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Evaluation of protective effects of costunolide and dehydrocostuslactone on ethanol-induced gastric ulcer in mice based on multi-pathway regulation

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ABSTRACT

The aim of the present study was to evaluate the anti-ulcerogenic activity of costunolide (Co) and dehydrocostuslactone (De) on ethanol-induced gastric ulcer in mice and to elucidate the potential mechanisms of the action involved. Mice were pretreated orally with Co (5 or 20 mg/kg), De (5 or 20 mg/kg) and omeprazole (OME, 20 mg/kg) for 7 consecutive days, followed by ulcer induction using absolute ethanol (0.2 mL/20 g body weight). Treatment with Co had a remarkable gastroprotection compared to the ethanol-ulcerated mice that significantly reduced the ulcerative lesion index (ULI) and histopathological damage. Daily intragastric administration of Co exerted a powerful anti-inflammatory activity as evidenced by the suppression of nuclear factor (NF)-κB, tumor necrosis factor (TNF)-α, nitric oxide (NO), inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, as well as increased interleukin (IL)-10. Also, pretreatment with Co effectively inhibited ethanol-induced malondialdehyde (MDA) overproduction, increased the depleted superoxide dismutase (SOD) and promoted gastric mucosa epithelial cell proliferation by up-regulating proliferating cell nuclear antigen (PCNA) expression. Similarly, De had a protective effect on ethanol-induced ulcer, which was dependent on the inhibition of inflammatory cytokines and MDA generation, but independent of IL-10, SOD and PCNA improvement. Conclusively, the results have clearly demonstrated the anti-ulcerogenic potential of Co and De on ethanol-induced gastric ulcer; nevertheless, the gastroprotective activity of Co was superior to De due to more multi-pathway regulation than De. These findings suggested that Co or De could be a new useful natural gastroprotective tool against gastric ulcer, which provided a scientific basis for the gastroprotection of sesquiterpene lactones.

1. Introduction

Gastric ulcer is still a serious medical problem with approximately 5–10% of the population worldwide suffering from it [1]. The disease occurs due to an imbalance between defensive factors and aggressive factors in the digestive tract [2]. It can be caused by some endogenous etiologies like gastric acid hypersecretion, pepsin activity, gastric contractions, gastric mucosa ischemia and particularly in the infection of bacterium (*Helicobacter pylori*) [3]. Furthermore, exogenous damaging agents such as alcohol consumption, stress, smoking, and prolonged ingestion of non-steroidal anti-inflammatory drugs (NSAIDs) are all relevant the

Abbreviations: Co, costunolide; De, dehydrocostuslactone; OME, omeprazole; TNF-α, tumor necrosis factor alpha; NF-κB, nuclear factor kappa B; IL-10, interleukin-10; COX-2, cyclooxygenase-2; NO, nitric oxide; iNOS, inducible nitric oxide synthase; SOD, superoxide dismutase; MDA, malondialdehyde; PCNA, proliferating cell nuclear antigen; NSAIDs, non-steroidal anti-inflammatory drugs; ROS, reactive oxygen species; ULI, ulcerative lesion index; H&E, hematoxylin and eosin; ELISA, enzyme-linked immunosorbent assay; TBARS, thiobarbituric acid reactive substance; PBS, phosphate-buffered saline; AOD, average optical density.

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formation of gastric ulcer [4]. Especially for the over-consumption of alcohol, it not only increases the risk of gastritis but one of main causes of hepatitis, cirrhosis and pancreatitis [2,5]. Therefore, it is greatly urgent to find the prevention or treatment strategies for ethanol-induced diseases. Ethanol given orally is absorbed rapidly into the blood stream from the stomach and intestine [6,7]. The high-concentration of ethanol directly erodes gastric mucosa and causes acute gastritis. Excessive ethanol ingestion predisposes to acute gastric ulcer formation through the neutrophil infiltration, the over-expression of nuclear fact-κB (NF-κB), and the release of pro-inflammatory cytokines [8,9]. Additionally, inflammatory responses accompanied by the production of reactive oxygen species (ROS) are responsible for cell damage and death. ROS causes oxidative bursts in essential cellular constituents, including proteins, lipids and nucleic acids, which further amplify inflammatory signals, resulting in tissue injury [10].

Current medicinal treatments of gastric ulcer generally rely on the inhibition of gastric acid secretion by H_2-antagonists, such as ranitidine; or proton-pump inhibitors, such as omeprazole [11]. However, most of these agents induce adverse generate side effects such as hypersensitivity, arrhythmia, impotence, gynecomastia, and hematopoietic changes [12], which highlight the need for safer and more effective antiulcer agents. Recently, many studies have focused on novel approaches for improving the resistance of the gastric mucosa to injury. A variety of chemical compounds, herbs and plant extracts have been proved to possess therapeutic properties in experimental models of gastric ulcer. Gastroprotective effect of these compounds or extracts has been attributed to antisecretory, cytoprotective, anti-inflammatory and antioxidant properties [13–15].

