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ORIGINAL ARTICLE

Effect of Decursinol on the Aspirin-induced Gastric Ulcer in Mice

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Abstract Decursinol is one of the coumarins purified from the dried roots of *Angelica gigas* Nakai (Umbelliferae) and has various pharmacological effects including an analgesic property. Although aspirin is widely used to reduce pain and inflammation, aspirin-induced gastric damage remains the major limitation to its use. Therefore, the anti-ulcer activity of decursinol in aspirin-induced gastric ulcer in mice was examined. One group of mice was treated orally once daily with aspirin (300 mg/kg) and another group was co-administered with decursinol (10, 25, 50, and 100 mg/kg) and aspirin (300 mg/kg) orally once daily for 5 consecutive days. On day 6, the gastric mucosae were examined. Animal groups co-administered with decursinol and aspirin exhibited a dose-dependent reduction of gastric damage against aspirin-induced gastric ulceration. The extent of inhibitions for the respective doses employed was 11.1, 15.8, 50.3, and 70.4%, respectively. These results suggest that combination therapy with aspirin and decursinol may be useful to alleviate pain and inflammation without major gastrointestinal side effects.

Keywords *Angelica gigas* Nakai · aspirin · combination therapy · decursinol · ulcer · Umbelliferae

Introduction

Decursinol is one of the coumarins purified from the dried roots of *Angelica gigas* Nakai (Umbelliferae), which has been used in oriental traditional medicine as a treatment regimen for anemia and some circulatory disorders. Previous reports have revealed that decursinol shows various pharmacological effects, such as anticancer (Ahn et al., 1997), antiplatelet (Lee et al., 2003), neuroprotective (Kang and Kim, 2007), and analgesic effects (Choi et al, 2003; Seo et al, 2009). Choi et al. (2003) have found an antinociceptive property of decursinol and proposed possible mechanisms using various pain models.

Aspirin is widely used oral analgesic drug and has profound anti-inflammatory effect. Seo et al. (2009) characterized the possible interaction between decursinol and aspirin or acetaminophen in the mouse writhing test. Because the association of gastric damage with the use of aspirin remains the major limitation for use (Wallace, 1997), combination therapy with aspirin and an analgesic agent possessing the anti-ulcer activity may be applicable to alleviate pain and inflammation effectively without major gastrointestinal side effects. Thus, in the present study, anti-ulcer activity of decursinol in the aspirin-induced gastric ulcer in mice was examined.

Materials and Methods

The animal experiments were approved by the University of Hallym Animal Care and Use Committee. All procedures were conducted in accordance with the 'Guide for Care and Use of Laboratory Animals' published by the National Institutes of Health.

Experimental animals. Male ICR mice (MJ Co., Korea) weighing 20–25 g were used for all experiments. Animals were housed 5 per cage in a room maintained at 22±0.5°C with an alternating 12 h light-dark cycle. Food and water were available *ad libitum*. The animals were allowed to adapt to the laboratory

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for at least 2 h before testing and were only used once. Experiments were performed during the light phase of the cycle (10 : 00–17 : 00).

Aspirin-induced ulcerogenesis and experimental protocols. Aspirin-induced ulcerogenesis was performed following the method of Szelenyi and Thiemer with slight modification (1978). In brief, a group of mice was treated orally once daily with aspirin (300 mg/kg) for 5 consecutive days to induce ulcer. To examine the effect of decursinol on aspirin-induced gastric ulcer, another group of mice was co-administered with decursinol (10–100 mg/kg) and aspirin (300 mg/kg) orally once daily for 5 consecutive days. Oral administration was performed with gavage in a volume of 500 μL/25 g body weight. Control group received orally once daily a similar volume of vehicle or decursinol (100 mg/kg) for 5 consecutive days. All treatment doses were determined from previous studies (Szelenyi and Thiemer, 1978; Choi et al, 2003; Seo et al, 2009).

