Preventive Effects of Chinese Herb Chai-Hu-Gui-Zhi-Tang Extract on Water Immersion Restraint Stress-Induced Acute Gastric Ulceration in Rats

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ABSTRACT. It is well known that maintenance therapy using Chai-hu-gui-zhi-tang (CHGZT), a traditional Chinese medicine, has been proven to prevent the recurrence of peptic ulcers. However, little is known as to whether or not it has protective effects against acute gastric injury. In the present study, we investigated the preventive effects of pretreatment with CHGZT extract on the development of water immersion restraint stress-induced acute gastric ulceration in male Wistar rats. The CHGZT extract (50, 250, 500 mg/kg b.w., p.o.) was given to rats before they were exposed to 2 or 4 hr of water immersion restraint stress; they were then were sacrificed immediately after stress exposure. Gastric mucosal lesions were evaluated macroscopically, and the gastric mucosal and hepatic non-protein sulphydryls (NP-SH) were measured simultaneously. The results indicate that exposure to water immersion restraint stress resulted in the development of acute gastric stress erosions. Pretreatment with CHGZT extract caused a significant reduction of stress lesions and an increase in the gastric mucosal NP-SH and hepatic NP-SH concentrations. We conclude that the anti-ulcer response and extensive antioxidant effect of Chai-hu-gui-zhi-tang may be valuable in prevention of experimental gastric mucosal lesions in rats because it possesses preventive and gastroprotective effects.

KEY WORDS: antioxidant effect, Chai-hu-gui-zhi-tang (CHGZT), gastric ulcer, non-protein sulphydryls, water immersion restraint stress.


Chai-hu-gui-zhi-tang (CHGZT), a traditional Chinese herbal prescription, is a mixture of nine herbs, Bupleuri radix, Scutellariae radix, Ginseng radix, Cinnamomi ramulus, Paeoniae radix, Zingiberis rhizome, Pinelliae tuber, Glycyrrhizae radix and Zizyphi fructus, has shown a good antioxidant effect and has been used to prevent the formation of nervous lesions, such as epilepsy, Alzheimer’s disease and developmental defects of neurons during pregnancy and after birth [12, 25]. In traditional Chinese medicine, CHGZT has been used in therapies for gastric ulcers and gastritis, but the underlying mechanisms are not well understood. Among the herbal drugs contained in GHGZT, Scutellariae radix [6] and Panax ginseng [11] have been used extensively, and their clinical efficacies have been documented in regard to anti-ulcer activity. The flavonoids of Scutellariae radix have been found to be free radical scavengers [3]; free radicals play an important role in ulcerative and erosive lesions of the gastrointestinal tract. Glycyrrhizae radix water extract and its two major constituents, glycyrrhizin and 3-glycyrrhetinic monodesmoside, may be promising for amelioration of hypoxia (ischemia)-reoxygenation (reperfusion) injury and improvement of renal function by acting as antioxidant and oxygen radical-scavenging agents [32].

It is well known that stress plays a major role as a risk factor in the occurrence of stomach erosion and ulcers [22].

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Water immersion restraint stress (WIRS) mimics the clinical acute gastric ulcerations caused by trauma, surgery or sepsis [8] and has been widely accepted for studying stress ulcers [5, 26, 27]. Involvement of central nervous stem compounds such as the limbic system, hypothalamus and brain stem nuclei has been considered [9]. Mucosal ischemia and enhanced back diffusion of hydrogen ions have been proposed as major local mechanisms for these stress-induced injuries [29]. Gastric acid secretion is remarkably enhanced under water immersion stress without significant alteration in gastric mucosal blood flow [10]. Ohta et al. have shown the therapeutic effect of Oren-gedoku-to extract on stress-induced acute gastric mucosal lesions in rats and indicated preventive actions on lipid peroxidation and sulphhydryl oxidation via oxygen free radicals generated by the xanthine-XO system and infiltrated neutrophils in the gastric mucosa and on neutrophil infiltration into the tissue [17]. Yoshikawa et al. indicated that Saiko-keishi-to (Chai-hu-gui-zhi-tang) extract not only had an ability to scavenge oxygen free radicals, but also a protective effect against ischemia-reperfusion-induced gastric mucosal lesions mediated by oxygen free radicals and lipid peroxidation in rats [34].

