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Auraptenol attenuates vincristine-induced mechanical hyperalgesia through serotonin 5-HT$_{1A}$ receptors

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Common chemotherapeutic agents such as vincristine often cause neuropathic pain during cancer treatment in patients. Such neuropathic pa[illegible]s refractory to common analgesics and represents a challenging clinical issue. Angelicae d[illegible]urica[illegible]adix is an old traditional Chinese medicine with demonstrated analgesic efficacy in huma[illegible]. Hov[illegible]ever, the active component(s) that attribute to the analgesic action have not been identified. This work[illegible]scribed the anti-hyperalgesic effect of one coumarin component, auraptenol, in a m[illegible]se model of chemotherapeutic agent vincristine-induced neuropathic pain. We reported that aur[illegible]aptene[illegible]lose-dependently reverted the mechanical hyperalgesia in mice within the dose range of 0.05–0[illegible]mg/kg. I[illegible]addition, the anti-hyperalgesic effect of auraptenol was significantly blocked by a selective s[illegible]rotonin 5-HT$_{1A}$ receptor antagonist WAY100635 (1 mg/kg). Within the dose range studied, auraptenol did not si[illegible]ificantly alter the general locomotor activity in mice. Taken together, this study for the firs[illegible]time identified an active component from the herbal medicine angelicae dahuricae radix that possesses ro[illegible]ust analgesic efficacy in mice. These data support further studies to assess the potential of auraptenol as a n[illegible]l analgesic for the management of neuropathic pain.

Chemotherapy-induced peripheral neuropathy has been increasingly recognized as a serious side effect associated with several commonly used chemotherapeutic agents, including taxanes, platinum agents, and vinca alkaloids (e.g., vincristine) during cancer treatment. Depending on the treatment regimens, chemotherapy-induced neuropathic pain can occur in 30–40% of patients and even as high as 75% under certain regimens. Common peripheral sensory symptoms include paresthesias and dysesthesias, pain, numbness and tingling, and sensitivity to touch and temperature. Motor symptoms include weakness and gait and balance disturbances[1]. In most cases, this kind of neuropathic pain is only partially reversible with cessation of treatment and in the worst cases damage can be permanent. To date, there is no one drug or drug class that is considered safe and effective for treatment of chemotherapy-induced neuropathic pain, making the development of alternative effective analgesics a crucial clinical need.

Angelicae dahuricae radix is a perennial plant that grows naturally in broad areas of China. Angelicae dahuricae radix has a strong scent and its leaves are used to make incense. In addition, the roots of angelicae dahuricae radix (also known as Bai Zhi) are used in traditional Chinese medicine to treat harmful external influences on the skin, such as cold, heat, dampness and dryness[2]. Modern pharmacological studies on angelicae dahuricae radix have reported that crude extracts of angelicae dahuricae radix possesses anti-inflammatory, analgesic and antipyretic actions and acute toxicity as a guideline for clinic application[2]. Essential oil of angelicae dahuricae radix has analgesic effect in rat models of pain, and the antinociceptive effects have been linked to the facilitated release of endogenous opioids such as beta-endorphin[3]. More importantly, clinical studies have demonstrated that angelicae dahuricae radix has significant analgesic effect in humans, supporting the clinical utility of angelicae dahuricae radix as an alternative medicine for pain control[4]. However, the crude extract and essential oil of angelicae dahuricae radix include multiple potentially active chemical compounds and the active ingredient(s) of angelicae dahuricae radix that are responsible for its analgesic activity are currently unknown. Recent phytochemical research has purified and identified several active coumarin components of angelicae dahuricae radix[5],

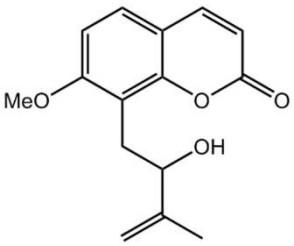

Figure 1 | Chemical structure of auraptenol.

and further pharmacological studies are needed to identify the active coumarin component underlying the antinociceptive actions of angelicae dahuricae radix.

