Food and Chemical Toxicology 80 (2015) 32–40

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Dose-related neuropathic and anti-neuropathic effects of simvastatin in vincristine-induced neuropathic pain in rats

Shrutya Bhalla, Nirmal Singh, Amteshwar Singh Jaggi *

Department of Pharmaceutical Sciences and Drug Research, Punjabi University Patiala, 147002, India

ARTICLE INFO

Article history:
Received 22 July 2014
Accepted 20 February 2015
Available online 26 February 2015

Keywords:
Cholesterol
Vincristine
Neuropathic pain
Inflammation

ABSTRACT

The present study explores the role of simvastatin in vincristine-induced neuropathic pain, which was induced by administering vincristine (100 μg/kg *i.p.*) for 10 days (two 5 day cycles with 2 days pause). Pain was assessed by determining mechanical hyperalgesia, mechanical dynamic allodynia, heat hyperalgesia and cold allodynia. Biochemically, myeloperoxidase (MPO) activity was measured along with serum cholesterol levels. Simvastatin (7.5, 15 and 30 mg/kg) was administered for 14 days after administration of vincristine. Simvastatin (7.5 and 15 mg/kg) reversed vincristine-induced neuropathic pain and attenuated vincristine-induced increase in MPO, without altering cholesterol levels. Simvastatin at higher dose (30 mg/kg) did not alter neuropathic pain despite decreasing MPO levels. Furthermore, administration of simvastatin (30 mg/kg *i.p.*) in vincristine treated rats as well as it's *per se* administration in normal rats reduced cholesterol levels. *Per se* administration of simvastatin in normal rats produced neuropathic pain. It is concluded that simvastatin attenuates neuropathic pain only at lower doses with no reduction in cholesterol levels and anti-inflammatory effects may possibly reverse neuropathic pain. However, despite reducing inflammation, simvastatin did not confer beneficial effects at higher doses at which there is reduction in cholesterol levels, suggesting the critical role of cholesterol in neuropathic pain induction.

1. Introduction

Neuropathic pain is a debilitating condition caused by a lesion or disease of the somatosensory nervous system. Peripheral neuropathic pain may manifest itself as stimulus-independent pain (spontaneous pain) or stimulus-evoked pain that is generated after damage to sensory neurons (Woolf and Mannion, 1999). Peripheral nerve injury is followed by a series of events in the primary afferents which are characterised by decreased threshold (allodynia) and increased response to supra-threshold stimuli (hyperalgesia) (Jaggi and Singh, 2011, 2012; Kukkar et al., 2013). Vincristine is a chemotherapeutic agent that has been used extensively for the treatment of several malignancies including breast cancer, leukaemia, lymphomas, and primary brain tumours (Ito et al., 2002). However, treatment with vincristine is primarily involved in the generation of neurotoxicity of the peripheral nerve fibre leading to sensory-motor neuropathy. This has led to the development of rodent models of vincristine-induced neuropathic pain to unravel mechanisms behind toxicity and also to discover novel therapeutic agents (Jaggi et al., 2011).

The effective management of neuropathic pain is difficult and currently involves the use of few effective therapies including opiates, tricyclic anti-depressants, anti-epileptic agents like lamotrigine and gabapentin, N-methyl-D-aspartate (NMDA) receptor antagonist, sodium channel blockers, and cannabinoid receptor agonists. Although many of these approaches provide limited relief in a subpopulation of patients with neuropathic pain, it is important to understand that the key issue is the therapeutic window, *i.e.* the relationship in clinical efficacy in controlling the pain state and the ability of the patient to tolerate often serious side-effects. Thus, neuropathic pain represents a substantial unmet medical need for the development of novel therapies or exploiting the therapeutic potential of clinically available drug (Jaggi and Singh, 2011).

