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Effects of Traditional Herbal Medicine on Gastric Mucin against Ethanol-Induced Gastric Injury in Rats

Y. Goso,[1] Y. Ogata,[2] K. Ishihara[1] and K. Hotta[1]

[1]Departments of Biochemistry and [2]Internal Medicine,
Kitasato University School of Medicine, Sagamihara 228, Japan

ABSTRACT. The effect of the traditional herbal medicine, Rikkunshi-to and its component crude drugs, Zingiberis Rhizoma and Glycyrrhizae Radix, on the gastric mucin was studied using a method developed to separate and quantify the mucin localized in the different layers of rat gastric mucosa. The oral administration of spray-dried extract to Rikkunshi-to (1000 mg/kg), Zingiberis Rhizoma (500 mg/kg) and Glycyrrhizae Radix (500 mg/kg) significantly prevented gastric mucosal damage induced by 70% ethanol in rats. In ethanol-treated rats the mucin content of the deep mucosa was reduced, and the reduction of the deep corpus mucin content was significantly inhibited by pretreatment of Rikkunshi-to and Zingiberis Rhizoma. Rikkunshi-to and Glycyrrhizae Radix pretreatment increased the surface mucin content by 140 and 146%, respectively. The effect on the gastric mucin by each drug differed in the different layers of the gastric mucosa. COMP BIOCHEM PHYSIOL 113C, 17–21, 1996.

KEY WORDS. Deep mucin, ethanol injury, gastric mucin, glycyrrhizae radix, rikkunshi-to, surface mucin, traditional herbal medicine, zingiberis rhizoma

INTRODUCTION

Traditional herbal medicines (traditional Chinese medicines) have been widely used as important drugs in Japan and China (20). Rikkunshi-to, which is composed of eight herbal components, is known to have preventive effects against gastric diseases (21). The antiulcer and cytoprotective properties of Rikkunshi-to on experimental models have been studied (3). However, little is known about which is the most effective drug component of Rikkunshi-to for antiulcer properties and how the gastric mucus, an important factor in the gastric defense mechanism, participates by this drug administration.

In the present study, we compared the effect of each drug composing Rikkunshi-to against ethanol-induced gastric mucosal damage. Among the eight components, Zingiberis Rhizoma and Glycyrrhizae Radix were selected because of their intense antiulcer effects. The effect of Rikkunshi-to and the two drugs on the distributional changes of gastric mucin content in the ethanol-induced gastric damage was determined using our novel method developed to separate and quantify the mucin localized in the different layers of rat gastric mucosa (11).

Address reprint requests to: Kyoto Hotta, Department of Biochemistry, Kitasato University, School of Medicine, Sagamihara 228, Japan.
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MATERIALS AND METHODS

Animals

Seven-week-old male Wistar rats (SLC, Shizuoka, Japan), each weighing approximately 160 g, were used. All were fasted for 24 hr before the experiments and had free access to water during this time. Each study was carried out using 5–13 rats per group. During the study estimating mucin contents, 18–24 rats per group were used.

Drug Administration

Rikkunshi-to and its herbal components, Zingiberis Rhizoma, Glycyrrhizae Radix, Pineliae Tuber, Zizyphi Fructus, Hoelen, Aurantii Nobilis Pericarpium, Atracylodis Lanceae Rhizoma and Ginseng Radix, in the form of a splay-dried powder of the extract were supplied from Tsumura Co. (Tokyo, Japan). Each drug powder was suspended in 0.5% carboxymethylcellulose at a concentration of 100 mg/ml and orally administered to the animals at a dose of 10 ml/kg for Rikkunshi-to or 5 ml/kg for the herbal component. For dose-dependent experiment, drug suspension of 100 ng/ml was diluted with 0.5% carboxymethylcellulose to an appropriate concentration, and the rats were orally given the drug at 5 ml/kg body weight.

Study of Ethanol-Induced Gastric Injury

Each animal was orally given 70% ethanol (5 ml/kg) and killed 1 hr later. Rikkunshi-to or the component was orally

TABLE 1. Protective effect of Rikkunshi-to against ethanol-induced gastric mucosal damage.

Group	Ulcer Index
70% EtOH treated group	4.10
Rikkunshi-to pretreated group	1.67*

Rikkunshi-to (1000 mg/kg) was given orally 30 min before 70% ethanol administration.

Severity of gastric mucosal damage was graded as follows: 0 = no lesion; 1 = hemorrhagic erosions (less than 5); 2 = hemorrhagic erosions (more than 5) or one small linear ulcer (shorter than 2 mm); 3 = many small linear ulcers or single linear ulcer of marked size (longer than 2 mm); 4 = multiple marked ulcers (less than 5); 5 = multiple marked ulcers (more than 5).