In this study, we described the potent antinociceptive effects of one of the coumarin components of angelicae dahuricae radix, auraptenol (8-(2-hydroxy-3-methylbut-3-en-1-yl)-7-methoxy-2*H*-chromen-2-one, Fig. 1), in a mice model of vincristine-induced neuropathic pain. We also found that a selective serotonin 5-HT_{1A} receptor antagonist, WAY100635, significantly antagonized the antinociceptive effect of auraptenol, suggesting that the observed antinociceptive effect of auraptenol was partially mediated by 5-HT_{1A} receptors.

Results

Daily vincristine treatment (0.5 mg/kg) for 5 days led to marked mechanical hyperalgesia in mice as measured by von Frey filament (Fig. 2). Paired t-test revealed that vincristine treatment produced a significant decrease in the paw withdrawal threshold ($t(7) = 12.56$, $P < 0.0001$). In addition, repeated test every 10 min over a period of 100 min did not alter the hyperalgesic condition, which remained significantly lower than the baseline measurement prior to vincristine treatment (Fig. 3). Two-way ANOVA revealed a significant main effect of vincristine treatment ($F [1, 63] = 87.28$, $P < 0.0001$). Post hoc analysis found that throughout all the time points the paw withdrawal threshold was significantly lower after vincristine treatment ($P < 0.05$). Auraptenol dose-dependently increased the paw withdrawal threshold in mice (Fig. 3). A smaller dose of auraptenol (0.05 mg/kg) did not significantly elevate the paw withdrawal threshold. Two-way ANOVA revealed no significant main effect of auraptenol treatment ($F [1, 63] = 0.72$, $P > 0.05$). A larder dose of auraptenol (0.2 mg/kg) markedly and significantly increased the paw withdrawal threshold. Two-way ANOVA revealed significant main effect of auraptenol treatment ($F [1, 63] = 24.36$, $P < 0.0001$). Multiple comparison analysis found that the paw withdrawal threshold was significantly increased throughout the 20–80 min time period. When the dose of auraptenol was further

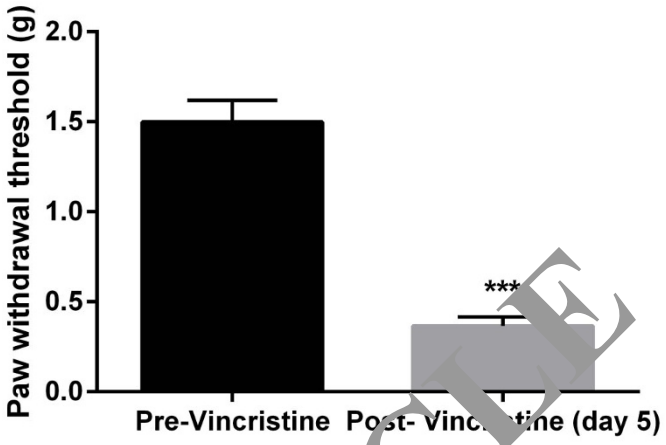

Figure 2 | Paw withdrawal thresholds before and after 5 days of daily 0.5 mg/kg vincristine treatment in mice (n = 8 per group). *** $P < 0.001$ as compared to pre-vincristine measurements.

increased to 0.8 mg/kg, the paw withdrawal threshold was significantly increased the pre-vincristine treatment level (Fig. 3).Two-way ANOVA revealed a significant main effect of auraptenol treatment ($F [1, 63] = 87.28$, $P < 0.0001$). Multiple comparison analysis found that the paw withdrawal threshold was significantly increased throughout the 10–90 min time period.

In order to understand the receptor mechanism underlying the anti-hyperalgesic actions of auraptenol, a dose of the selective serotonin 5-HT_{1A} receptor antagonist WAY-100635 was studied in combination with 0.8 mg/kg auraptenol (Fig. 4). WAY100635 significantly attenuated the anti-hyperalgesic effects of auraptenol. Two-way ANOVA revealed that there were significant main effects of WAY100635 treatment ($F [9, 126] = 47.52$, $P < 0.0001$) and time ($F [9, 126] = 22.15$, $P < 0.0001$). Post hoc analysis found that the anti-hyperalgesic effect of auraptenol was significantly decreased across the 10–90 min time period.

