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Tetrahydrocurcumin exerts protective effect on vincristine induced neuropathy: Behavioral, biochemical, neurophysiological and histological evidence

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ABSTRACT

Hyperalgesia, allodynia, delayed motor nerve conduction velocity, oxidative stress and axonal damage are signs and symptoms of chemotherapy induced peripheral neuropathy (CIPN). Present treatment/preventive strategies of CIPN are futile and the neuropathy may even lead to discontinuation of chemotherapy. In this study, we evaluated the protective effect of tetrahydrocurcumin (THC) 40 and 80 mg/kg in experimental vincristine induced neuropathy in rats. Hyperalgesia was assessed by hot plate (thermal), Randall–Selitto (mechanical) test, allodynia was assessed by cold plate (thermal) test, functional loss was measured by sciatic function index, nociception was evaluated by formalin test. Neurophysiological recordings were carried out to assess motor nerve conduction velocity. Total calcium levels, oxidative stress and TNF-α was measured in sciatic nerve tissue homogenate to assess neuropathy. Histopathological changes was observed on sciatic nerve to assess the protective effect of THC against the vincristine. Pregabalin was used as a standard in this study. Rats administered with THC at 80 mg/kg significantly attenuated the vincristine induced neuropathic pain manifestations which may be due to its multiple actions including anti-nociceptive, anti-inflammatory, neuroprotective, calcium inhibitory and antioxidant effect. This study delineates that THC can be a promising candidate for the prevention of CIPN by chemotherapeutic agents.

1. Introduction

Cancer associated pain is a major clinical problem that will likely only rise in its magnitude as the average life span continues to increase and cancer therapies continue to progress. Hence, with the increasing survival proportion of cancer patients, these formerly lethal diseases have often been changed into more chronic disease states. The two key causes of cancer associated pain are that from the malignancy itself and from the treatments employed to alleviate the cancer (e.g. surgery, radiation and chemotherapy) [22]. Vincristine is one of the most common chemotherapeutic agents used to treat several types of malignancies, such as leukemias, lymphomas, and sarcomas. However, chemotherapy induced peripheral neuropathy (CIPN) is a common side effect of anti-cancer treatment with vinca alkaloids, platinum drugs, taxanes, and other chemotherapeutic drugs affecting up to 30–40% of patients [42]. Unfortunately, vincristine is a unique chemotherapeutic agent that is predictably and uniformly neurotoxic to all who receive it. In therapeutic doses, it causes dose related neuropathy, very early in the course of treatment. Thus, its anticancer efficacy is limited by the development of a mixed sensorimotor neuropathy [14].

At present, the clinical options for CIPN are limited to tricyclic anti-depressants, anti-convulsants and opioids. But these agents exhibit various adverse effects which restrict their full clinical exploitation [9]. Several herbal medicines such as *Phyllanthus emblica* Linn. (Family: Phyllanthaceae), *Cannabis sativa* Linn. (Family: Cannabaceae), *Nigella sativa* Linn. (Family: Ranunculaceae), *Ocimum sanctum* Linn. (Family: Lamiaceae), and *Ginkgo biloba* Linn. (Family: Ginkgoaceae) are shown to have potential in different types of experimentally induced neuropathic pain [19,26,41]. Some clinical reports have also advocated beneficial effect of drugs from plant origin in neuropathic pain conditions [10,43].

Curcumin, a dietary pigment from *Curcuma longa* L., (Zingiberaceae) is known to exert antinociceptive effects in different animal models of pain, which also includes neuropathic pain [2,36]. Conversely, poor oral absorption of curcumin has raised several concerns that this may limit its clinical impact. Curcumin possess two double bonds (dienone), which can undergo Michael

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addition, critical for some of the effects of curcumin but known to contribute chemical instability in aqueous solution. This lead to the hypothesis that *in vivo* efficacy of curcumin may come from a more bioavailable and/or potent metabolite, tetrahydrocurcumin (THC) [5,18]. Interestingly, THC has been reported earlier for its potent anti-inflammatory, anti-oxidant activity in comparison to curcumin [1]. With the above background, the present study was undertaken to investigate the effect of THC vincristine induced neuropathic pain in rat model. Pregabalin, a selective Ca_v 2.2 ($\alpha 2$-δ subunit) channel antagonist served as positive control in this study.

2. Materials and methods

2.1. Chemicals and preparation of drug solutions

Vincristine sulfate (Sun Pharma, Mumbai), pregabalin (Ranbaxy Research Laboratories, Gurgaon), THC (Sami labs, Bangalore), nitro blue tetrazolium (NBT), (Sigma Aldrich, USA), Folin Ciocalteu's phenol reagent (Merck Limited, Mumbai), 5,5′-dithio, bis (2-nitro benzoic acid) (DTNB), bovine serum albumin (BSA), (Sisco Res earch Laboratories Pvt. Ltd. Mumbai), reduced glutathione (GSH), nicotinamide adenine dinucleotide phosphate (NADPH) (Himedia Laboratories, Mumbai), thiobarbituric acid (TBA) (Sigma Aldrich, USA) were procured for the present study. All the reagents used in the present study were of analytical grade. Pregabalin and THC were suspended in 0.3% w/v carboxymethyl cellulose (CMC) solution and vincristine was diluted with normal saline.