Statins are competitive inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase which catalyse an early, rate-limiting step in cholesterol biosynthesis. They are used clinically to treat patients with cardiovascular disease like atherosclerosis, stroke and peripheral arterial disease due to their cholesterol lowering effect (Ali and Alexander, 2007; Golomb et al., 2008). However, statins exhibit umpteen pleiotropic cholesterol independent effects including anti-oxidants by reducing superoxide formation (Uekawa et al., 2014), anti-inflammatory by reducing macrophage migration

* Corresponding author. Department of Pharmaceutical Sciences and Drug Research, Punjabi University Patiala, Patiala-147002, India. Tel.: +9501016036; fax: +0175 2283073.
E-mail address: amteshwarjaggi@yahoo.co.in (A.S. Jaggi).

http://dx.doi.org/10.1016/j.fct.2015.02.016
0278-6915/

(Ma et al., 2014), anti-microbial effect in staphylococcus blood infections (López-Cortés et al., 2013), modulation of the neurotrophic levels (Wu et al., 2008) and up-regulation of L-type Ca^{2+} channel affecting smooth muscle contraction (Clunn et al., 2010). Due to these multiple activities, preclinical studies have suggested the potential beneficial role of statins in a number of other diseased states like cancer (Khurana et al., 2007; Kong et al., 2014; Mondul et al., 2011), arthritis (Gottschalk et al., 2014), chronic obstructive pulmonary disease (Criner et al., 2014), Alzheimer's disease and Parkinson's disease (Barone et al., 2014; Wolozin et al., 2007) and chronic kidney disease (Dasari et al., 2014).

Statins have also been explored for their beneficial effects in attenuation of neuropathic pain in various animal models (Shi et al., 2011; Xavier et al., 2012). Treatment with statins attenuated peripheral and central pain without altering the serum cholesterol levels in animal models of neuropathic pain like mouse partial sciatic nerve ligation and rat trigeminal neuralgia model (Shi et al., 2011), streptozotocin (STZ)-induced diabetic neuropathy (Cameron et al., 2003), and sciatic nerve crush injury model in Wistar rats (Xavier et al., 2012). However, the role of statins in vincristine-induced neuropathic pain is still not explored. In contrast to preclinical studies, there are a number of clinical evidences supporting that treatment with statins is associated with development of neuropathic pain (Gaist et al., 2001; Jeppesen et al., 1999). The exact mechanism responsible for this anomalous behaviour of statins in preclinical and clinical studies is not known. Preclinical studies document the pain attenuating effects of statins due to its anti-inflammatory actions independent of cholesterol reductions (Shi et al., 2011; Xavier et al., 2012). On the other hand, statins are used clinically due to their cholesterol lowering activity (Ali and Alexander, 2007; Golomb et al., 2008). Therefore, it may be possible that cholesterol may be a critical factor that modulates statin-mediated neuropathic pain attenuation/induction. However, there are no direct studies to understand the role of cholesterol in statin-mediated modulation of neuropathic pain. The present study was designed to explore: (a) the neuropathic pain resolving potential of simvastatin in vincristine-induced neuropathic pain in rats and (b) the role of cholesterol in statin-mediated modulation of neuropathic pain.

2. Materials and methods

2.1. Experimental animals

Wistar albino rats (GADVASU, Ludhiana, India) of either sex weighing 200–250 g, maintained at standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water, were employed in the present study. They were housed in the departmental animal house and were exposed to normal cycle of light and dark. The experimental protocol was duly approved by Institutional Animal Ethics Committee (Reg. No. 107/1999/CPCSEA/2013-07) and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. 107/1999/CPCSEA).

2.2. Drugs and chemicals

Vincristine sulphate (Biochem Limited, Mumbai, India) was dissolved in normal saline. Chemicals such as Folin–Ciocalteu's Phenol reagent (Merck Limited, Mumbai), BSA (Bovine Serum Albumin), (Sisco Research Laboratories Pvt. Ltd. Mumbai), o-dianisidine, and Cholesterol estimation kit (Erba Diagnostics Mannheim Gmbh, Mumbai, Maharashtra, India) were procured for the present study. All the reagents used in the present study were of analytical grade. Simvastatin was obtained from Ind. Swift Laboratories, India, and was prepared as a 10 mg/mL stock. Briefly, 500 mg was dissolved in 5 mL of ethanol and 7.5 mL of 0.1 N NaOH, kept at 50 °C for 2 h, and then pH was adjusted to 7 and volume was adjusted to 50 mL. Simvastatin is lipophilic in nature and was dissolved in ethanol and chemically activated to simvastatin hydroxyl acid by alkaline hydrolysis which is its pharmacologically active form. The stock solution was kept at 4 °C before use. The drug was administered through intraperitoneal injection (*i.p.*) for 14 days in the morning between 9:00 AM and 10:00 AM.