We also studied the anti-hyperalgesic actions of daily repeated auraptenol treatment (Fig. 5). Daily treatment with 0.8 mg/kg auraptenol, a dose that completely reversed mechanical hyperalgesia, maintained its anti-hyperalgesic effect and no significant antinociceptive tolerance was observed. Two-way ANOVA revealed a significant main effect of auraptenol treatment ($F [1, 7] = 464.8$, $P < 0.0001$), but no significant main effects of time or interaction were found. Post hoc analysis found that the paw withdrawal threshold after 0.8 mg/kg auraptenol treatment was significantly higher as compared to the daily pre-drug treatment baseline. In addition, the

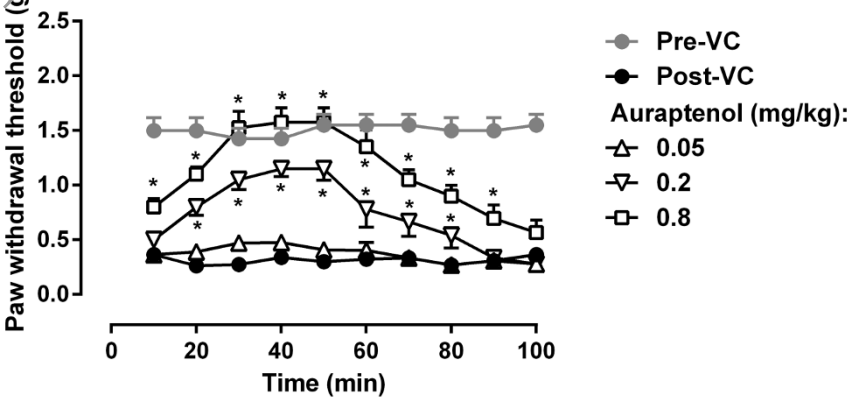

Figure 3 | Anti-hyperalgesic effect of auraptenol in mice (n = 8 per group). * $P < 0.05$ as compared to corresponding post-CV baseline data. VC, vincristine.

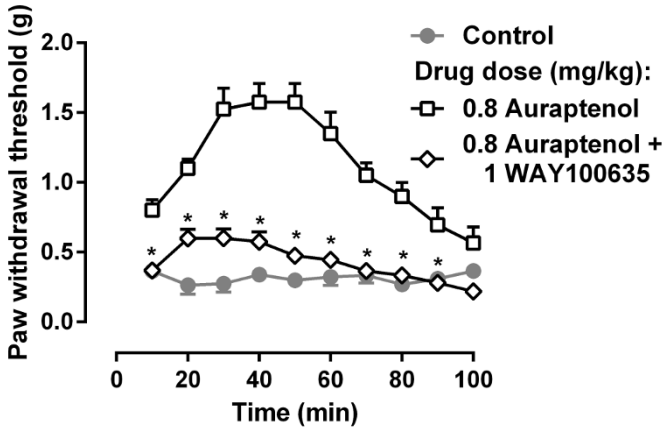

Figure 4 | Effect of WAY100635 on 0.8 mg/kg auraptenol-induced anti-hyperalgesia in mice (n = 8 per group). * $P < 0.05$ as compared to corresponding 0.5 mg/kg auraptenol data.

anti-hyperalgesic effect among the 7 daily treatments was not significantly different.

The potential effect of auraptenol on the general locomotor activity in naïve mice was examined with different doses of auraptenol (Fig. 6). It was found that auraptenol did not significantly alter the locomotor activity in mice across a dose range of 0.05–0.8 mg/kg. One-way ANOVA found no significant difference ($F [3, 31] = 0.21$, $P > 0.05$).

Discussion

In this study, we reported that an active component from the plant angelicae dahuricae radix, auraptenol, produced robust anti-hyperalgesic effect in a mouse model of chemotherapy-induced neuropathic pain. We also reported that the anti-hyperalgesic effect was at least partially mediated by 5-HT_{1A} receptors and the effect was not due to general behavioral impairment. Although angelicae dahuricae radix was used for the treatment of various diseases in traditional Chinese medicine, this is the first study that identified the antinociceptive active component that may explain the pain relieving effect of this plant and this important herbal medicine. In addition, these results encourage continued effort to further understand auraptenol, which may well serve as a potential novel analgesic for the control of chronic neuropathic pain.

