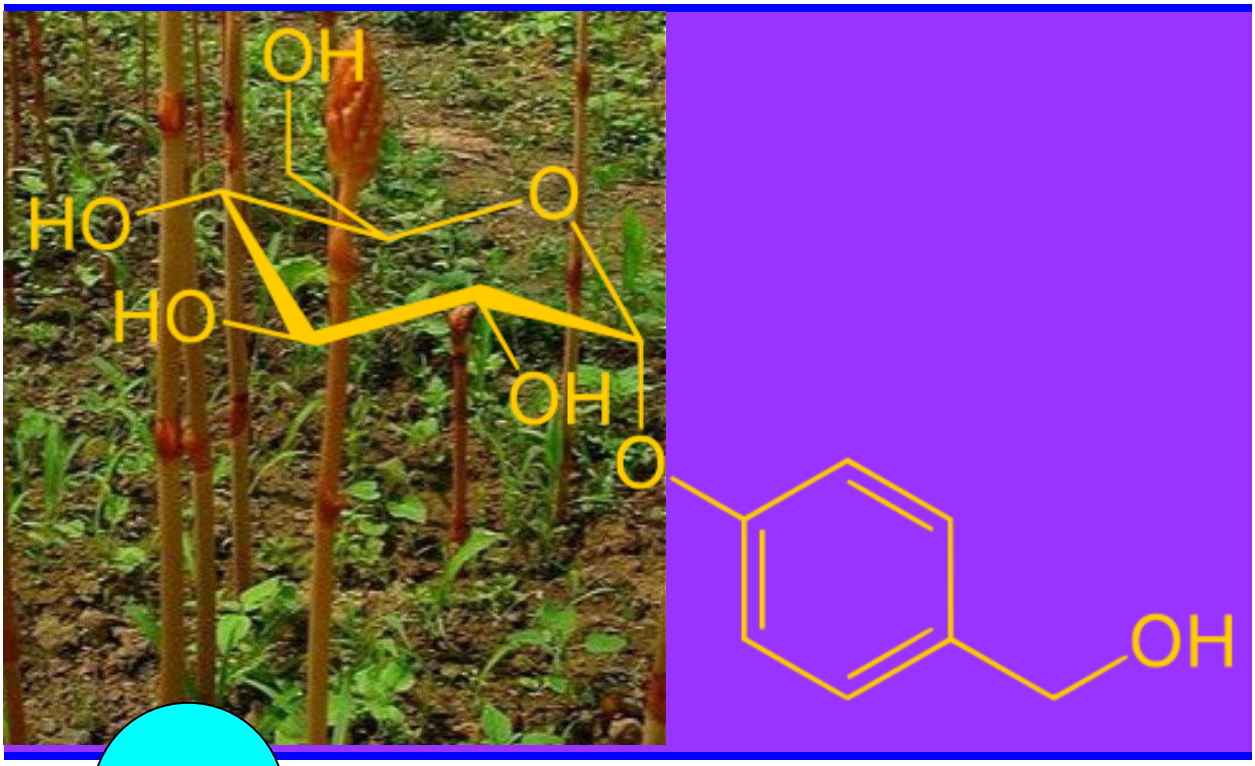

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Gastrodin attenuates vincristine-induced mechanical hyperalgesia through serotonin 5-HT_{1A} receptors

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Abstract

Gastrodia elata Blume (Orchidaceae) is an old traditional Chinese medicine with demonstrated analgesic efficacy in humans. However, the potential analgesic effect of its active component, gastrodin, has not been systematically studied. This work described the anti-hyperalgesic effect of gastrodin in a mouse model of chemotherapeutic agent vincristine-induced neuropathic pain. Gastrodin (0.05-0.8 mg/kg) dose-dependently reverted the mechanical hyperalgesia in mice. In addition, the anti-hyperalgesic effect of gastrodin was significantly blocked by a selective serotonin 5-HT_{1A} receptor antagonist WAY100635 (1 mg/kg). In contrast, gastrodin did not significantly alter the general locomotor activity in mice. Taken together, this study demonstrated that gastrodin possesses robust analgesic efficacy in mice and may be a novel analgesic for the management of neuropathic pain.

Introduction

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 inclu -de paresthesias and dysesthesias, pain, numbness and tingling, and sensitivity to touch and temperature. Motor symptoms include weakness and gait and balance disturbances (Visovsky et al., 2007). 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.

