerve cell bodies of dorsal root ganglia has been found (6). Administration of these chemotherapeutic agents induces neuropathic pain behaviour in rats which mimics that seen in patients (7).

Several compounds like nerve growth factor, amifostine, glutathione and glutamate have been tried to prevent the toxicities of chemotherapeutic agents (6). The symptomatic management of neuropathic pain due to chemotherapy mainly relies on the use of antidepressants, anticonvulsants, anaesthetics and opiates. However there is a clear medical need for new treatments to improve safety and efficacy (8).

There are reports suggesting the role of nitric oxide (NO) in pathogenesis of nerve conduction. Impaired NO production has been shown to be associated with the occurrence of various forms of neuropathic symptoms including hyperalgesia and spontaneous pain in animal models of CIPN (9-14). The effects of NO are mediated by cyclic guanosine monophosphate (cGMP), which is degraded by the enzyme phosphodiesterase-5 (PDE-5). Here, we aim to evaluate the role of sildenafil, which inhibits PDE-5 and thus potentiates NO, in alleviation of various parameters of peripheral neuropathy and in comparison to gabapentin in animal model of CIPN caused by vincristine.

Materials and Methods

Animals

Male albino rats weighing around 200-250 g were used. Animals were housed in polypropylene cages under standard laboratory conditions maintained at 25±2°C, humidity 65±5 and light and dark cycle of 12 hours. Animals had a free access to food and water.

The experiments were conducted between 0900-1600 hrs. The procurement, storage and experimentation of animals were done in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (15) after approval from Institutional Animal Ethics Committee.

Drugs

Vincristine (Cipla, India), Sildenafil (Cipla, India) and Gabapentin (Sun Pharma, India) were purchased from the pharmacy. NG-nitro-l-arginine methyl ester (L-NAME) was purchased from Sigma chemicals (Switzerland). Sildenafil-citrate and gabapentin tablets were finely crushed into powder form. The drugs were then dissolved in 0.9% NaCl (saline) and solution was vigourously shaken using a test-tube shaker in a tube to make it homogeneous, immediately before loading in a syringe. It was administered by intraperitoneal (i.p.) route in a volume of 1ml.

Induction of neuropathic pain with vincristine

Vincristine sulphate was dissolved in 0.9% NaCl and rats received injections at a dose of 200 µg/kg i.v. (1 ml/kg) on days 1, 4 and 6 (cumulative dose 600 µg/kg) (16).

Procedure

The vincristine-treated rats were administered following drugs daily on days 10-14: saline, sildenafil (0.5, 1 and 2 mg/kg), gabapentin 50 mg/kg (17), sildenafil 2 mg/kg + L-NAME 20 mg/kg (18).

L-NAME, a nitric oxide synthase (NOS) inhibitor, was administered 30 min prior to sildenafil. For assessment of parameters, administration of drugs and observations were performed blind by different persons.

Nociceptive tests

Tactile Allodynia

Tactile allodynia was measured by assessing rat hind

paw withdrawal thresholds in response to mechanical stimulation by a modified technique using von Frey filament (INCO). The rats were acclimatized in individual clear Plexiglass boxes on an elevated wire mesh platform to allow access to the plantar surface of the hindpaws. The filament was pressed perpendicularly against the mid-plantar surface of the right hindpaw from below the mesh floor and held for 3-5 s with it slightly buckled. The experiment was started using the greatest length of the filament i.e. 6 cm and gradually the length of the filament was shortened with successive attempts. 3 attempts were done at each length at intervals of 5 s. The length of the filament that caused the animal to flinch or move the paw away from the stimulus 2 out of 3 times was determined to be the mechanical threshold. The shorter the length of the filament, the greater was the mechanical threshold. Testing was performed on days 0, 3, 5, 7, 10, 11, 12, 13, 14.

Thermal allodynia

Thermal allodynia was assessed by hot-plate method. Animals were placed on a metal plate adjusted to 38°C (19) The latency of the first reaction was recorded (licking, moving the paws, little leaps, or a jump to escape the heat, with a cut-off time of 30 s. Testing was done before and 2 hours after administration of drugs on day 0 and days 10-14.

Tail-flick latency

The animals were positioned on a tail-flick analgesiometer (Techno), so that their tail was subjected to radiant heat from a heated filament (20). The latency to the flicking of the tail was recorded. Baseline values were determined on day 0. Testing was done before and 2 hours after administration of drugs on day 0 and days 10-14.

Statistical analysis: All values were expressed as Mean±S.D. Tactile allodynia: In each group, repeated measures ANOVA (RMA) followed by Scheffe's test was used to compare the mean of day 0 values with that of other days. In total, forty-eight pair-wise combinations were tested (eight in each group). One way ANOVA followed by Dunnett's *t*-test (2-sided) was used to compare the mean values for tactile allodynia of different treatment groups from day 10 – day 14.