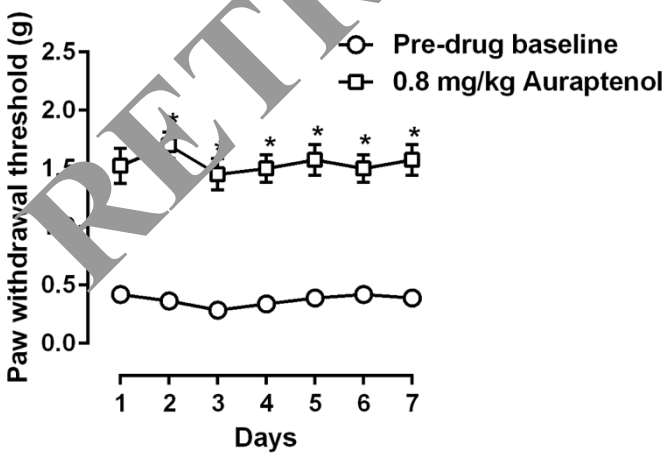

Figure 5 | Anti-hyperalgesic effect of daily 0.8 mg/kg auraptenol treatment in mice (n = 8 per group). * $P < 0.05$ as compared to corresponding daily baseline data as measured before vincristine treatment.

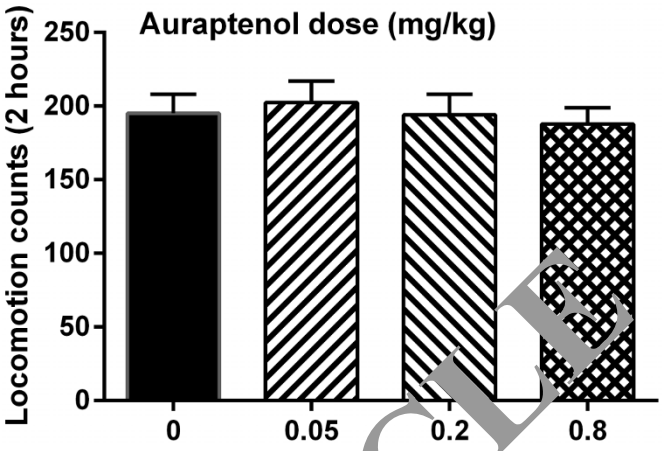

Figure 6 | Effect of auraptenol on general locomotor activity in mice (n = 8 per group).

Many microtubule-targeting cancer chemotherapeutic agents including vincristine are widely recognized to cause peripheral and cranial neuropathy[7–9]. In an effort to better understand this form of neuropathy and develop novel treatment for its management, several animal models of chemotherapeutic agent-induced neuropathy was developed[10–12]. Rodents treated with chemotherapeutic agents typically develop thermal and mechanical hyperalgesia. In consistency with the literature, we found that mice treated with 0.5 mg/kg daily for 5 days developed a reliable mechanical hyperalgesia as measured by von Frey filament test. Repeated measures within a short period of time (100 min) did not significantly change the test results, which offers an opportunity to determine the duration of actions of the study drug. We found that auraptenol produced a very robust effect in decreasing mechanical hyperalgesia. This effect was both dose-dependent and time-dependent and at larger doses it completely reversed the mechanical hyperalgesia. Although angelicae dahuricae radix was used in folk medicine for centuries, and modern pharmacological studies confirmed its analgesic actions[3,4,13], the active components have not yet been identified. This study clearly demonstrated that one major coumarin component from angelicae dahuricae radix, auraptenol, has very robust antinociceptive effect in a mouse model of chronic neuropathic pain, marking the first effort to decipher the phytochemical substrates of angelicae dahuricae radix-induced analgesia. More importantly, repeated treatment with auraptenol did not show evidence of tolerance development. Considering the long-term therapeutic need to treat neuropathic pain, this lack of tolerance development is significant and clearly puts auraptenol in an advantageous position as a potential analgesic.

Serotoninergic (5-HTergic) system is critically involved in pain modulation[14]. Indeed, the serotonin-norepinephrine reuptake inhibitor duloxetine has been approved to treat several chronic pain conditions including peripheral neuropathy and fibromyalgia[15,16]. In addition, 5-HT_{1A} receptor agonists demonstrate robust antinociceptive effect in animal models of chronic neuropathic pain[17–19]. This study found that a selective 5-HT_{1A} receptor antagonist, WAY-100635, significantly blocked the anti-hyperalgesic effect of auraptenol, suggesting that the anti-hyperalgesic action of auraptenol is primarily mediated by 5-HT_{1A} receptors. This dose of WAY-100635 (1 mg/kg) has been shown to significantly block 5-HT1A receptors in other studies[20,21].