Gastrodia elata Blume is (also known as Tian Ma) is used in traditional Chinese medicine to treat epilepsy, inflammation and pain (Ojemann et al., 2006; Jung et al., 2007; Xu and Guo, 2000). Modern phytochemical studies on *G. elata* have identified major active component of *G. elata* as gastrodin (4-hydroxybenzyl alcohol 4-O-beta-D-glucopyranoside). Recent *in vitro* studies found that gastrodin exerts a neuroprotective action by attenuating glutamate accumulation during transient focal cerebral ischemia (An et al., 2003; Yong et al., 2009; Zeng et al., 2006). There are numerous reports showing that gastrodin may improve learning and facilitate memory consolidation and retrieval (Hsieh et al., 1997; Wu et al., 1996). Gastrodin was also reported to display anti-inflammatory properties by inhibiting the expression of pro-inflammatory cytokines in microglial cells (Dai et al., 2011). However, relatively little is known regarding the analgesic effects of gastrodin.

In this study, we described the potent antinociceptive effects of gastrodin 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

gastrodin, suggesting that the observed antinociceptive effect of gastrodin was partially media-ted by 5-HT_{1A} receptors.

Methods and Methods

Animals

Male C57BL/6 mice weighing 16-22 g (Weitong Lihua, Beijing, China) were acclimated to the temperature, humidity and lighting (12 hours 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.

Drugs

Vincristine sulphate injection was purchased from Haimen Pharmaceutical Co. (Zhejiang, China). Gastrodin (4-hydroxybenzyl alcohol 4-O-beta-D-glucopyranoside) was purchased from Shanghai Lei Yun Shang Pharmaceutical Co. (>95% purity, Shanghai, China). WAY100635 was purchased from Sigma-Aldrich (USA). Gastrodin and WAY100635 were 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 hyperalgesia measurement

Mechanical hyperalgesia 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 sec using the up-down method (Dixon, 1980). 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 gastrodin, 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 gastrodin 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 gastrodin 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 4 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 groom-ing 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 paired 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 × gastro-din 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.

Results

Daily vincristine treatment (0.5 mg/kg) for 5 days led to marked mechanical hyperalgesia in mice as measured by von Frey filament (Figure 1). Paired t-test revealed

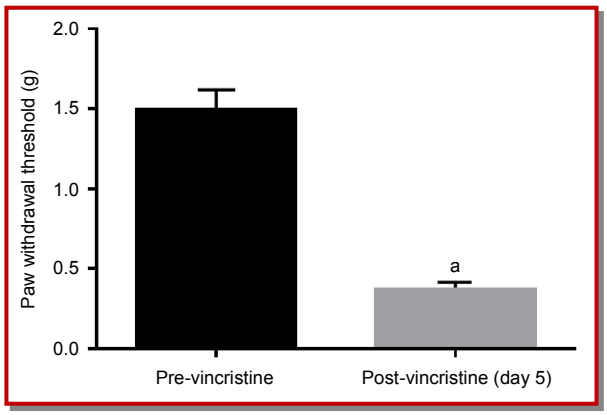

Figure 1: Paw withdrawal thresholds before and after 5 days of daily 0.5 mg/kg vincristine treatment in mice (n = 8 per group). [a]p<0.001 as compared to pre-vincristine measure-ments

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 (Figure 2). 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).

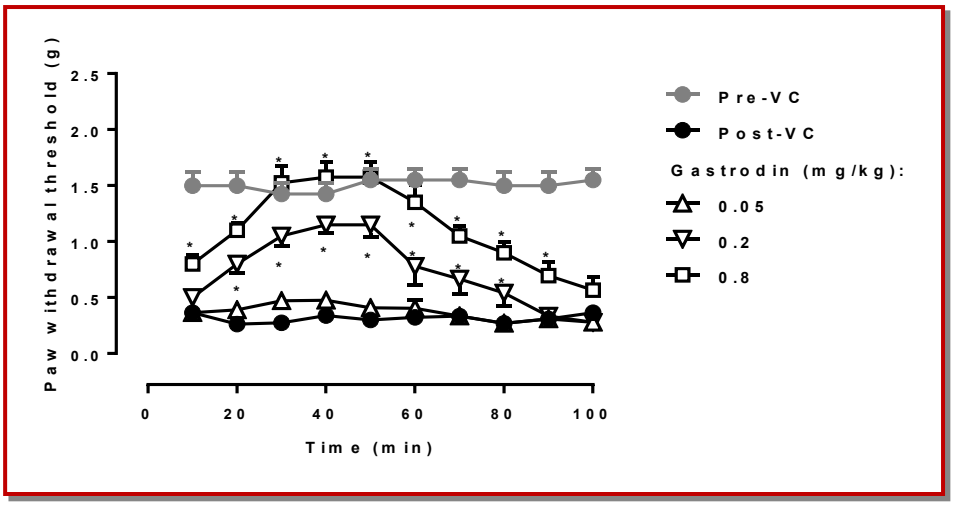

Figure 2: Anti-hyperalgesic effect of gastrodin in mice (n=8 per group). *p<0.05 as compared to corresponding post-CV baseline data. VC, vincristine

