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The protective effects of sumatriptan on vincristine - induced peripheral neuropathy in a rat model

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ABSTRACT

Clinical use of vincristine (VCR), an effective chemotherapeutic agent, has been limited due to its peripheral neuropathy toxicity. Sumatriptan, which is an anti-migraine agent is a specific agonist for 5-hydroxytryptamine 1B, 1D (5HT1B, 1D) receptors. Several studies have shown that sumatriptan exerts anti-inflammatory and immunomodulatory properties. This study aimed to investigate the effects of sumatriptan on VCR-induced peripheral neuropathy in a rat model. Male Wistar rats were intraperitoneally injected with VCR and normal saline four times per week for 2 weeks. In the treatment group, sumatriptan (1 mg/kg) was administered intraperitoneally 30 min prior to VCR injection every day. Mortality rate, weight variations and histopathological changes were monitored. Hot plate, tail flick and motor nerve conduction velocity (MNCV) tests were used to evaluate sensory and motor neuropathy. Levels of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and caspase-3 in the dorsal ganglion root were assessed by quantitative reverse transcription-PCR (qRT-PCR). Moreover, the protein levels of p65 nuclear factor kappa B (NF- < kappa > B) and phospho-p65 NF- < kappa > B were examined by Western blot analysis. Co-administration of sumatriptan with VCR significantly reversed alterations in the hot plate, tail flick threshold and sciatic MNCV induced by VCR and also prevented mixed sensory-motor neuropathy, as indicated by better general conditions, behavioral and electrophysiological results. In addition, sumatriptan improved the body weight loss caused by VCR. The mRNA levels of TNF-α, IL-1β and caspase-3 were significantly diminished in the treatment group. These findings were confirmed by histopathological analysis. In conclusion, this study demonstrated that sumatriptan significantly attenuated VCR-induced neuropathy and could be considered as a neuroprotective agent to prevent the VCR-induced neuropathy.

1. Introduction

Vincristine (VCR), one of the most commonly prescribed chemotherapeutic agent, has been used to treat various cancers such as Hodgkin's lymphoma, non-Hodgkin's lymphoma and leukemia (Dorchin et al., 2013)Unfortunately, clinical application of VCR is limited due to a progressive painful neuropathy, which leads to severe motor and sensory peripheral neuropathies. This dose-limiting neurotoxicity of VCR can be devastating due to the subsequent dose reduction, treatment delays and affecting the quality of life (Quasthoff and Hartung, 2002; Mora et al., 2016). Previous studies have shown that VCR causes neuropathy by disturbing the microtubulation of the exons and disrupting the transmission of the nerve in the peripheral nervous system (Gomez-Nicola et al., 2008; Schiavetti and Frascarelli, 2004). Although the exact mechanism for the VCR-induced neuropathy is not clearly understood, a few studies have found out that up-regulation of pro-inflammatory interleukins and tumor necrosis factor-alpha (TNF-α) in the injured region of spinal cord plays a critical role in

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the neuropathic pain (Scholz and Woolf, 2007). Also, a significant elevation in the levels of TNF-α and interleukin-1β (IL-1β) in the dorsal root ganglia (DRG) and sciatic nerves is associated with the induced neuropathy in rat models (Michalski et al., 2013; Muthuraman et al., 2011). Both IL-1β and TNF-α mediate their proliferative effects through activation of the nuclear factor kappa B (NF- < kappa > B) (Tak and Firestein, 2001). NF- < kappa > B is a factor in the nucleus of B cells, which binds to a kappa-like immunoglobulin chain and its deregulated activation promotes inflammation. (Aggarwal, 2004). There is evidence that cytokine chemotherapy and radiation agents activate the NF- < kappa > B signaling module (Cata et al., 2006; Kumar et al., 2004).

Sumatriptan is the first clinically available triptan used for the treatment of migraine which has been shown as a selective agonist of serotonin 5-hydroxytryptamine 1B, 1D (5HT1B, 1D) (Castro et al., 1997; Jennings et al., 2004). Sumatriptan decreases release of neurotransmitters and neuropeptides such as calcitonin gene-related peptide (CGRP) and substance P (SP) by acting on the serotonin receptors at the terminal end of neurons (Moskowitz et al., 1983; Vause and Durham, 2012). Both neuropeptides are effective against the inflammatory factors. Previous studies have illustrated that both 5HT1B and 5HT1D receptor subtypes in humans and rats are located mainly in the trigeminal ganglia and sensory neurons (Hou et al., 2001; Smith et al., 2002; Wotherspoon and Priestley, 1999). Furthermore, the presence of 5HT1D receptor in DRG implies for its role in regulating the pain pathway of non-trigeminal origin (Potrebic et al., 2003).