Significant difference was achieved between the two groups with or without Rikkunshi-to pretreatment (*$p < 0.01$); $n = 24$.

given 30 min before the administration of ethanol. The animals were killed under CO_2, and then their stomachs were removed, inflated by injecting 5 ml of 10% formalin, and allowed to stand for 15 min to fix the inner surface of the gastric tissue. After opening the stomach along the greater curvature, the length (cm) of the visible necrotic lesions in the glandular area was measured macroscopically, and the lesion index was scored by adding the lengths of the lesions in the stomach. In case of Rikkunshi-to, the mucosal lesions were macroscopically observed and scored on a 0–5 scale, as shown in Table 1.

Preparation of Stomach Specimens

Immediately after the sacrifice of the rats, the stomach was excised and cut along the greater curvature. The gastric content was gently rinsed with phosphate-buffered saline. After macroscopical observation, the surface mucosa along with the mucus gel attached to the mucosal surface was collected by scraping with a soft silicone rubber spatula as previously described (11). The remaining deep mucosa of the stomach was subsequently separated into the corpus and antrum regions. Specimens from each of three animals were pooled and lyophilized.

Separation and Quantification of Mucin

The extraction of mucin present in the powder of each layer of the gastric mucosa was performed according to a previously described method (1,15) The powdered samples were dissolved in 50 mM Tris-HCl, pH 7.2, containing 2% Triton X-100 and homogenized using a Polytron homogeneizer. After centrifugation at 10,000 rpm at 4°C for 30 min, the supernatant was collected. An aliquot of the supernatant was applied onto a Bio-Gel A-1.5m column and eluted with the 2% Triton-Tris buffer. The void volume fraction (Fr-1) monitored by hexose measurement was collected as mucin. The hexose content in this fraction was measured using the phenol-sulfuric acid method (2) with galactose as the standard. The mucin content (Fr-1 hexose value) was expressed as mg hexose per stomach of one animal.

Statistical Analysis

The total lesion length (cm) was expressed as the scored lesion index. The results of the ulcer index were expressed as the mean ± SD. The statistical evaluation for multiple group comparison of the mucin content of each experimenal group was conducted using a one-way analysis of variance (ANOVA) with Dunnett's test. The Mann-Whitney U test was used for damage assessment data of ulcer grading. A difference of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of Rikkunshi-to Pretreatment on Ethanol-Induced Gastric Damage and Changes in Mucin Content

Macroscopic observation of hemorrhagic lesions was made one hour following the treatment with 70% ethanol with or without Rikkunshi-to pretreatment. Administration of Rikkunshi-to prior to ethanol ingestion strongly inhibited the hemorrhagic lesions ($p < 0.01$ vs. control) as shown in Table 1.

Table 2 shows the effects of Rikkunshi-to administration on the gastric mucin content expressed as the change in hexose content. The deep corpus mucin content was significantly increased by the drug administration. A remarkable decrease in the deep corpus and antral mucin content was noted after ethanol treatment. On the other hand, Rikkunshi-to pretreatment significantly inhibited ethanol-induced mucin reduction in the deep corpus mucosa, and increased the surface mucin content to 140%.

Effect of Herbal Component Pretreatment on Ethanol-induced Gastric Injury

Table 3 shows the antiulcer effect of the herbal component pretreatment scored as the total lesion length. Pretreatment with Zingiberis Rhizoma, Glycyrrhizae Radix, Pineliae Tuber, Atracylodis Lanceae Rhizoma or Hoelen significantly ($p < 0.01$) prevented any gastric damage. The degree of prevention was different among the five drugs, and the strongest one was Zingiberis Rhizoma.

Effect of Zingiberis Rhizoma and Glycyrrhizae Radix Pretreatment on Ethanol-Induced Gastric Damage and Changes in Mucin Content

Development of the lesions with Zingiberis Rhizoma and Glycyrrhizae Radix was achieved using a dose from 0.5 to 500 mg/kg as shown in Fig. 1. The ulcer length was 5.95 ± 3.12, 2.74 ± 1.35, 0.98 ± 0.78 and 0.08 ± 0.17 cm with Zingiberis Rhizoma pretreatment at dose of 0.5, 5, 50 and 500 mg/kg, respectively. Significant differences from the control were achieved in a dose-dependent manner from 5, 50 and 500 mg/kg. The total mucin content was decreased to 72% with 70% ethanol treatment. With Zingiberis Rhizoma pretreatment the total mucin content recovered 83, 90, and 99% of the control at dose of 5, 50 and 500 mg/kg, respectively, but not at dose

TABLE 2. Effect of Rikkunshi-to on gastric mucin content in ethanol-induced injury.