Data of thermal allodynia and tail flick test (pre and post-treatment) was compared using Student's *t* test; $p<0.05$ was considered statistically significant.

Results

Tactile allodynia

A progressive decrease in the threshold stimulus in comparison to day 0 values was noted in all animals which started building up from day 3 and fully established at day 7 [$F(8,45)= 31.3$ in saline group; 44.9 in gabapentin group; 12.8 in sildenafil 0.5 mg/kg group, 31.9 in sildenafil 1 mg/kg group 27.6 in sildenafil 2 mg/kg group, and 10.9 in sildenafil 2 mg/kg + L-NAME 20 mg/kg group ($p<0.001$ on day 7 in all the groups)]. It remained significantly low in comparison to day 0 values up to 8 days after the last injection of vincristine in vehicle-treated animals (days 7-14; $p<0.001$ for each comparison). Mean threshold stimulus was significantly higher in sildenafil 0.5 mg/kg than saline (control) on day 10 [$F(5,30) = 17.4$ ($p<0.05$ using Dunnett's test)] but not on days 11-14. Sildenafil 1 and 2 mg/kg and gabapentin 50 mg/kg significantly reversed vincristine-induced allodynia 2 h after i.p. injection from day 10 to day 14 compared with vehicle-treated animals {[$F(5,30) = 17.4$(day 10), 18.2 (day 11), 48.3 (day 12), 27.4 (day 13), 17.5 (day 14); ($p<0.001$ for each)]; $p<0.05$ for each using Dunnett's test}. Administration of L-NAME 20 mg/kg before sildenafil (2mg/kg) reversed its attenuation of vincristine-induced tactile allodynia (Fig. 1, 2).

Thermal allodynia

No significant change in threshold to thermal stimulation was noted on days 10-14 following vincristine treatment. None of the drugs-sildenafil (2 mg/kg), gabapentin or sildenafil+L-NAME combination-showed any significant change in threshold to thermal stimulation by the hot plate test or tail-flick test after 2 h of i.p. injection (Table I, II).

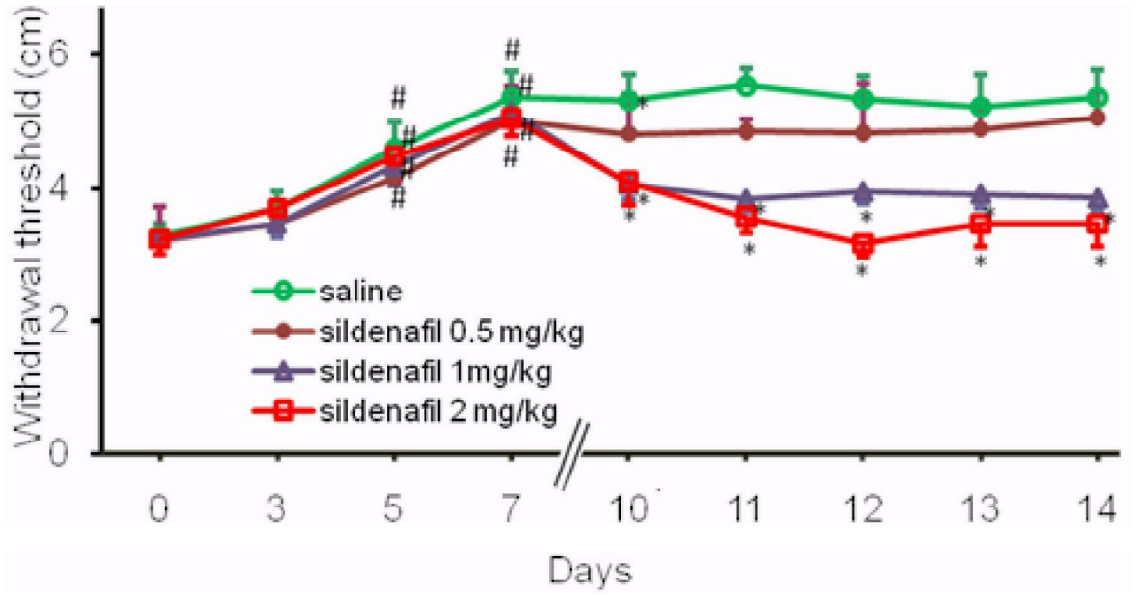

Fig. 1 : Effect of sildenafil (0.5, 1 and 2 mg/kg) on vincristine-induced tactile allodynia in rats. Day-0 represents values before administration of vincristine. Data are expressed as Mean±SD, (n=6 in each group). #p<0.05 compared versus day-0; *p<0.05 compared with vehicle-treated rats on same day. A progressive decrease in the threshold stimulus in comparison to day-0 values was noted in all animals which fully established after the third injection on day-7 and remained significantly low up to day-14 in vehicle-treated animals (repeated measures ANOVA followed by Scheffe's test). 0.5 mg/kg dose of sildenafil significantly reversed vincristine-induced allodynia 2 h after i.p. injection on day-10 while 1 and 2 mg/kg dose significantly reversed the allodynia on days 10-14 compared with vehicle-treated animals (one way ANOVA followed by Dunnett's t-test).