On day 6, mice were sacrificed by cervical dislocation. Immediately, stomach was dissected out, opened along the greater curvature, and stretched on cork plates. The inner surface was rinsed with iced cold normal saline to remove blood contaminant, if any. The gastric mucosae were examined with binocular stereomicroscope under 20× magnification. Severity of gastric mucosal lesions was graded for each animal as: (I) petechiae and erosions <1 mm; (II) erosions 1–3 mm; (III) erosions >3 mm. Ulcer index was calculated as follows: [(1 x number of lesion I) + (2 x number of lesion II) + (3 x number of lesion III)] / number of animals (Szelenyi and Thiemer, 1978; Bacchi and Sertie, 1994).

Drugs. Aspirin was purchased from Sigma Chemical Co. (USA). Decursinol (M.W.: 246) was isolated from the dried root of *A. gigas* Nakai in keeping with the method published in a previous report (Kang et al., 2001), and its purity was about 99% (Fig. 1). Aspirin and decursinol (stock solution) was dissolved in 20% dimethyl sulfoxide (DMSO) for oral administration. All drugs were prepared just before use. The final concentration of DMSO did not cause any effect '*per se*'.

Statistical analysis. Data were presented as the means ± SEM (Standard error of the mean). The statistical significance of differences between groups was assessed with one-way ANOVA with Tukey's post-hoc test using GraphPad Prism version 4.0 for Windows XP (GraphPad Software, USA); $p < 0.05$ was considered significant.

Results

The effect of decursinol (Fig. 1) on gastric damage induced by aspirin is presented in Fig. 2. Oral administration of 300 mg/kg of aspirin only for 5 consecutive days induced gastric lesions in the form of edema, hyperemia, petechiae, and erosion (ulcer index 68.00±13.72; Fig. 3). Animal groups co-administered orally with the decursinol (10, 25, 50, and 100 mg/kg) and aspirin exhibited a dose-dependent reduction of gastric damage against aspirin-induced gastric ulceration. The extent of inhibitions for the respective doses employed were 11.1, 15.8, 50.3, and 70.4%, respectively. Decursinol as well as vehicle itself did not affect the gastric mucosae significantly.

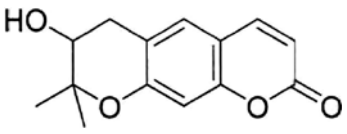

Fig. 1 Structure of decursinol isolated from *Angelica gigas* Nakai (Korean angelica).

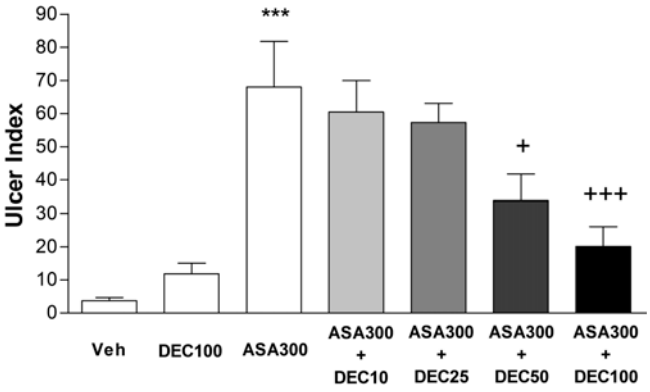

Fig. 2 Effect of co-administrated decursinol on aspirin-induced gastric ulcer in mice. Mice were treated orally once daily with aspirin (ASA, 300 mg/kg) for 5 consecutive days to induce ulcer. Another group of mice was co-administered with decursinol (DEC, 10–100 mg/kg) and aspirin (300 mg/kg) orally once daily for 5 consecutive days. Control group received orally once daily a similar volume of vehicle (Veh) or decursinol (100 mg/kg) for 5 consecutive days. On day 6, the severity of gastric mucosal lesions was graded for each animal as: (I) petechiae and erosions <1 mm; (II) erosions 1–3 mm; (III) erosions >3 mm. Ulcer index was calculated as follows: [(1 x number of lesion I) + (2 x number of lesion II) + (3 x number of lesion III)] / number of animals. Ulcer index was analyzed by one-way ANOVA with Tukey's post-hoc test. The vertical bars denote the standard error of the mean. The number of animals used for each group was 7–10. (***$p < 0.001$ compared to the group of mice treated with vehicle. $^{+}p < 0.05$ and $^{+++}p < 0.001$ compared to the group of mice treated with aspirin.)

