Protective and therapeutic effects of Hange-shashin-to on water-immersion restraint stress induced gastric ulcers

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Abstract

Hange-shashin-to (HST, 半夏瀉心湯) is a traditional Chinese herbal prescription (Banxia Xiexin Tang) which has long been used in the therapy of gastric functional disorders. In this report, the effects of HST on the changes in gastric mucin content and ulcerations induced by water-immersion restraint stress in rats were investigated. Ulcer index measured microscopically by the sum total of the lengths of ulcers and gastric mucin content determined by a PAS-staining method were examined for both the formation and healing process of stress induced gastric ulcers in rats. HST extract was orally administered at 1.5, 3.0, and 4.5 g/kg for three days before and after stress. As a result, stress decreased the mucin content and produced ulcers in a time dependent manner. In the HST pretreatment group, three different doses of HST markedly inhibited the reduction of mucin content and the development of gastric ulcers. In the HST post-treatment group, significant increases in mucin content and decreases in the ulcer index, in dose dependent manners, were detected. These results suggest that the therapeutic and prophylactic effects of HST on stress induced gastric ulcers may be associated with an increase in gastric mucin content.

Key words Hange-shashin-to (Ban-Xia-Xie-Xin-Tang), water-immersion restraint stress, gastric ulcers, mucin, Periodic Acid/Schiff staining.

Introduction

Hange-shashin-to (HST, 半夏瀉心湯), Ban-Xia-Xie-Xin-Tang in Chinese, is an herbal medicine which has long been used mainly in the therapy of gastric functional disorders. In recent years, some Chinese herbs including HST have been used clinically to treat chronic gastritis, even with erosion and ulcers of the gastric mucosa, because of their reduced side-effects, availability and cost effectiveness.[1,2] In fundamental studies, HST has also been reported to prevent gastric ulcers in rats induced by chemical stimulation through inhibiting decreases in the mucin content in gastric mucus.[3] However the pharmacological effects of HST on gastric ulcers are still poorly understood.

In general, medicinal therapy for peptic ulcer diseases has been targeted at inhibiting acid secretion, based on the belief that ulcers occur as a result of an imbalance between luminal aggressive and mucosal defensive factors.[4] Recently, the mucosal defensive mechanism in gastric ulcer formation has attracted much attention, and it has been demonstrated by many researchers that gastric mucus is an important mucosal defensive factor.[5-7] The well known antiulcer medicine, sucralfate, has been reported to protect against mucosal injury by stimulating the release of mucus.[8] Since mucin is the major component of gastric mucus, its analysis is important for evaluating the protective mechanism of gastric mucus. Besides the biochemical determination of hexosamine and fucose content in gastric mucus,[9] histochemical studies have shown that the PAS-positive reaction area is mainly mucosal surface mucin.[10] Therefore,

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we used a PAS - staining method (Periodic Acid / Schiff)[11] to measure gastric mucin content in the present study because of its ease and specificity.

Complementary psychic factors induced by numerous stressors cause different types of diseases including stress-induced gastric ulcers.[12, 13] It is therefore important that more appropriate therapies are developed to better treat the increasing trends in stress related diseases. A water-immersion restraint stress rat model, which has been widely used for studying the antiulcer activity of drugs,[14] was employed in the present study.

This study was carried out to investigate the correlation between quantitative changes in gastric mucin content and water-immersion restraint stress induced gastric ulcers. In addition, the protective and therapeutic effects of HST on stress induced gastric ulcers were also examined.

Materials and Methods

Drug Preparation: HST granular extract was purchased from Shinwa Pharmaceutical Company (Tokyo, Japan). It was suspended in boiled distilled water and shaken for several minutes. The solution was then centrifuged at 3,000 rpm for 10 minutes. The aqueous HST extract was adjusted to the following appropriate dosages: 1.5g/kg, 3.0 g/kg, and 4.5 g/kg, used in the present experiment.

Animals: Male Wistar rats weighing about 200-250 g were deprived of food for 24 hours prior to the experiment, but were allowed free access to water during this time. The animals were divided into two groups which underwent the following treatments:

1. Pretreatment Group: Animals were divided into three subgroups of 8 rats each; animals in each of the three subgroups were orally administered one of the three dosages of HST noted above, in a volume of 1 ml/100 g of body weight for three consecutive days before 24-hour water-immersion restraint stress.
2. Post-treatment Group: Animals were also divided into three subgroups of 8 rats each; animals in each of the three subgroups were orally administered one of the three dosages of HST described above, in a volume of 1 ml/100 g of body weight for three consecutive days after 24-hour water-immersion restraint stress.

