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Cinobufacini protects against paclitaxel-induced peripheral neuropathic pain and suppresses TRPV1 up-regulation and spinal astrocyte activation in rats

Xiyuan Ba[a,b], Jiali Wang[a], Shiyang Zhou[a], Xinxin Luo[a], Yun Peng[a], Shimin Yang[a], Yue Hao[a,*], Guangyi Jin[a]

[a] School of Pharmaceutical Sciences, Health Science Center, Shenzhen University, Shenzhen, 518060, China
[b] Department of Pain Medicine and Shenzhen Municipal Key Laboratory for Pain Medicine, Shenzhen Nanshan People's Hospital, 6th Affiliated Hospital of Shenzhen University Health Science Center, Shenzhen 518060, China

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ABSTRACT

Chemotherapy-induced peripheral neuropathic pain is a major limiting factor affecting cancer patients. No effective treatment is currently available. Cinobufacini, an aqueous extract from toad skin, is a widely used anticancer drug in China. Clinical evidence has demonstrated the safety and effectiveness of cinobufacini in combination with chemotherapy to promote the therapeutic efficacy while alleviating side effects, especially cancer-related pain symptoms. In this study, the effects of cinobufacini were investigated in a rat model of paclitaxel-induced peripheral neuropathic pain (PIPNP) to better understand and expand its clinical application. A single injection of cinobufacini (2.5 g/kg, i.p.) alleviated pre-established PIPNP, as indicated by decreased mechanical and thermal hypersensitivity compared with paclitaxel-treated rats. Repeated cinobufacini (1.25 and 2.5 g/kg, i.p.), given during the induction of PIPNP, prevented the establishment of paclitaxel-induced mechanical and thermal hypersensitivity. This preventative effect was associated with suppressed paclitaxel-induced TRPV1 up-regulation and spinal astrocyte activation, as well as decreased production of spinal TNF-α and IL-1β. These findings reveal cinobufacini as a therapeutic potential to treat and prevent paclitaxel-induced peripheral neuropathic pain.

1. Introduction

Paclitaxel is a widely used cancer chemotherapy agent that frequently causes severe peripheral neuropathic pain in many treated patients [1]. This side effect profoundly impairs the patient's quality of life, limits or even leads to discontinuation of the application of paclitaxel. The clinical management of paclitaxel-induced peripheral neuropathic pain (PIPNP) is difficult as current pain drugs are marginally effective and display unacceptable side effects [2]. To date, no effective therapy has been successful in treating or preventing of PIPNP [3].

Previous studies identified members of the transient receptor potential family of ion channels (TRP channels) as contributors to the induction of PIPNP [4–8]. Selective inhibition of TRPV1 with capsazepine or TRPV4 with HC-067047 reduced PIPNP in rodents [6]. In addition, paclitaxel causes robust activation of astrocytes, which triggers release of inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6 [9]. These increased cytokines lead to sensitization of nociceptive neurons and neurogenic inflammation which contribute to the induction of PIPNP.

Chansu, the dried secretion from the skin glands of *Bufo bufo gargarizans* Cantor, has been used clinically as an analgesic, local anesthetic, cardiotonic and antineoplastic agent for over a millennium in China [10]. Cinobufacini (Hua-chan-su), an injectable form of chansu, is a water extract of the dried toad skin. As a widely used prescription drug (approximately 160,000 patients received cinobufacini treatments per year in China), cinobufacini has been officially approved by the Chinese Food and Drug Administration (ISO9002) as a regimen for treating HBV infection and several types of cancer since 1991 [11]. The

Abbreviations: PIPNP, paclitaxel-induced peripheral neuropathic pain; TRP channels, transient receptor potential family of ion channels; TNF-α, tumour necrosis factor-α; IL, interleukin; DRGs, dorsal root ganglia; ELISA, enzyme linked immunosorbent assay; CIPNP, chemotherapy-induced peripheral neuropathic pain; CRF, corticotropin releasing factor

* Corresponding author.
E-mail address: yuehao@szu.edu.cn (Y. Hao).

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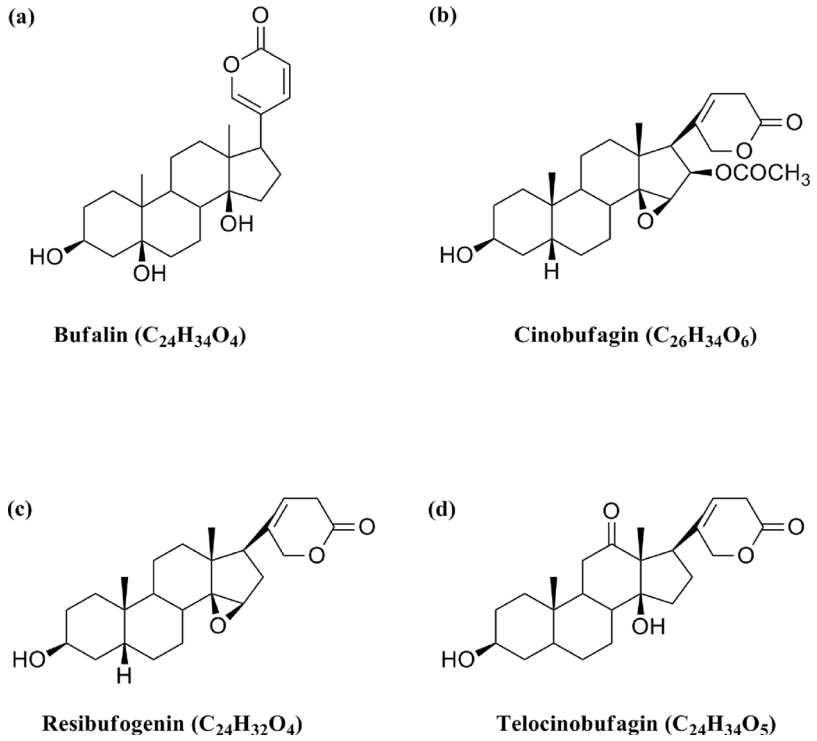