	Hexose Value (% of control)		
	Corpus	Antrum	Surface
Vehicle	536.6 ± 31.7 (100%)	113.7 ± 13.8 (100%)	312.4 ± 61.7 (100%)
Rikkunshi-to	613.2 ± 36.4 (114%)†	117.9 ± 36.6 (104%)	341.2 ± 98.7 (109%)
70% EtOH	318.9 ± 55.0 (59%)†*	39.3 ± 14.1 (35%)†	347.0 ± 56.7 (111%) *
Rikkunshi-to + EtOH	406.6 ± 43.2 (76%)†	55.5 ± 17.2 (49%)†	436.8 ± 112.0 (140%)†

1000 mg/kg Rikkunshi-to was given orally.
Hexose value corresponding to mucin content is in micrograms of Fr-1 hexose per stomach and expressed as means ± SD (n = 8).
*$p < 0.05$; †$p < 0.01$, as compared with control or 70% ethanol.

TABLE 3. Protective effect of herbal components against ethanol-induced gastric mucosal damage.

	Ulcer Length (cm)
70% EtOH	7.19 ± 2.37
Hoelen	1.31 ± 1.62*
Aurantii Nobilis Pericarpium	6.14 ± 2.63
Pineliae Tuber	0.75 ± 0.72*
Glycyrrhizae Radix	0.22 ± 0.24*
Ginseng Radix	2.84 ± 1.47
Zingiberis Rhizoma	0.17 ± 0.17*
Atracylodis Lanceae Rhizoma	0.68 ± 0.68*
Zizyphi Fructus	4.87 ± 4.61

Each drug (500 mg/kg) was given orally 30 min before administration of ethanol. Values are expressed as the mean ± SD (n = 7).
*$p < 0.01$, as compared with 70% ethanol.

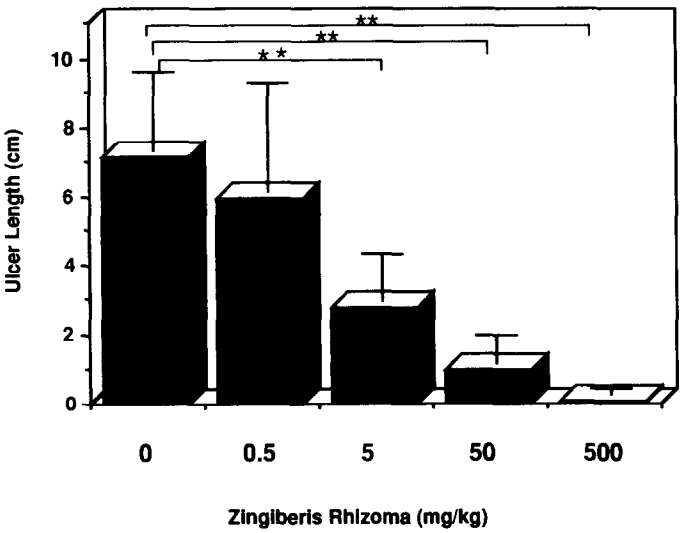

FIG. 1. Effect of Zingiberis Rhizoma pretreatment on ethanol-induced gastric damage. Zingiberis Rhizoma (0, 0.5, 5, 50 or 500 mg/kg) was given orally 30 min before 70% ethanol administration. Values are expressed as the mean ± SD. **Significantly different from the control group at $p < 0.01$, n = 13.

of 0.5 mg/kg (71% of the control). A similar result was obtained with Glycyrrhizae Radix (data not shown).

The changes in mucin content localized in the different layers by Zingiberis Rhizoma and Glycyrrhizae Radix administration at dose of 500 mg/kg each are shown in Table 4. Although the single administration of each drug did not affect the total mucin content, but its distribution in the three different layers was noted to have changed; the deep corpus mucin content by Zingiberis Rhizoma and the surface content by Glycyrrhizae Radix significantly increased as a consequence of its decrease in the other layers. The total mucin content in the entire stomach essentially attained the control level by Zingiberis Rhizoma and Glycyrrihizae Radix pretreatment on ethanol damage. The reduction in the mucin content of the deep corpus mucosa with ethanol treatment (64%) was significantly inhibited to 92% of the control value with Zingiberis Rhizoma pretreatment and the surface mucosa mucin content was selectively and significantly increased to 146% of the control value with Glycyrrhizae Radix pretreatment.

TABLE 4. Effect of Zingiberis Rhizoma and Glycyrrhizae Radix on gastric mucin content in ethanol-induced injury.