The present study was performed to investigate the preventive effects of CHGZT against acute gastric mucosal lesion development as a result of water immersion restraint stress in rats. The effect of pretreatment of CHGZT extract on the progression of gastric mucosal lesions in rats subjected to stress exposures for 2 and 4 hr periods and the gastric mucosal lesions were evaluated macroscopically (ulcer
index). The gastric mucosal and hepatic non-protein sulphydryls (NP-SH), which are a major source of cellular reducing substances, were measured for the purpose of evaluating the preventive and gastroprotective effects of gastric mucosal injury.

MATERIALS AND METHODS

Materials: Chai-hu-gui-zhi-tang (CHGZT) was provided by Kaiser Pharmaceutical Co., Ltd. (Taipei, Taiwan) and was prepared from boiled water extracts of the following herbs: 1.5 g of Bupleuri radix, 0.6 g of Scutellariae radix, 0.6 g of Ginseng radix, 0.6 g of Cinnamomi ramulus, 0.6 g of Paeoniae radix, 0.6 g of Zingiberis rhizome, 0.9 g of Pinelliae tuber, 0.4 g of Glycyrrhizae radix and 0.4 g of Zizyphi fructus. The total weight was 6.2 g. All chemicals were ACS reagent grade. Butylated hydroxytoluene (BHT), 5,5'-dithiobis (2-nitrobenzoic acid; DTNB), ethylenediamine-tetracetic acid disodium (EDTA-Na2) and trichloroacetic acid were purchased from Sigma (St. Louis, MO, U.S.A.). Methanol and other solvents were analytical grade and supplied by J.T. Baker (Phillipsburg, NJ, U.S.A.). Deionized water was obtained from a Milli-Q Plus analytical deionization system (Bedford, MA, U.S.A.).

Induction of gastric ulceration: Male Wistar rats weighing 260–280 g were purchased from the Laboratory Animal Center, College of Medicine, National Taiwan University (Taipei, Taiwan). All experimental procedures were approved by the Institutional Animal Ethics Committee of National Taiwan University and conducted in accordance with the internationally accepted principles for laboratory animal used and care. Rats were starved for 18 hr but were allowed free access to water before the induction of WIRS or sampling of their gastric mucosa. Each rat was subjected to WIRS as described previously by Takagi and Okabe [26]. The rats were restrained in firmly fitted restraint cages (6 × 7 × 20 cm) that were immersed vertically in a water bath kept at 23°C to the level of the xiphoid process for 2 or 4 hr to induce gastric mucosal lesions (Fig. 1). The histological examination showed that all of the abnormal gastric tissues were fixed in 10% formaldehyde, routinely dehydrated, cleaned, infiltrated with wax, embedded and made into serial 4-μm thick sections. The sections were dewaxed and stained using the hematoxylin and eosin technique.

Biochemical assays: Samples used for assays of gastric mucosa and hepatic non-protein sulphydryls (NP-SH) were prepared with Ellman’s reagent [19] using 5,5'-dithiobis (2-nitrobenzoic acid) as described previously by Sedlak and Lindsay. The gastric mucosa were collected with a glass slide on an ice bath, and the liver was quickly removed from the rat after sacrifice. Two hundreds milligrams of gastric mucosa or liver tissue was immediately homogenized with 2 ml 0.02 M EDTA-Na2 containing 0.002% BHT in a Teflon-glass homogenizer on an ice bath.

Statistical evaluation: All results were presented as the mean ± SD. The paired data were compared by Student’s t-test, and the significant level was set at P<0.05.

RESULTS

Assessment of stress-induced gastric mucosal damage: As the period of stress was increased from 2 to 4 hr, the values of the ulcer index and severity of the ulcers increased as shown in Fig. 2. No macroscopic lesions were observed in untreated rats. Afterwards, extensive stress-induced superficial erosion of varying lengths and congestion of the surrounding portion were observed in the gland area of the stomach. When the appearances of the gastric ulcers in the glandular area were examined, blood coagulums could be seen at the bases of the ulcers.