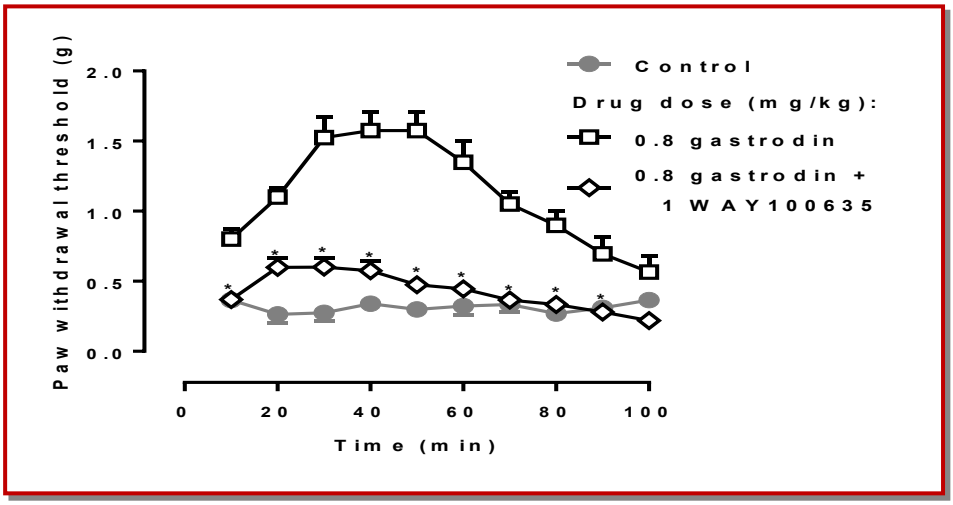

Figure 3: Effect of WAY100635 on 0.8 mg/kg gastrodin-induced anti-hyperalgesia in mice (n = 8 per group). *p<0.05 as compared to corresponding 0.5 mg/kg gastrodin data

Gastrodin dose-dependently increased the paw withdrawal threshold in mice (Figure 2). A smaller dose of gastrodin (0.05 mg/kg) did not significantly elevate the paw withdrawal threshold. Two-way ANOVA revealed no significant main effect of gastrodin treatment ($F [1, 63]=0.72$, $p>0.05$). A larder dose of gastrodin (0.2 mg/kg) markedly and significantly increased the paw withdrawal threshold. Two-way ANOVA revealed significant main effect of gastrodin 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 gastrodin was further increased to 0.8 mg/kg, the paw withdrawal threshold was significantly increased the pre-vincristine treatment level (Figure 2). Two-way ANOVA revealed significant main effect of gastrodin 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 gastrodin, a dose of the selective serotonin 5-HT_{1A} receptor antagonist WAY-100635 was studied in combination with 0.8 mg/kg gastrodin (Figure 3). WAY100635 significantly attenuated the anti-hyperalgesic effects of gastrodin. 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 gastrodin was significantly decreased across the 10-90 min time period.

We also studied the anti-hyperalgesic actions of daily repeated gastrodin treatment (Figure 4). Daily treatment with 0.8 mg/kg gastrodin, 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 gastrodin treatment ($F [1, 7]=$

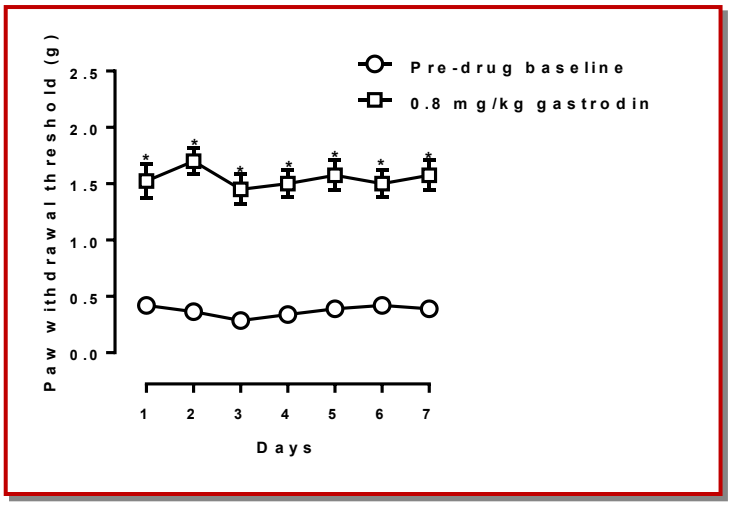

Figure 4: Anti-hyperalgesic effect of daily 0.8 mg/kg gastrodin treatment in mice (n = 8 per group). *p<0.05 as compared to corresponding daily baseline data as measured before vincristine treatment

