Pharmacological Properties of Traditional Medicine (XXXII)¹: Protective Effects of Hangeshashinto and the Combinations of Its Major Constituents on Gastric Lesions in Rats

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The protective effect of Hangeshashinto (HST) and its major constituents, baicalin (BA), berberine (BE), saponin fraction of ginseng (GS) and glycyrrhizin (GL) on rat gastric lesion induced by ethanol was examined to clarify its active ingredients and action mechanism. Oral treatment with HST at the doses of 125 and 250 mg/kg suppressed ethanol-induced gastric lesions. The mixture of BA, BE, GL and GS (4M), each of BE, GL and GS at the dosage corresponded to HST (125 mg/kg) also suppressed the ethanol-induced gastric lesion in rats, but BA did not. Treatment of ethanol augmented the activity of myeloperoxidase (MPO) in the stomach, which was significantly suppressed by the administration of HST, BE, GL and GS. These results suggest that the protective effect of HST on ethanol-induced gastric lesion was depended on BE, GL and GS, by, in part, the reduction of MPO activity in stomach.

Key words Hangeshashinto; ethanol-induced gastric lesion; berberine; baicalin; glycyrrhizin; ginsenoside

Hangeshashinto (HST), one of the Kampo formula in Japanese traditional herbal medicine, is the mixture of seven herbs including pinellia tuber, scutellaria root, glycyrrhiza, jujube, ginseng, processed ginger, and coptis rhizome, which are registered in Japanese Pharmacopoeia XV. According to the Golden Cabinet (Jin gui yao lue), this formula is recommended for the patients with vomiting, borborygmus, and focal distention of the epigastrum. The later physicians have used it to regulate stomach, to purge stagnation and flatulence in traditional Chinese medicine. In modern medicine, HST has been used for acute or chronic gastrointestinal catarrh fermentative diarrhea and acute gastroenteritis, and clinically evaluated as anti-ulcer drugs. Ethanol-induced gastric lesions are associated with peptic ulcer disease. Protection of the gastric mucosal cell and to relieve stagnation and flatulence in the gastrointestinal system. It is reported that HST is effective against this type of gastric lesions.

Materials and Methods

Materials Pinellia tuber (半夏, tuber of Pinellia ternata BREITENBACH, Araceae), scutellaria root (黄芩, root of Scutellaria baicalensis GEORG, Labiatae), ginseng (人参, root of Panax ginseng C.A. MEYER, Araliaceae), jujube (大枣, fruit of Zizyphus jujuba var. inermis REHDER, Rhamnaceae), glycyrrhiza (甘草, root or stolon of Glycyrrhiza uralensis FISHER, Leguminosae), coptis rhizome (黄连, rhizome of Coptis japonica MAKINO, Ranunculaceae), processed ginger (乾姜, steamed rhizome of Zingiber officinale ROSCOE, Zingiberaceae), which are standardized by Japanese Pharmacopoeia XV, respectively, and were purchased from Tochimoto-tenkai-do (Osaka). Baicalin (BA), berberine (BE), glycyrrhizin (GL), ginsenoside Rg1, were supplied by Wako Pure Chemical Industries, Ltd. (Osaka). The saponin fraction of ginseng (GS), containing 4.64% of ginsenoside Rg1, were prepared by the methods of our previous report. Cetraxate (CET) were bought from Daiichi Pharmaceutical Co., Ltd. (Neuer, Tokyo, Japan).

Animals Male Wistar/ST rats (6 week-old) were purchased from Japan SLC Co., Ltd. (Hamamatsu). They were housed in small groups (n=4–5) in the breeding room and fed a commercial diet (MF, Oriental Yeast Co., Tokyo) and allowed tap water ad libitum. Housing conditions were thermostatically maintained at 24±1°C with the humidity of 50±1% under 12 h dark–light cycle. All procedures involving the rats were performed using protocol approved by our Institutional Animals Care and Use Committee.

Preparation of HST Seven crude herbal drugs (human daily dose: pinellia tuber 5.0 g, scutellaria root 2.5 g, ginseng 2.5 g, jujube 2.5 g, glycyrrhiza 2.5 g, coptis rhizome 1.0 g and processed ginger 2.5 g) were decocted with a 20-fold amount of distilled water until the filtered decoction was reduced by half. The decoction was lyophilized and the obtained powder

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(HST, 25.2 w/w% of the crude drugs) was stored in a refrigerator. The contents of maker compounds in HST used in the present study were quantitated by the methods of our previous report, and it contained 3.49 (w/w)% of BA, 0.378% of BE, 0.938% of GL, and 0.073% of ginsenoside Rg1.

**Ethanol-Induced Gastric Lesions** The experiment was carried out according to the method of Robert et al. Rats, fasted for 24 h, received an oral dose of HST, its constituents (each of BA, BE, GL and GS and their mixture, 4M). All these samples were suspended in 0.5% tragacanth gum solution to prepare a dosage of 10 ml/kg b.w. Thirty minutes later, 1 ml of 99.5% ethanol was orally administered to the rats. One hour after the ethanol administration, the animals were sacrificed under over-anesthesia with ether. The injury observed in the stomach was photographed with a digital camera and its area was measured using computer software, Scion Image (Frederick, MD, U.S.A.).