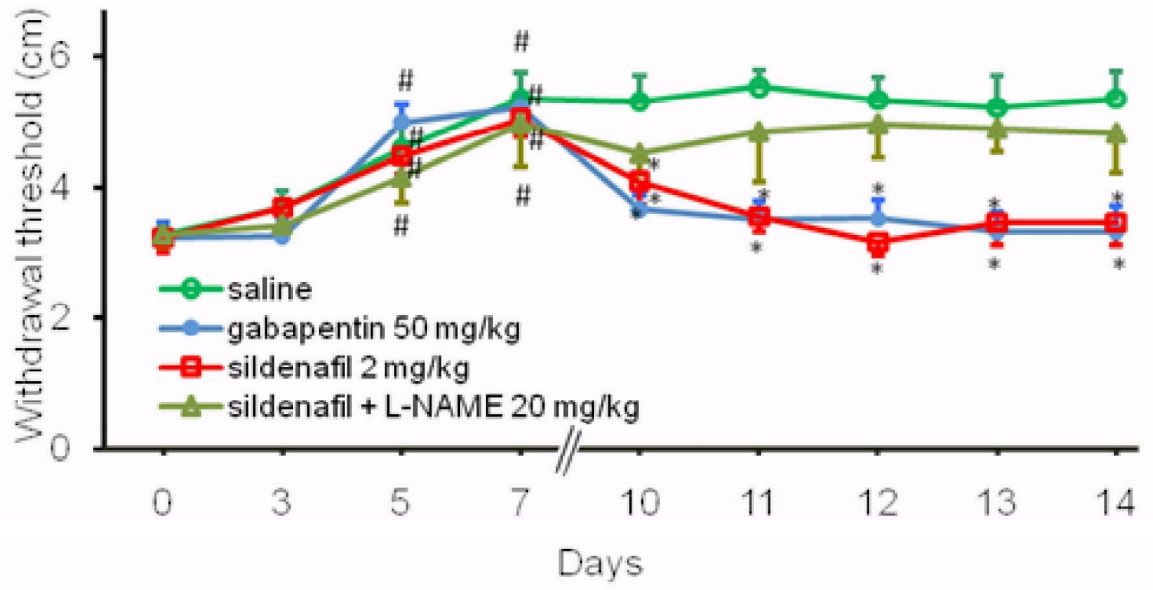

Fig. 2 : Effect of saline, sildenafil, gabapentin, sildenafil+L-NAME on vincristine-induced tactile allodynia in rats. Day-0 represents values before administration of vincristine. Data are expressed as Mean±SD, (n=6 in each group). #p<0.05 compared versus day-0; *p<0.05 compared with vehicle-treated rats on same day. A progressive decrease in the threshold stimulus in comparison to day-0 values was noted in all animals which fully established after the third injection on day-7 and remained significantly low up to day-14 (repeated measures ANOVA followed by Scheffe's test). Both sildenafil and gabapentin significantly reversed vincristine-induced allodynia 2 h after i.p. injection from days 10-14 compared with vehicle-treated animals; sildenafil+L-NAME significantly reversed tactile allodynia only on day 10 (one way ANOVA followed by Dunnett's t-test).

TABLE I: Thermal allodynia in vincristine-treated rats.

	Day 0	*Day 10 before drug*	*Day 10* after drug
Group I (0.9% NaCl)	30±0	29.56±0.19	29.21±0.07
Group II (Gabapentin)	30±0	29.43±0.26	28.95±0.19
Group III (Sildenafil)	30±0	29.73±0.18	29.41±0.48
Group IV (Sildenafil + L-NAME)	30±0	29.58±0.19	29.55±0.30

Values are expressed in seconds as Mean±S.E.M. (n=6)
*p<0.05, significant in comparison to day 0.
[a]p<0.05, significant in comparison to Group I.

TABLE II: Tail-flick test in vincristine-treated rats.