In both the pretreatment group and post-treatment group, nontreatment (control) animals were given distilled water orally in a comparable volume (1 ml/100 g) before and after stress loading, rather than one of the HST doses.

Water - immersion restraint stress: Rats were placed in a stress cage and immersed to the level of the xiphoid in a water bath at 23±1°C for 6, 8, 16 and 24 hours according to the previously described method by Takagi and Okabe.[15]

Calculation of ulcer index: Rats were killed by cervical dislocation before and after stress loading. The stomach was quickly removed after clamping the esophagus and pylorus, then the gastric lumen was inflated with 10 ml of 0.9 % saline. After being placed in 1 % formalin solution for 10 minutes, the stomach was opened along the greater curvature and examined stereomicroscopically for lesions in the glandular portion. The ulcer index was calculated as the sum of the lengths (mm) of all lesions in the stomach.

Measurement of gastric mucin content: Gastric mucus glycoprotein (mucin) content was determined according to the Periodic Acid/Schiff (PAS) staining method described by Mantle and Allen.[11] Briefly, gastric mucus was obtained by gentle scraping of the mucosa with a glass slide and immediately homogenized in 2 ml of 0.2M NaCl. The homogenized sample was incubated for 2 hours at 37°C with 0.2 ml of a freshly prepared periodic acid solution (10 μl of 50% periodic acid and 10 ml of 7 % acetic acid, both obtained from Wako Pure Chemical Corp, Tokyo, Japan) and then centrifuged at 0°C and 10.000 rpm for 10 minutes. Then 0.2ml of Schiff reagent was added to the supernatant (aqueous phase). These solutions were left standing for 30 minutes at room temperature and then examined spectrophotometrically at 555 nm in comparison with a calibration curve obtained from ten different concentrations of glycoprotein standard solutions.

Statistical analysis: Experimental results are expressed as mean ± S.E. and statistical analysis was conducted by employing the Newman-Keuls multiple-comparison test. Values of $p<0.05$ were considered statistically significant.

Results

Ulcer index after water-immersion restraint stress

The changes in ulcer index response to the duration of water-immersion restraint stress are shown in Table I. The ulcer index increased in a time dependent manner. After 6 hours of stress, lesions of various lengths with haemorrhage were observed in about 80 % of the rats examined. After 8 hours of stress, spotted or linear ulcers appeared in all of the rats. The ulcer index obtained after 24 hours of stress was 6 times higher than that obtained after 6 hours of stress.

Mucin content after water-immersion restraint stress

The changes in gastric mucin content following water-immersion restraint stress are shown in Table II. After 16 hours of stress, the mucin content decreased by 20 % compared with the normal level (at 0 hrs, 1.25 μg/mg). Mucin levels continued to decrease with increasing duration of stress. After 24 hours of stress, a significant decrease of approximately 50 % in mucin content was demonstrated ($p<0.05$), compared with the normal level.

Table I Changes in ulcer index induced by water-immersion restraint stress.

Restraint time (hr)	Number of rats	Ulcer index (mm)
0	27	0
6	6	9.70±3.75
8	10	28.57±3.65
16	10	36.45±4.62
24	10	57.09±6.72

Values represented are mean values±S.E.

Table II Changes in gastric mucin content after stress loading.

Restraint time (hr)	Number of rats	Mucin content (μg/mg)
0	27	1.25±0.10
8	26	1.23±0.09
16	10	0.97±0.08
24	10	0.71±0.08*

Values represented are mean values±S.E.
*: Significantly different from normal group (0 hr) ($p<0.05$)

Ralation between gastric mucin content and ulcer index

Fig. 1 compares the changes in gastric mucin content and ulcer index after 24-hour water-immersion restraint stress. From day 0 to day 3 after stress, a significant reduction of gastric mucin content was measured compared to the normal level of 1.25 μg/mg. The reduction in this content, however, recovered to normal levels by day 7. The ulcer index was markedly reduced at the third day after stress, and then recovered to the normal level by day 7.