Fig. 1. Representative bufadienolides: bufalin, cinobufagin, resibufogenin and telocinobufagin, the marker active compounds in cinobufacini injection.

major constituents in cinobufacini include steroidal cardiac glycosides, peptides and indole alkaloids [12]. Bufadienolides in steroidal cardiac glycosides, a class of cardioactive C-24 steroids with a characteristic α-pyrone ring at C-17, are the marker active compounds of cinobufacini [13,14]. More than 28 of bufadienolides have been identified, including bufalin, cinobufagin, resibufogenin and telocinobufagin (Fig. 1).

Cinobufacini used alone or in combination with other chemotherapeutic agents had significant activity against several cancers, including liver cancer, pancreatic cancer, non-small cell lung cancer, and gallbladder carcinoma [11,15]. Its anti-tumor effects originate from the ability to inhibit cancer cell proliferation and differentiation, induce apoptosis, and enhance immune responses against cancer [15,16]. However, due to the complicated mechanisms of traditional medicine, they were usually used as a constituent part of a combination therapy, but not monotherapy [17].

Clinical studies have demonstrated that cinobufacini was safely combined with chemotherapeutic agents (paclitaxel, cisplatin and oxaliplatin) to treat cancer. When combined with chemotherapy, cinobufacini not only enhances the antitumor efficacy, but reduces the side effects of chemotherapeutic agents, such as pain, nausea and vomiting, and leukocytopenia. Cinobufacini also improves the performance status and the overall quality of life of patients [18–20]. In particular, clinical data showed that cinobufacini has the analgesic effect on a variety of cancer-related pain symptoms with a total effective rate of 50%–97% [21]. Together with its anti-inflammatory and anesthetic action, cinobufacini would be an ideal therapeutic strategy to treat and prevent PIPNP. Supporting this hypothesis, cinobufacini, in combination with gemcitabine-oxaliplatin, achieved improved median overall survival and quality of life in patients with advanced gallbladder carcinoma with pain relief observed 2 days after cinobufacini injection [22]. A meta-analysis regarding the efficacy of cinobufacini combined with chemotherapy in treating non-small-cell lung cancer demonstrated significant improvements in objective tumor response, one year survival, Karnofshy performance status, and alleviation of nausea and vomiting, and pain symptoms compared with the mono-chemotherapy group [19].

In contrast with the accumulating clinical evidence, no experimental study was carried out to investigate the effects and mechanisms of action of cinobufacini as adjuvant treatment during chemotherapy. In this study, we examined the effect of a single injection of cinobufacini on pre-established PIPNP, as well as the effect of repeated cinobufacini on the prevention of PIPNP. To further elucidate its underlying mechanisms of action, we also tested whether cinobufacini reversed paclitaxel-induced alternations of TRPV1 and 4 in the dorsal root ganglia (DRGs) and the spinal astrocytes and microglial activation.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (200–250 g; Guangdong province Laboratory Animal Center, Guangzhou, China) were maintained on a 12 h/12 h light/dark cycle with food and water available ad libitum. All procedures were conducted in strict adherence to the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Health Science Center, Shenzhen University.

2.2. Treatment and experimental design

Paclitaxel (2.0 mg/kg dissolved in DMSO and serially diluted in saline, Dalian Meilun Biology Technology Co. Ltd, China) was injected intraperitoneally on four alternate days (2 mg/kg on days 1, 3, 5, and 7 with a final cumulative dose of 8 mg/kg) to induce peripheral neuropathic pain as described previously [23].

Cinobufacini (Anhui Huaren Jinchan Biochemistry Company Ltd, PR China; lot 160327-2) was tested on both pre-established paclitaxel-induced PIPNP and as a preventative agent during the induction of PIPNP. In the first experiment, cinobufacini 1.25 or 2.5 g/kg (based on raw material; doses were chosen based on its clinical application on human and previous report [24]) was i.p. injected on day 14 post the first injection of paclitaxel in rats with confirmed PIPNP (Fig. 2a). *von Frey* tests were conducted before and at 60, 120 and 240 min after cinobufacini injection; Hot plate tests were conducted before and at 90, 150 and 270 min after cinobufacini injection. In the second experiment,