In summary, this study for the first time identified an active component of angelicae dahuricae radix, auraptenol, which may be responsible for the analgesic actions of angelicae dahuricae radix. In a mouse model of chemotherapeutic agent-induced neuropathic pain, auraptenol demonstrated excellent analgesic activity with no apparent adverse effects. Although more studies are needed to examine the

generality of these findings and to better understand the potential toxicology of this compound, the current data do suggest that auraptenol could be a potential novel analgesic for pain management.

Methods

Animals. Male C57BL/6 mice weighing 16–22 g (Weitong Lihua, Beijing, China) were acclimated to the temperature, humidity and lighting (12 h light/dark cycle, lights on at 7:00 AM) controlled vivarium and housed in groups of four for at least one week before behavioral studies began. The animals had free access to dietary food and water except during the test sessions. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee, Xinxiang Medical University. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th edition, Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences, Washington DC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs. Vincristine sulphate injection was purchased from Haimen Pharmaceutical Co. (Zhejiang, China). Auraptenol (8-(2-hydroxy-3-methylbut-3-en-1-yl)-7-methoxy-2*H*-chromen-2-one, Fig. 1) was purchased from Shanghai Lei Yun Shang Pharmaceutical Co. (>95% purity, Shanghai, China). WAY100635 was purchased from Sigma-Aldrich (St. Louis, MO, USA). Auraptenol was suspended in 5% DMSO. WAY100635 was dissolved in 0.9% saline. All injections were given intraperitoneally in a volume of 1 ml/100 g of body weight. Vincristine was administered at a dose of 0.5 mg/kg daily for 5 days to establish vincristine-induced neuropathy.

Mechanical allodynia measurement. Mechanical allodynia was assessed prior to and after 5 days of vincristine treatment daily using Von Frey filaments of varying forces (0.07–4.0 g) applied to the mid-plantar surface of the right hind paw, with each application held until curved for 6 s using the up-down method[6]. Mice were placed in individual Plexiglas compartments atop of a wire grid floor suspended 50 cm above the laboratory bench top and acclimated to the environment for 30 min prior to each test session. For the time course studies, baseline von Frey filament measurement was immediately followed by an injection of auraptenol, and then the paw withdrawal threshold was measured every 10 min until the drug effect dissipated to a point that the paw withdrawal threshold was not significantly different from the pre-drug data. In studies that test the effect of the antagonist WAY100635, drug was administered 5 min prior to auraptenol treatment and a time course measurement was followed. For repeated treatment studies, mice were measured daily before drug treatment and 40 min after drug treatment for 7 days.

Locomotor activity test. The locomotor activity of naïve mice treated with vehicle or auraptenol was measured automatically with a Small Animal Locomotion Recording Apparatus (Shandong Academy of Medical Sciences, China), which consisted of six acrylic boxes and in each box there was one pyroelectric infrared sensor 5 cm above the floor. The sensor could detect the movements of the mice through infrared radiation. The apparatus recorded only gross movements of the mice, whereas small movements such as gnawing or grooming could not be differentiated and recorded.

Data analyses. For the mechanical hyperalgesia test prior to and 5 days after vincristine treatment, data were analyzed using a *t*-test. For the antinociceptive studies, data were presented as paw withdrawal threshold (grams) plotted as a function of time (min or days), respectively. Data were analyzed by two-way repeated measures analysis of variance (ANOVA) (time × auraptenol treatment or time × vincristine treatment) followed by post hoc Bonferroni test. For the locomotion tests, data were analyzed with one-way ANOVA followed by post hoc Bonferroni test.

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Author contributions

X.Z. and P.L. designed the research; Y.W., S.C., J.T. and G.L. conducted the studies; X.Z. and P.L. analyzed the data and prepared the manuscript; all authors read and approved the manuscript.

Additional information

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Retraction Note: Auraptenol attenuates vincristine-induced mechanical hyperalgesia through serotonin 5-HT_{1A} receptors

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The editors are retracting this Article because the figures and the majority of the text are identical to those of another paper[1]. These issues undermine confidence in the validity of the study and the conclusions cannot be considered reliable.

The authors could not be reached.

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