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Protective effect of chelerythrine against ethanol-induced gastric ulcer in mice

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ABSTRACT

The quaternary benzo[c]phenanthridine alkaloid, chelerythrine (CHE), is of great practical and research interest because of its pronounced, widespread physiological effects, primarily antimicrobial and anti-inflammatory, arising from its ability to interact with proteins and DNA. Although CHE was originally shown to possess anti-inflammatory properties, its effects on acute gastric ulcer have not been previously explored. The aim of the present study is to evaluate the protective effect of CHE on ethanol induced gastric ulcer in mice. Administration of CHE at doses of 1, 5 and 10 mg/kg bodyweight prior to ethanol ingestion dose-dependently inhibited gastric ulcer. The gastric mucosal lesion was assessed by ulcer area, gastric juice acidity, myeloperoxidase (MPO) activities, macroscopic and histopathological examinations. CHE significantly reduced the gastric ulcer index, myeloperoxidase activities, macroscopic and histological score in a dose-dependent manner. In addition, CHE also significantly inhibited nitric oxide (NO) concentration, pro-inflammatory interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) level in serum and gastric mucosal in the mice exposed to ethanol induced ulceration in a dose-dependent manner. In addition, immunohistochemical analysis revealed that CHE markedly attenuated the overexpression of nuclear factor-κB in gastric mucosa of mice. It was concluded that CHE represents a potential therapeutic option to reduce the risk of gastric ulceration. In addition, acute toxicity study revealed no abnormal sign to the mice treated with CHE (15 mg/kg). These findings suggest that the gastroprotective activity of CHE might contribute in adjusting the inflammatory cytokine by regulating the NF-κB signalling pathway.

1. Introduction

Gastric ulcer is a common disease with multiple etiologies, defined as a discontinuity in the gastric mucosa penetration through the muscularis mucosa [1]. It was reported to be associated with the imbalance between the aggressive factors (physical, chemical or psychological) in the lumen and protective mechanisms in the duodenal mucosa represented by mucus and bicarbonate secretions, as well as by prostaglandins, sulphydryl compounds, polyamines, nitric oxide (NO) and dopamine [2], causing chronic inflammation that leads to a defect in the regulation of gastrin production [3]. Gastrointestinal problems have now become a global problem, and many studies were conducted towards fixing it. The ability of some ulcer models to suppress secretion of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and protect ethanol ulcer by scavenging of ROS and preventing apoptosis has provided a means for intense investigations [4,5]. It was suggested that, cytokines such as TNF-α, IL-6 and IL-10 play important roles in the acute phase inflammation as well as in maintenance and regulation of the severity of gastric ulcers [6]. Over expression and translocation of the NF-κB subunits (p65 and p50) to the nucleus promote the over expression of proinflammatory mediators such as TNF-α and IL-6 [7]. Increasing evidence reveals that the inhibition of NF-κB activity may lead to alleviating the severity of inflammatory diseases [8]. Therefore, understanding the molecular mechanisms involved in this pathway is an essential step towards countering the damaging effects of pro-inflammatory mediators in gastric ulcer. On the other hand, neutrophil infiltration into the gastric mucosa is also a critical process in the pathogenesis of a variety of gastric ulcers [9,10]. It has been shown that ethanol-induced neutrophil infiltration in the gastric mucosa

Abbreviations: TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; NF-κB, nuclear factor kappa B; IκB, inhibitor of κB; CHE, chelerythrine; NO, nitric oxide; MAPK, mitogen-activated protein kinase; CMD, cimetidine; ELISA, the enzyme immunosorbent assay; DAI, disease activity index; MPO, myeloperoxidase.

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is closely related to the genesis of lesions [11]. The neutrophil infiltration into the gastricmucosal tissues can be reflected by the determination of the activities of myeloperoxidase (MPO) and NO, both of which serve as key indicators of neutrophil infiltration in various experimental gastric injuries [12,13].

Chelerythrine (CHE) (Fig. 1), a quaternary benzo[c]phenanthridine alkaloid, is common in the Papaveraceae and Rutaceae families of plants. This alkaloid shows a broad range of biological activities, primarily antimicrobial and anti-inflammatory, arising from its ability to interact with proteins and DNA [14]. Mechanisms of the anti-inflammatory effect of CHE may include inhibition of 5-lipoxygenase [15], attenuation of the oxidative burst [16], and blocking the P2X7 receptor activity [17]. Besides, CHE affects various signaling pathways via the inhibition of protein kinase C and mitogen-activated protein kinase phosphatase-1 [18]. Our previous studies clearly suggested that CHE is a potent inhibitor of cyclooxygenase-2, which may be relevant to the inhibition of the release/production of exudates and prostaglandin E2 [19], and can inhibit the expression of pro-inflammatory cytokines in cultured cells and experimental models [20], implying that CHE may be developed to a potential strategy in treatment for gastric ulcers. Besides, our previous studies have also demonstrated that CHE exerted its anti-inflammatory effects by inhibiting proinflammatory cytokines (such as TNF-α) production and interfered with mitogen-activated protein kinase (MAPK) signaling pathways [20]. Since gastric ulcer is the inflammatory disease, we supposed that CHE can be used to treat gastric ulcers. Unfortunately, to date, there has been no information on whether CHE is therapeutic for gastric ulcers. Therefore, we hypothesized that CHE could exert its gastroprotective and ulcer healing actions effect on gastric ulcers by inhibiting proinflammatory cytokines production and the activation of NF-κB signaling pathway. Here, we investigated the effect of CHE on a murine model of ethanol-induced gastric ulcer in order to provide experimental evidence that CHE serves as a possible treatment for patients with gastric ulcers.