The histological examination showed that all of the lesions were erosion of mucosa and that they extended down to submucosa with neutrophils infiltration. Obvious blood stasis was observed in the stressed rats (Fig. 3).
Changes in ulcer index in stress-induced gastric mucosal damage by CHGZT pretreatment: When rats were subjected to WIRS for 2 and 4 hr, the values of the ulcer index increased dramatically after stress as compared with the untreated rats (Fig. 4). The value of the ulcer index with 4 hr of stress was 1.43 ± 0.21 cm, and this value was 2.2-fold larger than that with 2 hr of stress. When rats were pretreated with 50, 250 and 500 mg/kg b.w., p.o. of CHGZT before the onset of WIRS, administration of each dose of the medicine significantly prevented the progression of gastric mucosal lesions. The values of the ulcer index decreased markedly in the stress-loaded rats (P<0.01).

Changes in non-protein sulfhydryls in stress-induced gastric mucosal damage by CHGZT pretreatment: The effects of CHGZT pretreatment followed by the onset of WIRS (2 and 4 hr) and changes in the gastric mucosal and hepatic NP-SH concentrations in the rats are shown in Fig. 5. The gastric mucosal NP-SH concentrations in the rats with 2 and 4 hr of WIRS were significantly lower than that in the control rats without WIRS. The decreased concentrations of gastric mucosal NP-SH with WIRS after 2 and 4 hr were recovered by pretreatment with CHGZT. When administered at a dose of 50, 250 or 500 mg/kg b.w., p.o, the average recovered gastric mucosal NP-SH concentrations were 1.2-, 1.5- and 1.5-fold, respectively, higher than the concentration with 2 hr of stress. Similarly, the data showed that the recovered gastric mucosal NP-SH concentrations were 1.2-, 1.8- and 1.5-fold, respectively, higher than the concentration with 4 hr of stress. As the hepatic NP-SH concentrations of the rats subjected to WIRS for 2 and 4 hr were slightly decreased compared with those of the control rats without the stress, the decreased concentrations of hepatic NP-SH with WIRS were also recovered pretreatment with CHGZT. This pre-administration of CHGZT prevented not only gastric mucosal lesion development in the rats subjected to stress, but also increased the gastric mucosal and hepatic NP-SH concentrations in the rats subjected to 2 and 4 hr of stress.

DISCUSSION

The Chinese formula Chai-hu-gui-zhi-tang (CHGZT) has...
been used to treat such things as influenza, pleurisy, stomach ache, gastritis (hyperacidity), stomach ulcer, duodenal ulcer, epilepsy [12, 25], hepatitis [21], pancreatitis [24], jaundice, pain between the ribs, and pain of sciatica. The results from animal models indicated that CHGZT extract has antioxidant effects and scavenging activity for free radicals generated within iron-induced epileptogenic regions of the rat’s brain [12]. CHGZT can inhibit pepsin and stomach acid secretion, enhance the secretion of stomach mucous, promote blood circulation in the stomach and improve the protection of the stomach [7]. Recently, Yasukawa et al. reported that reactive oxygen species (ROS) are associated with gastric ulcer [31]. Uteshev et al. indicated that a stronger intensity of lipid peroxidation and less activity of the antioxidant system of the blood are correlated with gastroduodenal ulcerous hemorrhages [28]. Previous studies in experimental gastric ulcers show that the elimination of free radicals by an anti-ulcer agent that has antioxidant and free-radical scavenging activities may contribute to reduce the severity of ulcer recurrence [15, 18].

The present study provided evidence that orally administered CHGZT extract at a dose of 50, 250 or 500 mg/kg b.w., p.o., can prevent the progression of acute gastric mucosal lesions in rats subjected to WIRS for 2 and 4 hr periods. According to a report by Nishida et al., the progression of WIRS induced acute gastric mucosal lesions in rats is mainly related to enhanced gastric mucosal sulfhydryl oxidation and lipid peroxidation [16]. In the present study, the decreased gastric mucosal non-protein sulfhydryl concentrations found at the second and fourth hours after WIRS were significantly recovered by oral administration of CHGZT extract (250 or 500 mg/kg b.w., p.o.), and the recovered concentrations were higher than the concentrations found with 2 and 4 hr of stress. It has previously been suggested sulfhydryls in the stomach are important for maintenance of gastric mucosal integrity [14].