Costunolide (Co) and dehydrocostuslactone (De), the sesquiterpene lactones, are the major bioactive ingredients of *Radix Aucklandiae* (the dried root of *Aucklandia lappa* Dence belonging to family Asteraceae). Our previous study suggested that Co and De were both absorbed well into rat plasma after oral administration of *Radix Aucklandiae* extract [16]. It has been convincingly demonstrated the abilities of Co and De to exhibit anti-inflammation, anti-ulcer, anti-microbial and anti-carcinogenesis [17–20]. Further mechanisms implicated are mainly focused on the inhibition of ROS, inflammatory responses and the improvement of immune system. In more specific terms, Co and De exert inhibitory effect on the production of proinflammatory cytokines, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expression, as well as nitric oxide (NO) generation [21–23]. Guo et al. verified that Co and De could improve gastrointestinal function and relieve the spasm of smooth muscle [24]. Consequently, as an inflammatory disease accompanied by gastric contractions, it is remarkable to explore the pharmacological protection of Co or De against ethanol-induced gastric ulcer.

In view of the potential anti-inflammatory advantage and gastric protection, we set forward this study to clarify the protective mechanisms of Co and De against ethanol-induced gastric ulcer, specially focused on the role of anti-inflammatory, anti-oxidative and proliferative activities.

2. Materials and methods

2.1. Animals

Adult Kunming mice (22–25 g) [License No. 039545; SCXK (Jin)-2012-004] were obtained from the Laboratory Animal Center of Health Science, Peking University, Beijing, China. All animals were housed at the Animal Breeding Laboratory of Tianjin Institute of Pharmaceutical Research, and kept at 25 ± 1 °C under a 12 h light/dark cycle condition with free access to standard pellet food and water. This study was carried out in compliance with the Institutional Animal Care and Use Committee (IACUC) of China, and followed institutional guidelines for animal welfare and experimental conduct (Permit Number: IACUC2014-010).

2.2. Chemicals

Co and De (>98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Omeprazole (99%; OME; a well-known antiulcer drug) was used as the positive control and was obtained from Sigma Company (St. Louis, MO, USA). Analytical grade of ethanol was purchased from Tianjin Fengchuan Chemical Reagent Science And Technology Co., Ltd. (Tianjin, China). Other chemicals were of analytical grade.

2.3. Ethanol-induced gastric ulcer

Gastric ulcer were induced by intragastric administration of ethanol according to the previously method described [25]. KM mice were randomly divided into 7 experimental groups with 12 mice in each group as follows: normal control (normal vehicle), ethanol model control (vehicle), positive control (OME, 20 mg/kg) and treatment (Co or De, 5 and 20 mg/kg) groups. Mice were continuously pretreated with drugs once daily at the same time by gavage for 7 days. On the 7th day, after overnight fasting, mice received ethanol (0.2 mL/20 g body weight) orally to induce acute gastric ulcer. One hour after treatment, all mice were anaesthetized with ether and blood samples were collected by retro orbital puncture for biochemical estimation [26]. Stomachs in mice were rapidly removed and immersed in 4% neutral buffered formalin for 30 min after being euthanized, and then opened along the greater curvature for evaluating the severity of gastric mucosal lesions mice.

2.4. Macroscopic gastric damage

The severity of gastric mucosal lesions was examined macroscopically under the light microscopy and was rated for gross pathology expressed as an ulcerative lesion index (ULI). The ULI was according to the method of Amirshahrokhi et al. [9] with a modified scoring system. ULI = Σ(a) + (2b) + (3c) (scoring the ULI consistency: a, the number of small erosions up to 1 mm; b, the number of linear erosions up to 3 mm; and c, the number of linear erosions greater than 3 mm). The results were shown as average scores for each group. Macroscopic scoring was performed by an observer unaware of the treatment groups. Furthermore, gastroprotection (%) was calculated as follows:

$$\text{Gastroprotection (\%)} = (\text{ULI model control} - \text{ULI pretreatment/ULI model control}) \times 100\%$$

2.5. Histopathological analysis

For histopathological analysis, the stomach segments were fixed in 10% neutral buffered formalin solution for 24 h. Tissues were embedded in paraffin and 5 μm-thick cross-sections were papered for hematoxylin and eosin (H&E) staining. The specimens were then assessed under the light microscopy by an experienced pathologist according to the method previously reported [27], briefly, the presence of edema (score: 0–4), hemorrhagic damage (score: 0–4), epithelial cell loss (score: 0–3) and inflammatory cell infiltration (score: 0–3).

2.6. Determination of cytokines levels

The involvement of inflammatory responses in ethanol-induced