2. Materials and methods

2.1. Drugs

CHEs were purchased from Xi'an Honson Biotechnology Co., Ltd. (Shannxi, China) and identified by the Pharmacognosy Laboratory, School of Medicine, Xi'an Jiaotong University (Xi'an, China). As the positive control, Cimetidine (CMD) was supplied by Shanghai Xinyi Jiahua Pharmaceutical Company Limited (Shannxi, China). The enzyme immunosorbent assay (ELISA) kit for mouse TNF-α and IL-6 was purchased from R&D Systems (Minneapolis, MN, USA). The kits for biochemical analysis of MPO and nitrites were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). NF-κB p65 polyclonal antibody and anti-Histone (H)4 antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Other reagents were of commercially available analytical grade.

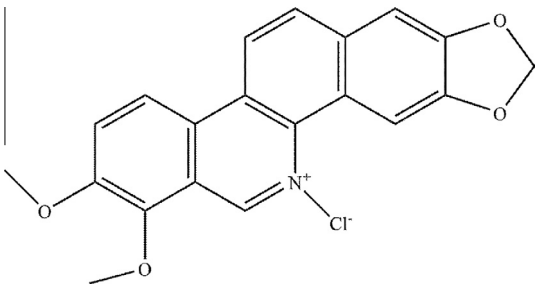

Fig. 1. Structure of CHE.

2.2. Animals

All experimental procedures utilizing mice were in accordance with National Institute of Health guidelines.

Male (25–30 g) Kunming mice were obtained from the Experimental Animal Center, Xi'an Jiaotong University (Xi'an, China). They were maintained in our animal house controlled at constant temperature of 23 ± 2 °C with a relative humidity of 55 ± 5% and a 12 h light/dark cycle. The animals were allowed free access to tap water and standard laboratory chow.

2.3. Ethanol-induced gastric mucosal lesions

This is a widely used model that seems to cause gastric ulcer, independently from the acid secretion. Acute gastric lesions were induced by intragastric administration of absolute ethanol in accordance with a previously described method [21]. Animals were randomized into six groups, each consisting of 12 animals. Groups 1 (normal control) and 2 (gastric ulcer control) received 0.9% saline at a dose of 50 ml/kg, groups 3, 4 and 5 were given CHE 1, 5, 10 mg/kg, respectively, and the last group obtained cimetidine (CMD, 100 mg/kg), an antagonist of H_2 receptors, was used as the reference drug. All drugs were administered once daily for 4 days. Drugs were given by gastric gavage, once daily and were suspended in saline. On the last day of treatment, 90 min after drugs administration, absolute ethanol (0.2 ml/animal) was administrated orally to all mice expect normal control group. 4 h after ethanol administration, the animals were anaesthetized with ether and blood was collected by retro orbital puncture for biochemical estimation. The animals were sacrificed by cervical dislocation, and the stomach was removed and opened along the greater curvature, and rinsed gently in PBS. The stomach was stretched on a piece of cork with the mucosal surface facing up and was examined in a standard position to assess the degree of gastric mucosal lesions. The hemorrhagic erosions in the stomach were photographed with a Lecia digital camera. The total and injured gastric lesions were measured using an image analyzer (Leica Micro systems Imaging Solutions Ltd, Cambridge, UK) and are expressed in terms of the percent (%) of the gastric area with lesions. After photo-graphing the gastric lesions, the stomach was stored at −70 °C for later biochemical analysis.

2.4. Determination of macroscopic gastric damage

Immediately after the animals were killed, their stomachs were removed, cut along the greater curvature, rinsed with ice-cold isotonic saline, and the mucosal lesions were examined macroscopically. Macroscopic scoring of tissue samples was performed by an observer unaware of the treatment groups. The degree of gastric mucosal damage was evaluated from digital pictures, and rated for gross pathology according to the ulcer score scale described by Schiantarelli et al. [22] using the following scale: 0 = normal mucosa; 1 = hyperemic mucosa or up to 3 small patches; 2 = from 4 to 10 small patches; 3 = more than 10 small or up to 3 medium-sized patches; 4 = from 4 to 6 medium-sized patches; 5 = more than 6 medium-sized or up to 3 large patches; 6 = from 4 to 6 large patches; 7 = from 7 to 10 large patches; 8 = more than 10 large patches or extensive necrotic zones. "Small" was defined as up to 2 mm across (max. diameter), "medium-sized" as between 2 and 4 mm across and "large" as more than 4 mm across.