Many studies have suggested that oxygen free radicals act as mediators of gastric mucosal injury. The mucosal availability of the antioxidant reduced glutathione (GSH, γ-glutamyl-cysteinyl glycine), which is an important protective factor against the development of gastric mucosal ischemia/reperfusion injury [23]. Body et al. reported on gastric glutathione depletion and acute ulcerogenesis after subcutaneous administration of diethyl maleate to rats [4]. In regard to oxidation of reduced glutathione and formation of mixed disulfides between protein and non-protein sulfhydryls, it is well known that non-protein sulfhydryls (NP-SH) are a major source of cellular reducing substances. Recently, Nagy et al. indicated that endogenous sulfhydryls (SH) play an important role in the maintenance of gastroduodenal integrity and in protection against chemically-induced lesions in cells, tissues and organs [14]. Further-
more, Ohta et al. suggested that the therapeutic effect of Oren-gedoku-to extract (Huanglian-Jiedu-Tang), a traditional Chinese herbal medicine, could be due to the preventive actions on lipid peroxidation and sulfhydryl oxidation via oxygen free radicals generated by the xanthine-XO system [17]. An antioxidant defense mechanism may therefore be of critical importance in protecting against the development of acute gastric mucosal injury.

Fig 5. Effect of pretreatment with Chai-hu-gui-zhi-tang extract on the changes in the gastric mucosal NP-SH (a) and hepatic NP-SH (b) concentrations in rats with water immersion restraint stress over a 2- or 4-hr period. Rats received oral administration of CHGZT (50, 250, 500 mg/kg) prior to water immersion restraint induction. Rats without CHGZT pretreatment received oral administration of an equal volume of deionized water. These rats were subjected to water immersion restraint stress for 2 or 4 hr. Each value represents the mean ± SD for 10 animals. Significant differences were calculated by the Student's t-test. * and **: P<0.05 and P<0.01 versus the controls, respectively. * and **: P<0.05 and P<0.01 versus the control stressed rats with WIRS treatment, respectively.
It was widely accepted that a major portion of phospholipid peroxidation occurs due to the generation of oxygen-derived free radicals. Previous investigations demonstrated the prevention of oxidative damage of the gastric mucosa by a significant blockage of lipid peroxidation and by scavenging the endogenous hydroxy radical \( (\cdot OH) \), which is the major causal factor for the formation of an ulcer [2]. However, it is also known that \( \cdot OH \)-mediated oxidation causes damage to the human gastric mucosal DNA. Yoshikawa et al. have previously reported that the gastric mucosal blood flow decreases even in the early phases of WIRS [33]. Shian et al. pointed out the probable role of lipid peroxidation in the pathogenesis of gastric injury induced by WIRS [20].

As described above, the herbal remedies contained in GHGZT were shown to have anti-ulcer and anti-inflammatory responses. Additionally, the constituents of \textit{Paenoniae radix} exhibited a significant oxygen radical scavenging activity and had an inhibitory effect on lipid peroxidation [13]. Ginger is known to stimulate digestion beneficially and has anti-ulcer effects [30]. The cytoprotective and anti-ulcerogenic effects of ginger have been shown to prevent the occurrence of gastric ulcers induced by non-steroidal anti-inflammatory drugs and hypothermic restraint stress [1].

The results in the present study indicated that orally administered CHGZT exerts a preventive effect on water immersion restraint stress-induced acute gastric ulceration in rats. Pretreatment with CHGZT markedly reduced gastric mucosal lesions in the stress-loaded rats, and the decreased gastric mucosal and hepatic NP-SH concentrations after the onset of WIRS were recovered. The endogenous sulfhydryls play an important role in the maintenance of gastroduodenal integrity; the relatively high concentration of non-protein sulfhydryls in the gastric mucosa also indicates their possible implications for gastroprotection. Since an antioxidant defense mechanism may be critically important in protecting against the development of acute gastric mucosal injury, the anti-ulcer response and extensive antioxidant effect of CHGZT may be valuable in prevention of experimental gastric mucosal lesions in rats because they exert preventive and gastroprotective effects in rats.

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