Effect of HST on gastric ulcers

1. *Effect of HST on mucin content in normal rats*

In order to examine the effect of HST on mucin content in normal rats, the three different concentrations of HST were administered to normal rats for three consecutive days. The mucin content in the nontreatment rats was 1.25±0.10 μg/mg (n=27). Following repeated administration of HST at 1.5 g/kg, 3.0 g/kg and 4.5 g/kg, the mucin content was 1.16±0.06 μg/mg (n=6). 1.66±0.24 μg/mg (n=8), 2.09±0.08 μg/mg (n=6), respectively. These results show that the treatment with HST at 3.0 g/kg and 4.5 g/kg increased the mucin content compared with that of the nontreatment group, and that a significant increase was detected in the group administered 4.5 g/kg ($p<0.01$).

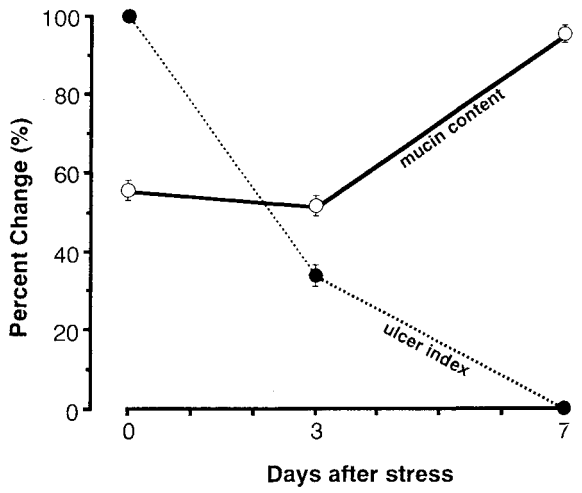

Fig. 1 Relation between gastric mucin content and ulcer index after water-immersion restraint stress (24 hrs) in rats.
Each value is shown as a percentage of recovery. In gastric mucin content (straight line), the normal level was calculated as 100 %. In ulcer index (dotted line), the most severe damage was calculated as 100 %.

2. *Effect of pretreatment with HST on gastric ulcers induced by stress*

Fig. 2 shows the protective effect of pretreatment with HST on gastric ulcers induced by 24-hour water-immersion restraint stress. The nontreatment group (shown as the black column, n=10) represents the ulcer index (57.09±6.72 mm) and gastric mucin content (0.71 ±0.08 μg/mg) at the end of the stress period. HST was administered to three groups (8 rats each) before inducing stress at the three different doses employed. In each of the groups, the ulcer index was prominently reduced by about 40 % ($p<0.01$) compared with that of the nontreatment group, but did not differ substantially from one group to another. Furthermore, the reduction of gastric mucin content was significantly inhibited by the pretreatment with HST ($p<0.01$)

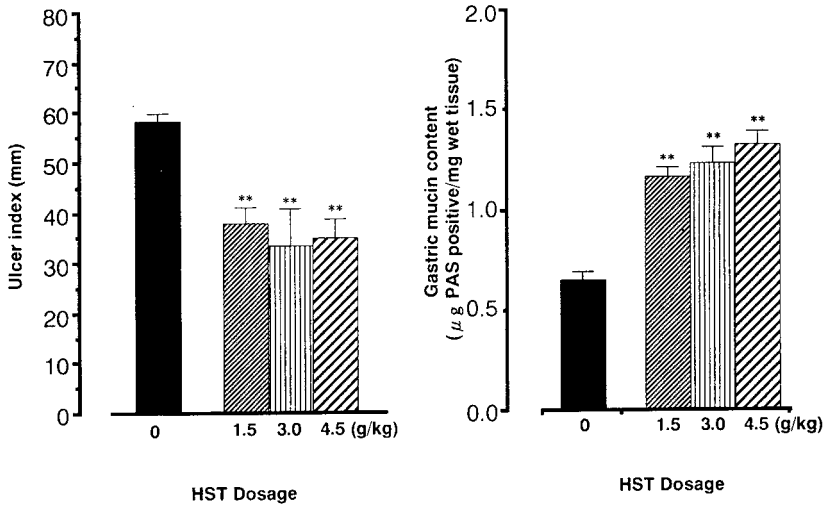

Fig. 2 Effect of pretreatment with Hange-shashin-to on gastric ulcers induced by water-immersion restraint stress.
Three day consecutive treatment with HST, administered orally, 24 hrs before water-immersion restraint stress (24 hrs).
**: Significantly different from nontreatment group (black column), $p<0.01$.