	Hexose Value (% of control)		
	Corpus	Antrum	Surface
Vehicle	558.5 ± 55.5 (100%)	120.8 ± 32.2 (100%)	328.9 ± 40.2 (100%)
Zingiberis Rhizoma	631.6 ± 53.5 (113%)*	100.6 ± 15.2 (83%)	286.8 ± 44.8 (87%)
Glycyrrhizae Radix	511.8 ± 128.7 (92%)	109.7 ± 14.5 (91%)	441.6 ± 33.7 (134%)*
70% EtOH	356.8 ± 25.2 (64%)†*	90.9 ± 5.9 (75%)	326.6 ± 26.5 (99%) *
Zingiberis Rhizoma + EtOH	510.4 ± 70.1 (92%)	96.4 ± 15.7 (80%)	388.3 ± 73.6 (118%)
Glycyrrhizae Radix + EtOH	373.1 ± 52.0 (67%)†	82.9 ± 30.3 (67%)	481.1 ± 51.5 (146%)*

Each drug (500 mg/kg) was given orally.
Hexose value corresponding to mucin content is in micrograms of Fr-1 hexose per stomach and expressed as mean ± SD (n = 8).
*$p < 0.05$, †$p < 0.01$; as compared with control or 70% ethanol.

DISCUSSION

Many kinds of traditional herbal medicines have long been clinically accepted as effective drugs for many diseases (19). These drugs are composed of many crude drug components, and the cooperative and mutual action of these components have been thought to be essential for the efficacy of these drugs (21). On the other hand, the pharmaceutical role of each component drug is not well understood. It seems very important to determine the mechanism of action of each crude drug.

Rikkunshi-to is composed of eight herbal components, and markedly prevented the development of ethanol-induced gastric damage in rats. The five crude drugs composing Rikkunshi-to showed a significant protective effect against 70% ethanol-induced gastric mucosal damage in rats, and the strikingly protective effect was achieved by Zingiberis Rhizoma and Glycyrrizae Radix. The protective effect of Zingiberis Rhizoma and Glycyrrhizae Radix against ethanol-induced gastric damage is obtained in a dose-dependent manner.

Gastric mucin, produced by mucus cells, is considered essential for providing protection to the gastric mucosa (14). Changes in the gastric mucin content have been shown to occur in association with the oral administration of ethanol or aspirin (1,9,13). It has been demonstrated that gastric mucin present in the different layers of gastric mucosa has distinct histochemical characteristics (18), and appears to have its own physiological role in the gastric mucosal defense mechanism (17). Recently, we developed a method to separate rat gastric mucosa into three layers, each containing a different mucin species (11,4). In this experiment the gastric mucosa was separated into the deep corpus, antral mucosa and the surface mucosa including the adherent mucus gel.

A single administration of Rikkunshi-to induced about an 11% increase in total mucin content and caused a significant increase in that of the deep corpus mucosa. In the case of Zingiberis Rhizoma and Glycyrrhiizae Radix administration, the total mucin content was almost unchanged, but the distribution of the mucin content in the three layers was somewhat changed.

Our previous study showed that several agents such as proton pump inhibitors (16,5), H2-blockers (6), prostaglandins (8,12) and other antiulcer drugs (7,10) affected the mucus metabolism of the gastric mucosa. Applying a newly developed method, it was demonstrated that Zingiberis Rhizoma accumulated mucin in the deep corpus mucosa and accelerated the secretion of the mucin during ethanol-induced gastric damage and Glycyrrhizae Radix accelerated the secretion of deep mucosal mucin and aided in its retention in the surface mucus during ethanol-induced gastric damage. On the other hand, Rikkunshi-to, which is a mixture drug of the crude components, indicated the cooperative and mutual action of these components. The increase in surface mucin content may have been compensated for by the loss of deep mucosal mucin and helped to conserve the stable structure of the covering mucus gel layer by being present with the surface mucosal cell-derived mucus.

The present study indicates the traditional herbal medicines affect the mucus metabolism of the gastric mucosa, and the antiulcerogenic effect on the ethanol-induced gastric lesion might be attributed to activation of the mucus metabolism.