2.3. Induction of peripheral neuropathy by vincristine

Peripheral neuropathy was induced in rats by administration of vincristine sulphate (100 μg/kg/day *i.p.*) for a period of 10 days (two 5 day cycles with 2 days pause between cycles) (Rahn et al., 2007; Weng et al., 2003). The pain assessment was done on different days, i.e. days 0 (before vincristine administration), 14 and 28 (Fig. 1).

2.4. Behavioural examination

2.4.1. Paw cold-allodynia (acetone drop test)

Cold-allodynia of the hind paw was assessed using acetone drop method as described by Choi et al. (1994), with slight modification, for assessing the reactivity to non-noxious cold chemical stimuli. The rats were placed on the top of a wire mesh grid, allowing access to the hind paws. Acetone (0.1 ml) was sprayed on the plantar surface of hind paw of rat and hind paw withdrawal duration from the mesh surface was noted. The duration of the withdrawal response was recorded with an arbitrary minimal value of 0.5 s and a maximum of 20 s. Acetone was sprayed thrice to the hind paw, with a gap of 5 min between acetone applications and individual withdrawal durations were summed up as measured with a stopwatch (Bennett et al., 2003).

2.4.2. Mechanical hyperalgesia (pin-prick test)

The mechanical hyperalgesia was assessed by the pin-prick test as described by Erichsen and Blackburn-Munro (2002). The surface of the injured hind paw was touched with the point of the bent gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response. The duration of the paw withdrawal was recorded in seconds with a stopwatch with an arbitrary minimal value of 0.5 s.

2.4.3. Paw heat-hyperalgesia (hot plate test)

The thermal nociceptive threshold, as an index of thermal hyperalgesia, was assessed by the Eddy's hot plate, maintained at a temperature of 52.5 ± 1.0 °C. The rat was placed on the hot plate and withdrawal latency, with respect to licking of the hind paw, was recorded in seconds. The cut-off time of 15 s was maintained (Jain et al., 2009).

2.4.4. Mechanical dynamic allodynia (paint-brush test)

The "paint-brush" behavioural test has been used to explore dynamic responses to a mechanical stimulus. The response to smooth paint-brush has been described as allodynia because normal rats never withdraw from this stimulus. It has been established that dynamic mechanical allodynia is mediated by peripheral low threshold, large myelinated Aβ-fibres (Gracely et al., 1992; Ochoa and Yarnitsky, 1993). The rat was placed in the cylinder with the wire mesh floor and a smooth paint-brush was used to rub the plantar area of hind paw from the heel to the toes as a stimulus. The stimulus was applied five times with a 5 s interval and the numbers of withdrawals were noted (between 0 and 5). The same procedure was repeated twice, with a gap of five minutes and the total number of withdrawals (in three tests) were added to obtain a single cumulative score of mechanical dynamic allodynia with a minimum value of 0 and maximum of 15 (Thibault et al., 2008; Weissman-Fogel et al., 2008).

2.5. Serum isolation procedure

On the 28th day, blood was collected from retro-orbital sinus using microhematocrit capillary tubes. The collected blood was allowed to coagulate at room temperature for 20 min and was centrifuged at 3000 *g* for 15 minutes to separate serum for further analysis.

2.6. Biochemical estimations

All animals were sacrificed on the 28th day of experimental protocol and the tissue beneath the sciatic nerve was isolated immediately for biochemical estimations:

2.6.1. Estimation of protein content

The protein concentration was estimated according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. The absorbance was determined spectrophotometrically at 750 nm.

2.6.2. Estimation of myeloperoxidase activity

The myeloperoxidase activity was measured by a method described by Grisham et al. (1990) and Jain et al. (2009). The inflammatory reactions are characterised by the recruitment of inflammatory cells from the blood capillaries to the connective tissue, adjacent to the point of injury. Tissue beneath the sciatic nerve was taken, rinsed with ice-cold saline, blotted dry and weighed. Minced tissue was homogenised in 10 volumes of ice-cold potassium phosphate buffer (pH 7.4), using a tissue homogeniser. The homogenate was centrifuged at 5000 *g* for 10 min at 4°C. The supernatant was discarded and 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyl trimethyl ammonium bromide (HETAB), and l0 mM EDTA was added to the pellet. It was then subjected to one cycle of freezing and thawing and a brief period (l5 s) of sonication. After sonication, the solution was