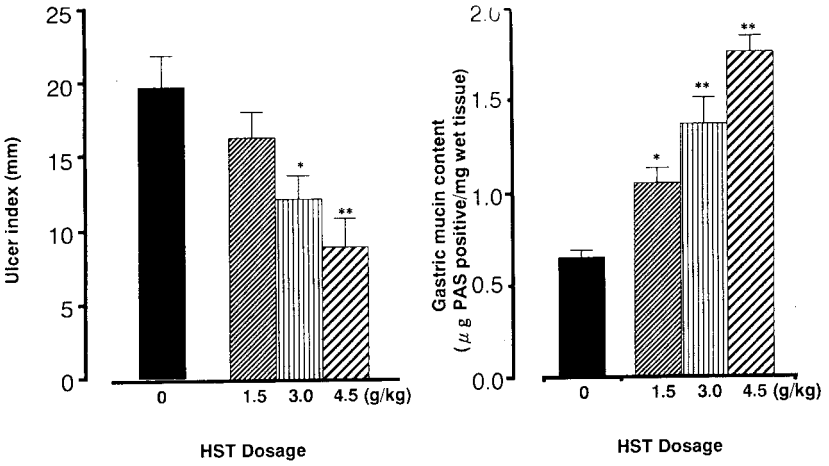

Fig. 3 Effect of post-treatment with Hange-shashin-to on gastric ulcers induced by water-immersion restraint stress.
Three day consecutive treatment with HST, administered orally, 24 hrs just after water-immersion restraint stress (24 hrs).
*,**: Significantly different from nontreatment group (black column), $p<0.05$ and $p<0;01$, respectively.

compared with that of the nontreatment group, which reached nearly normal levels in each of the three groups.

3. *Post-treatment effect of HST on stress induced gastric ulcers*

Fig. 3 shows the protective effect of post-treatment with HST on the gastric ulcers induced by 24-hour water-immersion restraint stress. The nontreatment group (shown as the black column, n=12) represents the ulcer index (19.65 ± 2.35 mm) and gastric mucin content (0.65 ± 0.05 μg/mg) after three days of stress. HST was administered to three groups (8 rats each) after inducing stress at the previously described doses. In the first group (1.5 g/kg), there was no significant decrease in the ulcer index, although a significant increase in mucin content was observed ($p < 0.05$) compared with that of the nontreatment group. However, in the other two groups (3.0 g/kg and 4.5g/kg), significant decreases in the ulcer index and increases in the mucin content ($p < 0.05$ and $p < 0.01$) were exhibited in dose dependent manners compared with the levels detected in the nontreatment group.

Discussion

In this study, the relationship between changes in gastric mucin content and water-immersion restraint stress induced gastric ulcers was investigated. In addition, the effects of HST on stress induced gastric ulcers were also examined. The results obtained are summarized as follows:

1. Water-immersion restraint stress produced gastric ulcers in a time dependent manner and decreased mucin content gradually, however, the reduction of mucin content did not parallel the development of ulcers. Also, the complete recovery of both ulcer index and mucin content took seven days, although significant differences in both factors, compared with the normal levels, remained until three days after stress (Tables I, II and Fig. 1).

2. Pretreatment with HST significantly decreased the ulcer index and inhibited the reduction of mucin content, however, these effects could not be detected in a dose dependent manner (Fig. 2). On the other hand, post-treatment with HST showed a decrease in the ulcer index and an increase in mucin content, each in a dose dependent manner (Fig. 3).

The relationship between decreases in mucin content and the development of gastric ulcers has been reported.[3, 9] Ogata, Y. *et al.*[3] reported that treatment with 70 % ethanol produced severe gastric ulcer as well as a remarkable reduction in mucin content. Menguy and Masters[9] observed that aspirin induced ulcers in rats decreased mucin contents when measured by PAS-staining to sections of gastric mucosa, and that reduced contents of hexosamine and fucose were detected by spectrophotometric determination. In our present study, however, weaker correlations were observed. These discrepancies can be explained by the numerous factors involved in the formation of ulcers induced by water-immersion restraint stress. Not only peripheral factors, such as gastric secretion,[16] motility,[17] mucosal microcirculation[18] and gastric mucus,[19] but also central factors such as variations of neuromodulators and/or neurotransmitters[20-22] could be related to ulcer formation induced by water-immersion restraint stress.