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References

1. Azuumi, Y.; Ohara, S.; Ishihara, K.; Okabe, H.; Hotta, K. Correlation of quantitative changes of gastric mucosal glycoproteins with aspirin-induced gastric damage in rats. Gut 21:533–536;1980.
2. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugars and related substances. Analyt. Chem. 28:350–356;1956.
3. Fukuda, T.; Arakawa, T.; Kobayashi, K. Mechanism of Rikkunshi-to. Prog. Med. 11:449–453;1991.
4. Ishihara, K.; Hotta, K. Comparison of the mucus glycoproteins present in the different layers of rat gastric mucosa. Comp. Biochem. Physiol. 104B:315–319;1993.
5. Ishihara, K.; Ichikawa, T.; Komuro, Y.; Ohara, S.; Hotta, K. Effect on gastric mucus of the proton pump inhibitor leminoplazole and its cytoprotective action against ethanol-induced gastric injury in rat. Arzneim.-Forsch./Drug Res. 44 (II):827–830;1994.
6. Ichikawa, T.; Ishihara, K.; Komuro, Y.; Kojima, Y.; Saigenji, K.; Hotta, K. Effects of the new histamine H_2 receptor antagonist, FRG-8813, on gastric mucin in rats with or without acidified ethanol-induced gastric damage. Life Sciences 54:159–164;1994.
7. Ishihara, K.; Komuro, N.; Nishiyama, N.; Yamasaki, K.; Hotta, K. Effect of rebamipide on mucus secretion by endogenous prostaglandin-independent mechanism in rat gastric mucosa. Arzneim.-Forsch./Drug Res 42(II):1462–1466;1992.
8. Ishihara, K.; Kuwata, H.; Ohara, S.; Okabe, H.; Hotta, K. Changes of rat gastric mucus glycoprotein in cytoprotectin: influences of prostaglandin derivatives. Digestion 39:162–171;1988.
9. Ishihara, K.; Ohara, S.; Azuumi, Y.; Goso, K.; Hotta, K. Changes of gastric mucus glycoproteins with aspirin administration in rats. Digestion 29:98–102;1984.
10. Kojima, Y.; Ishihara, K.; Ohara, S.; Saigenji, K.; Hotta, K. Effects of the M_1 muscarinic receptor antagonist pirenzepine on gastric mucus glycoprotein in rats with or without ethanol-induced gastric damage. Scand. J. Gastroenterol. 27:764–768;1992.
11. Komuro, Y.; Ishihara, K.; Ishii, K.; Ota, H.; Katsuyama, T.; Saigenji, K.; Hotta, K. A separation method for quantifying mucus glycoprotein localized in the different layer of rat gastric mucosa. Gastroenterol. Jpn. 27:466–472;1992.
12. Komuro, Y.; Ishihara, K.; Ohara, S.; Saigenji, K.; Hotta, K. A new method of separation and quantitation of mucus glycoprotein in rat gastric mucus gel layer and its application to mucus secretion induced by 16,16-dimethyl PGE2. Gastroenterol. Jpn. 26:582–587;1991.
13. Kuwata, H.; Ishihara, K.; Kakei, M.; Ohara, S.; Okabe, H.; Hotta, K. Correlation of quantitative changes of gastric mucus glycoproteins with ethanol-induced gastric damage in rats. Jpn. J. Gastroenterol. 82:28–33;1985.

14. Neutra, M.R.; Forstner, J.F. Gastrointestinal mucus: synthesis, secretion and function. In: Johnson, L.R., ed. Physiology of gastrointestinal tract, 2nd ed. New York: Raven Press; 1987: 975–1009.
15. Ohara, S.; Ishihara, K.; Kakei, M.; Azuumi, Y.; Hotta, K. Distribution of mucosal macromolecular glycoproteins in rat stomach. Comp. Biochem. Physiol. 72B:309–311;1982.
16. Ohara, S.; Okawa, H.; Ishihara, K.; Hotta, K.; Komuro, Y.; Okabe, H. Effect of the proton pump inhibitor, NC-1300 , on gastric mucus secretion in rats. Scand. J. Gastroenterol. 24 (suppl 162):186–189;1989.
17. Ota, H.; Katsuyama, T. Altering laminated array of two types of mucin in the human gastric surface mucous layer. Histochem. J. 24:86–92;1992.
18. Ota, H.; Katsuyama, T.; Ishii, K.; Nakayama, J.; Shiozawa, T.; Tsukahara, Y. A dual-staining method for identifying mucins of different gastric epithelial mucous cells. Histochem J. 23:22–28;1991.
19. Perry, L.M. Medical Plants of East and Southeast Asia. Boston: The MIT Press; 1980:443–448.
20. Takagi, K.; Kimura, M.; Harada, M.; Otsuka, K. Wakan Yakubutu Gaku. Tokyo: Nanzando;1982.
21. Takemoto, T.; Matsuda, K.; Tada, M.; Okazaki, Y.; Okita, K. Clinical evaluation of TJ-43 Tsumura Rikkunshi-to on gastritis with additional symptom. Shoukakika 12:223–224;1990.