Discussion

Previously, another species of angelica called *Angelica sinensis* dose-dependently prevented gastric mucosal damage induced by ethanol or indomethacin in rats (Cho et al., 2000). They also reported the increase of myeloperoxidase activity, a marker for inflammation and neutrophil infiltration, was prevented by oral treatment of polysaccharides isolated from the root of *A. sinensis*. It is well established that the neutrophils and neutrophil-endothelial cell interaction may play important roles in the pathogenesis of gastric ulceration induced by NSAIDs (Wallace et al., 1990; Wallace, 1997). In addition, Shin et al. (2009) reported that the carrageenan-induced inflammation and neutrophil

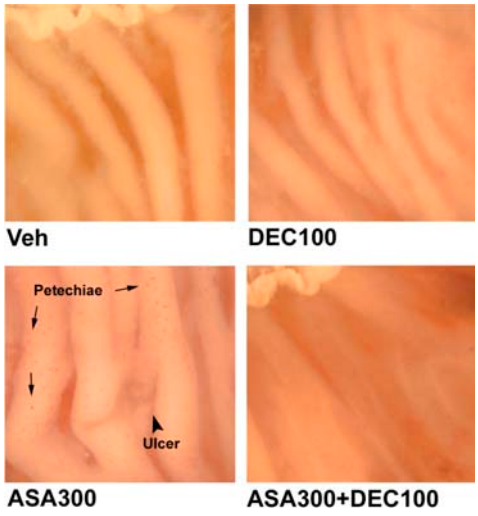

Fig. 3. Effect of decursinol on gastric ulcer induced by aspirin. The gastric mucosae were examined with binocular stereomicroscope under 20 × magnification. Oral administration of 300 mg/kg of aspirin (ASA) only for 5 consecutive days induced gastric lesions in the form of edema, hyperemia, petechiae, and ulcer. Animal groups co-administered orally with decursinol (DEC, 100 mg/kg) and aspirin reduced gastric damage against ASA-induced gastric ulceration. Decursinol as well as vehicle (Veh) did not affect the gastric mucosa

infiltrations were significantly attenuated by oral administration of an ethanol extract of *A. gigas* in mice. Furthermore, *in vitro* studies have found that several coumarin compounds (decursinol angelate, decursin, and decursinol) isolated from *A. gigas* inhibited the expression of pro-inflammatory cytokines (Kim et al, 2006; 2010).

On the other hand, prostaglandin is well known to be involved in the regulation of mucosal blood flow, epithelial proliferation, immune function, and secretion of mucus and bicarbonate (Chan and Leung, 2002). Generally, the effects of aspirin and other NSAIDs were assumed to be mediated by inhibition of COX-1 and COX-2 activities, which cause significant suppression of prostaglandin synthesis, thereby providing strong evidence of the ulcerogenic effect of NSAIDs (Wallace, 1997; Chan and Leung, 2002). However, oral treatment of an ethanol extract of *A. gigas* remarkably lowered blood level of prostaglandin in croton oil-induced mouse inflammation model (Shin et al., 2010). Taken together with the present findings, the gastric protective effect of decursinol on the ASA-induced ulceration is suggested to be involved in neutrophil but is not prostaglandin dependent, although the direct evidence of decursinol for the aspirin-induced gastric mucosal inflammation remains to be elucidated.

Previously, we have found that decursinol administered orally showed an antinociceptive effect in a dose-dependent manner in various pain models (Choi et al., 2003). In the present study, that co-administration of decursinol at the level of analgesic dose (50 and 100 mg/kg) with aspirin significantly was clearly demonstrated to reduce the gastric mucosal damage induced by aspirin in a dose-dependent manner. Even though further studies on other ulcer models, such as ethanol/HCl-induced ulcer models, evaluation of gastric secretion by the pylorus-ligated model, and determination of mucus in gastric content are needed to clarify the possible anti-ulcer mechanisms of decursinol, combination therapy with aspirin and decursinol may be useful to alleviate pain and inflammation without major gastrointestinal side effects.

Supporting information. The isolation of decursinol is available as Supporting Information.

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