Regarding its effects on gastric ulcers, HST has been reported to prevent acute gastric ulcers induced by chemical stimulation (e.g. 70 % ethanol, aspirin, and indomethacin) in the stomach of rats.[3, 23] In the present study, pretreatment with HST for three consecutive days showed a marked decrease in the ulcer index and increase in mucin content. Each of the three dosages of HST administered to the pretreatment group was effective in increasing the mucin level and was associated with lesser ulceration, compared with nontreatment group animals, which were not given HST. In contrast with the HST post-treatment group, the three dosages of HST used in the pretreatment experiment did not exhibit dose dependent pharmacological effects. This may be explained by the use of excessive dosages in the pretreatment group. Furthermore, these results may suggest that efficacious pretreatment doses are lower than doses required for treating established gastric ulcers. Also, possible immunological responses elicited by pretreatment with HST cannot be discounted.[24, 25] Further studies are warranted to better elucidate minimum effective dosages and the pharmacological mechanism of HST in the prophylaxis of gastric ulcers.

The effects of post-treatment with HST on the

healing process of ulcers, on the other hand, were demonstrated to be dose dependent. The pharmacological effects of post-treatment with HST on gastric ulcers after stress have not been well examined by animal studies, although its clinical efficacy in the treatment of gastritis even gastric ulcers has been proved.[1,2] In the current study, for the first three days after stress, severe gastric ulcers and a significant reduction in mucin content were maintained. Complete recovery of the ulcers took seven days, however, the healing process of gastric ulcers was accelerated by post-treatment with HST.

Previously, we have reported that benzodiazepine, a CNS depressant, inhibited ulcer formation induced by water-immersion restraint stress by preventing extraordinary changes of monoamines in brain and gut due to stress.[26] It has also been reported that anticholinergic and antacid drugs show excellent prophylactic effects on stress induced ulcers.[15] Therefore, the assumption that multiple factors are involved in the formation of ulcers is well supported. Furthermore, in this study, slightly higher doses of HST than those reported by other researchers[3,23] were employed because of different experimental conditions, and the effectiveness of these higher dosages was confirmed by the post-treatment experiment.

In conclusion, numerous factors are involved in the formation of water-immersion restraint stress induced gastric ulcers, of which mucin may play an important role in the process of ulcer formation and healing. Moreover, these experimental results indicate that HST protects the gastric mucosa, and this effect may be associated with an increase in the secretion of gastric mucin due to the augmentation of glycoprotein synthesis. Thus, HST could be an effective mucosal protective agent for the therapeutic and prophylactic treatments of stress induced gastric ulcers.

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和文抄録

水浸拘束ストレス下での胃潰瘍形成とムチン(mucin)量の経時的変動を指標としてストレス誘発による消化性潰瘍に対する半夏瀉心湯(HST)の予防ならびに治療効果を考究した。方法として,1) Wistar系雄性ラット(200～250 g)を23±1°Cの水温中で6～24時間の拘束を行った。2) 経時的に胃粘液中のムチン量をPeriodic Acid/Schiff staining方法で比色定量を行い,潰瘍係数は腺胃部での潰瘍の長さを基準とし測定した。3) HST(1.5, 3.0, 4.5 g/kg)は1日1回連続3日間の水浸拘束を行う24時間前(前処置群),あるいは24時間後(後処置群),の2群に経口投与を行った。結果として,1) ムチン量は拘束時間に伴った減少が認められ,24時間拘束では対照群と比較して約50％の減少が確認された。2) HSTの前および後処置群では,ムチン量の減少ならびに潰瘍係数の増加に対する抑制が認められた。3) 無拘束群では,HSTの連続投与により用量依存的なムチン増量が認められた。胃潰瘍形成とムチン量の変動を指標とした本研究成績から,HSTはストレス誘発消化性潰瘍に対してムチン分泌増加を主とする組織修復促進作用を介して,予防ならびに治療効果を有することが示唆された。

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