Despite previous studies in this field, the effects of sumatriptan on peripheral neuropathy have not been investigated yet. In addition, the exact mechanism of the VCR-induced peripheral neuropathy is not completely understood. This study aimed to investigate the efficacy of sumatriptan against the VCR-induced peripheral neuropathy through attenuation of the inflammatory responses.

2. Materials and methods

2.1. Ethics

All of our *in vivo* work were in accordance with the National Institute of Health (NIH) Guide for the Care and Use of Laboratory Animals (HHS publication 85-23, 1985) and legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU), and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran).

2.2. Animals

Adult male Wistar rats weighting on 270–300 g (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran) were used in this study. Animals were kept at the temperature of 21–23 °C under a twelve-hour regular light/dark cycle and were given access to food and water *ad lib.* Rats were randomly allocated into four groups and each group consisted of 8 rats as follows: 1. control 2. Sumatriptan 3. VCR, and 4. Sumatriptan + VCR.

2.3. Drug administration

VCR (Sigma) was dissolved in saline and was intraperitoneally (i.p.) administrated with the dosage calculated on daily body weight every other day at the dose of 0.1 mg/kg (total cumulative dose is 1.2 mg/kg) for two weeks. Sumatriptan (Sigma) was dissolved in saline and injected i.p. at the dose of 1 mg/kg 30 min prior to VCR injection. Rats in the control group received saline.

2.4. Survival study and general toxicity

General condition of animal including edema, cachexia, alopecia, and mortality rate were observed daily and the rats weighted weekly throughout the period of experiment for two weeks(Pourmohammadi et al., 2012).

2.5. Behavioral examinations

Destructive effects of VCR on the sensory nerve function were assessed using tail flick and hot plate tests at day 14.

Tail flick: A radiant heat automatic tail flick analgesiometer was applied to measure reaction latencies. Basal reaction time of rats to radiant heat was recorded by locating the tip (last 1–2 cm) of the tail on the radiant heat source. The tail removal from the radiant heat was taken as the end point. A cutoff time of 15 s was used to avoid tail injury by heat.

Hot plate: Animals were placed on a 52 ± 0.2 °C heated plate (Sorel Hot plate model DS37, Ugo Basile, Italy) and the elapsed time between contact with the heat source and first indication of heat sensitivity was measured. The observed indications included jumping and forepaw or hind paw licking. The cut of time was considered 20 s.

Von ferry: The mechanical threshold was evaluated by using von Frey filaments (Bioseb, USA). The threshold was determined when the hind paw was withdrawn four or five times out of the five applications from a particular hair filament. The value of threshold was documented as a gram. The tests were performed at day 1, 7 and 14.

2.6. Electrophysiological examination

After the last behavioral test, rats were anesthetized with thiopental (65 mg/kg, ip; Sigma) dissolved in saline. The body temperature was monitored and maintained within normal limit during the test. MNCV was measured in the left sciatic nerve according to the method described by Ja'afer et al. (Pourmohammadi et al., 2012; Ja'afer et al., 2006).

2.7. Gene expression analysis by qRT -PCR

RiboEx Total RNA (GeneAll Biotechnology, Seoul, Korea) was used to harvest total RNA from the DRG tissue. Changes in mRNA levels of TNF-α, IL-1β and caspase3 genes were measured by qRT-PCR on a StepOne Plus instrument (Applied Biosystems) using qRT-PCR Master Mix kit (Ampliqon, Copenhagen, Denmark). The activation step was 15 min at 95 °C followed by 40 cycles including a denaturation step for 10 min at 95 °C and a combined annealing/extension step for 1 min at 60 °C. The primers used are listed in Table 1. The target gene expression levels were normalized to < beta > -actin levels in the same reaction. For calculations, $2^{-\Delta\Delta C}{}_T$ formula was used, with $\Delta\Delta C_T = (C_{T\ target} - C_{T\ <beta>-actin})$ experimental sample $- (C_{T\ target} - C_{T\ <beta>-actin})$ control samples where C_T is cycle threshold.

Table 1
Primer sequences for qRT-PCR.

Name	Froward	Revers
IL-1 < beta >	GACTTCACCATGGAACCCGT	GGAGACTGCCCATTCTCGAC
TNF-α	GGCTTTCGGAACTCACTGGA	GGGAACAGTCTGGGAAGCTC
< beta > -actin	GCAGGAGTACGATGAGTCCG	ACGCAGCTCAGTAACAGTCC
caspase-3	TTGGAACGGTACGCGAAGAA	AGAGTCCATCGACTTGCTTCC