**Measurement of Myeloperoxidase (MPO)** Myeloperoxidase activity was determined according to the method of Krawise et al. Briefly, the stomach was incised along the greater curvature and washed gently with saline. The mucosa surface of the stomach was collected by scraping and homogenized in 1 ml of saline. The stomach without mucosa surface was also homogenized in 2 ml of saline. Each homogenate (0.1 ml) was mixed with 0.1 ml of 1% hexadecyltrimethyl ammonium bromide in 50 mM potassium phosphate buffer (pH 6.0). After freeze-thawing the homogenate for three times, they were centrifuged at 12500 g for 5 min. The supernatant (20 μl) was combined with 20 μl of 5.26 mM O-dianisidine hydrochloride (Wako), 20 μl of 1.5% hydrogen peroxide and 140 μl of 50 mM potassium phosphate buffer (pH 6.0). Change of absorbance at 450 nm was recorded, and MPO-activity was calculated by the slope of absorbance calibrated by standard MPO (Wako). The concentration of total protein was measured by BCA Protein Assay Kit (Pierce, Rockford, IL, U.S.A.), and the data of MPO activity were expressed as unit/protein mg.

**Statistical Analysis** All data are presented as means±S.E. The data were analyzed with one-way analysis of variance (ANOVA) followed by the Scheffe’s test. Differences with p<0.05 were considered as significant.

**RESULTS**

**Effects of HST on Ethanol-Induced Gastric Lesions in Rats** Oral treatment of ethanol to rats raised gastric lesion. Oral administration of HST significantly (p<0.01) suppressed this gastric lesion in a dose-dependent manner (55.9 and 38.0% of control at the doses of 125 and 250 mg/kg, respectively, Fig. 1). Oral treatment of CET at 100 mg/kg markedly suppressed these lesions (27.7% of control, Fig. 1).

**Effects of the Constituents of HST on Ethanol-Induced Gastric Lesions in Rats** HST (125 mg/kg) significantly suppressed the ethanol-induced gastric lesion in rats (41.7% of control, Fig. 2). Among the constituents of HST, BE, GL, GS and the mixture of 4 components (4M) at the dosage corresponded to 125 mg/kg of HST significantly suppressed the gastric lesion at the same level of original HST (33.9, 22.3, 36.5 and 53.1% of control, respectively), but BA did not (Fig. 2).

**Effects of HST and Its Constituents on MPO Activity** Myeloperoxidase (MPO) activity in (A) gastric mucosa, (B) stomach without mucosa and (C) whole stomach of ethanol-treated rats were shown. Data represent mean±S.E. (n=9—13). ∗p<0.01 vs. normal, ∗∗p<0.01 vs. control. Abbreviations and the dosages are as same as Fig. 2.

**in Stomach** The MPO activity in gastric mucosa, stomach without mucosa and whole stomach increased by 220, 186 and 245% after the administration of ethanol, respectively (Figs. 3A—C). The increase of MPO activity in gastric mucosa significantly suppressed by the administration of BE and GL (46.9 and 40.0% of control, respectively, Fig. 3A). The increase of MPO activity in stomach without mucosa...
significantly decreased by the treatment of GL (28.6% of control, Fig. 3B), and that in whole stomach significantly suppressed by the administration of HST, BE, GL and GS (48.9, 36.6, 29.9 and 45.1% of control, respectively, Fig. 3C).

DISCUSSION

In the present study, we experimented on the effect of HST and the combinations of its major constituents in ethanol-induced gastric lesion in rats to clarify its active ingredients and action mechanisms.

HST suppressed ethanol-induced gastric lesions of rats, that agree with the report of Kase et al.18) In order to reveal the active ingredients and their contribution to the effect of HST, we examined the effects of its 4 major constituents (BA, BE, GS and GL) or their mixture (4M) at the dosage corresponded to the dose of HST 125 mg/kg. The 4M significantly suppressed the ethanol-induced gastric lesion with the same levels of the original HST, suggesting that the protective effect of HST would be achieved by these 4 components. When these components were administered singly to the rats, BE, GL and GS suppressed the ethanol-induced gastric lesion, but BA did not. The effect of BE, GL and GS did not have significant difference from 4M or HST, suggesting that the protective effect of HST seems to be depended on BE, GL and GS. The effect of each BE, GL and GS was as the same level as that of 4M, that indicates that they would not have additive or synergistic effects.

In previous studies, it is reported that BE could significantly protect gastric mucosa damaged with ethanol by the induction of eNOS mRNA expression and the inhibition of iNOS mRNA expression in stomach.19) It is also reported that ginsenoside Rb1 has anti-ulcer effect through the induction of mucus secretion.20) These effects of BE and ginsenosides may contribute the anti-ulcerous effect of HST. The activity of MPO, a marker enzyme of leukocytes, was thought to represent leukocyte migration to the injured tissue.21—23) Therefore, we measured MPO activity in gastric mucosa, stomach without mucosa and whole stomach, in order to estimate the number of leukocyte. We found a significant increase in MPO activity in gastric mucosa, stomach without mucosa and whole stomach after ethanol-treatment. In whole stomach, HST, BE, GL and GS significantly inhibited MPO activity, though 4M did not. These finding indicates that BA might extinguish the effect of each BE, GL and GS, but that other constituents than 4M finally contribute to the suppressive effect of HST on MPO activity in the stomach. The protective effect of the each constituent in HST on ethanol-induced gastric lesion was not parallel to the effect on MPO activity in stomach, suggesting that HST suppressed gastric lesion by other unknown mechanisms as well as anti-oxidative effect. The combination of 7 herbs would be very important to show this effect of HST.

In conclusion, HST suppressed the gastric lesions induced with ethanol in rats. Protective effect of HST on ethanol-induced gastric lesion was depended on BE, GL and GS, by, in part, the reduction of MPO activity in stomach.

REFERENCES AND NOTES