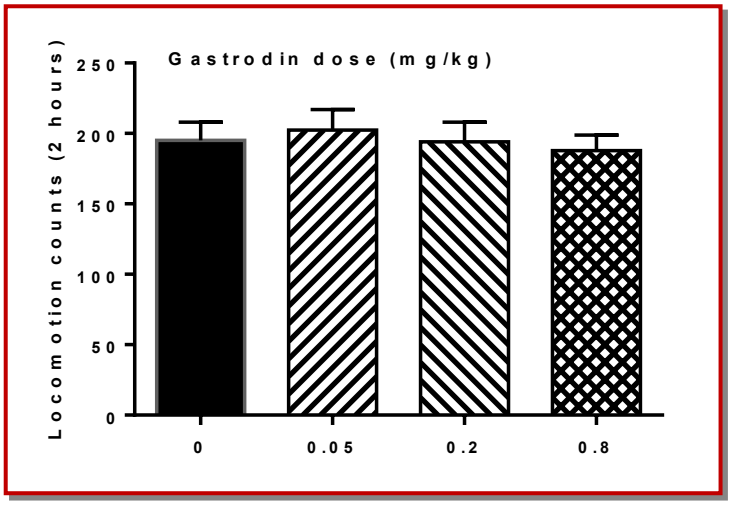

Figure 5: Effect of gastrodin on general locomotor activity in mice (n = 8 per group)

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 gastrodin treatment was significantly higher as compared to the daily pre-drug treatment baseline. In addition, the anti-hyperalgesic effect among the 7 daily treatments was not significantly different.

The potential effect of gastrodin on the general locomotor activity in naïve mice was examined with different doses of gastrodin (Figure 5). It was found that gastrodin 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 *G. elata*, gastrodin, 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 gastrodin has been reported to involve several disorders, this is the first study that identified the antinociceptive effects of gastrodin in a mouse model of chemotherapeutic agent-induced neuropathic pain. Taken together, these results encourage continued effort to better understand gastrodin, which may well serve as a potential novel analgesic for the control of chronic neuropathic pain.

Many microtubule-targeting cancer chemotherapeutic agents including vincristine are widely recognized to cause peripheral and cranial neuropathy (Dixit et al., 2012; Carlson and Ocean, 2011; Jaggi and Singh, 2012). 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 (Jaggi and Singh,

2012; Nativi et al., 2013; Contreras et al., 1997). 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 offer an opportunity to determine the duration of actions of the study drug. We found that gastrodin 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 gastrodin was shown to have neuroprotective action (An et al., 2003; Yong et al., 2009; Zeng et al., 2006) and improve learning and facilitate memory consolidation and retrieval (Hsieh et al., 1997; Wu et al., 1996), its antinociceptive actions has been rarely explored. This study clearly demonstrated that gastrodin has very robust antinociceptive effect in a mouse model of chronic neuropathic pain. More importantly, repeated treatment with gastrodin did not show evidence of toler -ance development. Considering the long-term therapeutic need to treat neuropathic pain, this lack of tolerance development is significant and clearly puts gastrodin in an advantageous position as a potential analgesic.

Serotoninergic (5-HTergic) system is critically involved in pain modulation (Millan, 2002). Indeed, the serotonin -norepinephrine reuptake inhibitor duloxetine has been approved to treat several chronic pain conditions including peripheral neuropathy and fibromyalgia (Ormseth et al., 2011; Pergolizzi et al., 2013). In addition, 5-HT_{1A} receptor agonists demonstrate robust antinociceptive effect in animal models of chronic neuropathic pain (Bardin et al., 2001; Colpaert et al., 2004; Colpaert, 2006). This study found that a selective 5-HT_{1A} receptor antagonist, WAY-100635, significantly blocked the anti-hyperalgesic effect of gastrodin, suggesting that the anti -hyperalgesic action of gastrodin 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 (Valhondo et al., 2013; Li et al., 2007).

Conclusion

This study for the first time identified gastrodin as a potent analgesic for the management of neuropathic pain. In a mouse model of chemotherapeutic agent-induced neuropathic pain, gastrodin demonstrated excellent analgesic activity with no apparent adverse effects.

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