2.2. Experimental animals

Thirty adult male Wistar rats with a body weight of 200–220 g were used for this study. Rats were group-housed ($n = 6$ per cage) in a room with controlled temperature (21 ± 2 °C), and 12 h light–dark cycle was maintained. All the rats had free access to food and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethical Committee and experiments were performed in accordance to the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for ethical use of animals.

2.3. Preselection of rats

The rota-rod test was performed to investigate the motor coordination of the rats. Rats were placed on the rota-rod treadmill at an accelerating speed from 6 round/min to 30 round/min for 3 min. The latency to fall was measured and three training sessions were given before experiment [33]. The animals performed three trials in a day. On the day of the test, only the rats that were able to stay balanced on rotating rod for 3 min were selected for further study.

2.4. Induction of peripheral neuropathy by vincristine

Peripheral painful neuropathy was induced in rats by intraperitoneal administration of vincristine sulfate (75 μg/kg) once per day for 10 consecutive days as per the method adopted by Muthuraman et al. [27].

2.5. Experimental design

Group I served as normal control in which rats received 0.3% w/v carboxy methyl cellulose (CMC) (p.o.) vehicle administration and saline (i.p. injection) for 14 days. Group II served as vincristine control in which rats were administered 0.3% w/v CMC (p.o.) 1 h before each vincristine injection (75 μg/kg for 10 days) for 14 consecutive days. Group III, IV and V rats received pregabalin (10 mg/kg/day p.o.); THC (40 mg/kg/day p.o.) and THC (80 mg/kg/-day p.o.) respectively 1 h before each vincristine injection (75 μg/kg for 10 days) for 14 consecutive days. The behavioral tests like thermal hyperalgesia (hot plate), thermal allodynia (cold plate), mechanical hyperalgesia (Randall and Selitto test), motor co-ordination (rota rod) and sciatic functional index were performed on different days, *i.e.*, day 0, 7, 10, and 14. On the 14th day rats were also subjected to neurophysiological study (nerve conduction velocity) and formalin test. Thereafter, all the rats were sacrificed under deep ether anaesthesia and subjected to biochemical analysis for estimating the total protein, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), lipid peroxide (LPO), nitric oxide (NO), total calcium and tumor necrosis factor-α (TNF-α) in sciatic nerve tissue samples. Histological analysis were also carried out in the sciatic nerve samples.

2.6. Behavioral assessment

2.6.1. Thermal hyperalgesia (hot plate test)

In this test, rats were individually placed on a hot plate (Eddy's hot plate) with the temperature adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or jump response to avoid the heat was taken as an index of the pain threshold; the cut off time was 10 s in order to avoid damage to the paw [39].

2.6.2. Thermal allodynia (cold plate test)

Rats were placed on an ice platform submerged approximately 1 cm below the surface of cold water (4 °C), such that the hairy and glabrous skin of the rat feet were in contact with the cold water. The latency prior to the first reaction (licking, moving the paws, leaps) was recorded with a cutoff time of 30 s [6].

2.6.3. Mechanical hyperalgesia (Randall and Selitto test)

Mechanical hyperalgesia was assessed with the Randall and Selitto test using a paw pressure analgesimeter, which applies a linearly increasing mechanical force to the dorsum of the rat's hind paw. The mechanical nociceptive threshold was defined as the force in grams at which the rat withdrew its paw. The cut-off pressure was set to 250 g. The duration of the paw withdrawal was recorded in seconds. A cut off time of 20 s was maintained.

2.7. Rota-rod test

Rats were subjected to rota-rod test to assess the motor coordination as described earlier.

2.8. Determination of sciatic functional index (SFI)

Rats were subjected to walking-track analysis and measurement of SFI using a method similar to that described by De Medinaceli et al. [8]. To brief, the hind paws of the rats were dipped in India-ink and then each animal was allowed to walk freely in the corridor. From the analysis of the footprints of the feet, the following lengths were obtained: (a) distance from the heel to the third toe, the print length (PL); (b) distance from the first to the fifth toe, the toe spread (TS); and (c) distance from the second to the fourth toe, the intermediary toe spread (ITS). All three measurements were taken from the experimental (E) and normal (N) sides. The factors were calculated as follows: (I) Print length factor (PLF) = (EPL − NPL)/NPL; (II) Toe spread factor (TSF) = (ETS − NTS)/NTS; (III) Intermediary toe spread factor (ITF) = (EIT − NIT)/NIT. These factors were then incorporated into the SFI formula derived by Bain et al. [3]: SFI = −38.3 PLF + 109.5 TSF + 13.3 ITF − 8.8. An SFI of 0 is normal. An SFI of −100 indicates total