	Day 0	*Day 10 before drug*	*Day 10* after drug
Group I (0.9% NaCl)	5.91±0.34	6.08±0.29	6.03±0.25
Group II (Gabapentin)	6.01±0.21	5.93±0.36	5.78±0.31
Group III (Sildenafil)	5.96±0.28	5.93±0.27	6.03±0.24
Group IV (Sildenafil + L-NAME)	5.58±0.41	6.16±0.32	5.92±0.36

Values are expressed in seconds as Mean±S.E.M. (n=6)
*p<0.05, significant in comparison to day 0.
[a]p<0.05, significant in comparison to Group I.

Discussion

Administration of vincristine, along with other antimitotic drugs produces neuropathy that can manifest as painful paresthesia. Models of vincristine-induced neuropathic pain have been developed in rats using daily i.v. injections of vincristine (16). Our results demonstrate that rats treated with vincristine in a dose of 600 µg/kg over a period of three days developed significant tactile allodynia without thermal allodynia. The decreased threshold to the mechanical stimulus was evident from day 3 itself as previously shown by Bordet et al. which remained significant till day 14 (16). This model thus would be useful for assessing treatment effects in early onset vincristine-induced neuropathic pain. Similar results have been observed in models developed by Aley et al., 1996 and Nozaki-Taguchi et al., 2001 whereby the animals displayed significant tactile allodynia without thermal allodynia (21,22). However rodent models described by Kamei et al., 2005, whereby, vincristine is administered in a dose of 0.05-0.125 mg/kg twice a week for 6 weeks, have shown to induce thermal hyperalgesia (13). Although the detailed reason is not clear, this discrepancy could be due to the differences between the dose and the treatment schedule of vincristine administration and longer duration of observation.

In the current study, it was demonstrated that tactile allodynia in rodents can be assessed using a single von Frey filament by altering its length - shorter length of the filament indicates greater mechanical threshold. Sildenafil (0.5, 1 and 2 mg/kg) showed a dose-response effect in attenuating the vincristine-induced allodynia. In a recent study, sildenafil given as a loading dose and continuous intravenous infusion did not show any beneficial effect in a chronic constriction injury animal model of neuropathic pain (23). In another study performed in the same model, sildenafil (100 and 200 µg, intrathecal) caused worsening of neuropathic hyperalgesia but in lower dose did not alter the nociceptive threshold (24). Possible explanation for this difference could be activation of different pathophysiologic events in the two models of neuropathic pain. A deficit in NO is shown to be involved in vincristine neuropathy (13). Aley and Levine (2002) suggested that increased activity of Protein Kinase C (PKC) might trigger vincristine-induced hyperalgesia, since PKC inhibitor reversed the vincristine-induced hyperalgesia in rats. Furthermore, it was also reported that activity of PKC lead to the reduction of neuronal NOS activity (25, 26). Sildenafil (1 and 2 mg/kg) and gabapentin significantly reversed tactile allodynia on all days from day 10 to day 14 in the present study. The effects of sildenafil (2 mg/kg) and gabapentin were comparable. The effects of sildenafil could have been mediated by NO-cGMP pathway. Sildenafil, as stated earlier, acts by inhibiting hydrolysis of NO and facilitates the NO-cGMP pathway. Several studies support the beneficial role of NO in allodynia due to neuropathic and central mechanisms (9-14).

Kamei et al., 2005 demonstrated that pre-treatment with L-arginine, an NO donor, dose-dependently reversed vincristine-induced thermal hyperalgesia in mice which was tested 6 weeks later (13). Our study shows that even early manifestations of vincristine-induced neuropathy such as tactile allodynia can be significantly attenuated by sildenafil. This beneficial

effect of sildenafil was antagonized by L-NAME, a NOS inhibitor, suggesting that a decrease in *de novo* neuronal NO synthesis could play a role in vincristine-induced early onset tactile allodynia. In an ex vivo study using human chorionic plate arterial rings, it was shown that vasodilatory effects of sildenafil citrate in the feto-placental circulation were not reversed by L-NAME, indicating that *de novo* nitric oxide generation is not required to produce sildenafil citrate mediated vasorelaxation in this circulation (27). Our results are in line with an earlier study in which beneficial effect of PDE-5 inhibitor tadalafil were shown in carrageenan- and diabetes-induced hyperalgesia also, which was reversed by L-NAME, suggesting a role of NO-cGMP pathway. Further, in a mouse model of diabetic peripheral neuropathy it was shown that hyperglycemia substantially upregulated PDE-5 in Schwann cells and sildenafil showed beneficial effects by activating the cGMP/PKG signaling pathway (28).

In conclusion, this study shows that vincristine-induced early onset tactile allodynia can be significantly attenuated with sildenafil. Addition of L-NAME reversed sildenafil's attenuation of tactile allodynia indicating a decrease in *de novo* neuronal NO synthesis could play a role in vincristine-induced early onset tactile allodynia

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