Effects of Sasa Senanensis Rehder Extract (SE) on Stress or Ethanol-induced Gastric Lesions in Rats

Takaaki Ohizumi, Sadao Nakayama and Katsuji Oguchi

Abstract: Effects of Sasa senanensis Rehder extract (SE) on gastric lesions induced by water-immersion and restraint (WIR) or ethanol administration and gastrointestinal transport of carbon powder were investigated in rats and mice. Mucosal lesions and hemorrhagic cicatrices in the region corpus ventriculi were produced by WIR. These gastric lesions were prevented by 5 and 10 ml/kg SE, p.o. In the pathological observations, SE suppressed the deep hemorrhagic necrosis of mucosa and deciduation of the mucosal layer induced by WIR. Increases in adrenal weight and the ratio of adrenal weight to body weight (mg%), and decreases in spleen weight and its mg% induced by WIR were inhibited by administration of SE. SE also prevented gastric lesions induced by ethanol. The degeneration of gastric mucosa by ethanol was suppressed by 5 and 10 ml/kg SE, p.o.; especially, the erosion of surface epithelium was markedly inhibited by SE. The gastrointestinal transport distance of carbon powder in mice was increased by 10 ml/kg, p.o. SE. The results suggest that the protective effect of SE on the gastric lesions induced by WIR or ethanol is due to the enhancement of mucosal resistance and mucosal protection.

Key words: Sasa senanensis Rehder extract, gastric lesion, water-immersion and restraint-stress, ethanol, anti-stress

Introduction

From ancient times, Sasa senanensis Rehder has been used as a popular remedy for burn, animal bite, hematemesis, melena and dysuria. A few preparations made of Sasa leaf extracts have been developed on the basis of therapeutic efficacy in the traditional therapy that uses Sasa senanensis Rehder.

It has recently been reported that Sasa leaf extracts demonstrate various pharmacological benefits such as antifatigue, enhancement of orexia and gastric secretion, and antitumor effects1-5). Alkaline hydrolytic extract of Sasa senanensis Rehder leaf (SE), is a traditional popular remedy and has been used for fatigue, anorexia, halitosis, body odor, periodontitis and gastritis. We have previously reported the anti-inflammatory effect, enhancement of phagocytic activity and membrane protective effect of SE6,7). However, the pharmacological effect of SE have not been investigated in detail.

In the present study, we investigated the effects of SE on gastric lesions induced by stress or ethanol, and its effectiveness in controlling the effects of stress.
Materials and Methods

Male Sprague-Dawley rats (6 weeks old, 200–230 g) were used in the experiment on stress removal and effects on gastric lesions. Male ddY mice (7 weeks old) were used in the experiment on gastrointestinal transport of carbon powder.

SE was kindly donated by Daiwa Biolaboratory (Kanagawa, Japan). Sasa leaves were crushed and processed for removal of resins and substitution of Mg++ for Fe++ in chlorophyll. The crushed Sasa leaves (1 kg) were prehydrolyzed in 151 of 0.07 N NaOH solution at 100°C for 20 min. Sasa leaves were separated by filtration and then washed 5 times with water. The prehydrolyzed Sasa leaves were heated in 2.7 l of 0.5 N NaOH solution at 100°C for 80 min. The pH of alkaline hydrolysate of Sasa leaf was adjusted to 7.6. The final yield of SE was 1 l.

1. Water-immersion and restraint (WIR)-induced gastric lesions

Gastric lesions were produced according to the method of Takagi and Okabe. Each rat was held in a restraint cage and kept immersed from the tail to the processus xiphoideus in a water bath at a controlled temperature (24 ± 1°C) for 16 hours. SE or distilled water was given orally 8 hours before and just before placement in the restraint cage. After stressor imposition, rats were sacrificed under sodium pentobarbital (30 mg/kg, i.p.) anesthesia.

2. Ethanol-induced gastric lesions

Gastric mucosal lesions were produced according to the method of Robert et al. SE or distilled water was given to rats orally at 24, 6 and 1 hour before oral administration of 1 ml of 99.5% ethanol. One hour after ethanol administration, rats were sacrificed under sodium pentobarbital (30 mg/kg, i.p.) anesthesia.

3. Estimation of gastric lesions

The rat stomach was removed and inflated by injecting 10 ml of 1% formalin into the gastric lumen for 10 min. Subsequently, the stomach was incised along the greater curvature and examined for lesions. The number and length (mm) of mucosal hemorrhagic cicatrices in the regio corpus ventriculi were measured under a dissecting microscope. The thickness of the mucosal layer in the regio corpus ventriculi was obtained from the magnified photograph of pathological specimens. The data of thickness represented the area (cm²) of the mucosal layer per cross length (cm) of regio corpus ventriculi in pathological specimens. The respective group sums of these lesion parameters (the number and length of lesions, and the thickness of mucosal layers) were calculated and used as lesion indices.

4. Pathological observation

Formalin-fixed stomach was embedded in paraffin, and sections of the stomach were stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS). Pathological observation was performed under a light microscope.

5. Gastrointestinal transport of carbon powder

Mice were fasted for 16 hours before the experiment, but were allowed free access to water. SE or distilled water was given orally 30 min prior to oral administration of 0.1 ml of 5% carbon powder suspended in 0.5% carboxymethylcellulose, and the final volume of SE or distilled water given to each animal was adjusted to 10 ml/kg body weight. Mice were sacrificed under sodium pentobarbital (30 mg/kg, i.p.) anesthesia 30 min after carbon powder administration and the gastrointestinal from the stomach to the rectum were removed. The transported distance of carbon powder in the gastrointestinal was measured.
6. Statistical analysis

The data represent the mean±S.D. Statistical analysis was performed using Student’s t-test; a p value of less than 5% was regarded as significant.

Results

1. Effect of SE on WIR-induced gastric lesions

The photographs of stomach specimens of WIR-imposed rats are shown in Fig. 1. The mucosal lesions and hemorrhagic cicatrices in the regio corpus ventriculi were caused by WIR (Fig. 1A). These gastric lesions were prevented by SE at 5 and 10 ml/kg (Figs. 1C and 1D).

The gastric lesion indices, number and length of mucosal hemorrhagic cicatrices and thickness of mucosa, are shown in Fig. 2. The number and length of hemorrhagic cicatrices caused by WIR were reduced by the administration of SE at 5 and 10 ml/kg, but SE at 1 ml/kg did not inhibit these mucosal lesions significantly. The thickness of mucosa was increased by the imposition of WIR. SE did not inhibit this increase.

In the pathological observation of regio corpus ventriculi, deep hemorrhagic necrosis of the mucosa and damage of the mucosal layer were found in the WIR rat (Fig. 3B). SE at 5 and 10 ml/kg suppressed the degeneration of gastric mucosa by WIR (Figs. 3C and 3D).

There was no remarkable difference in the body weight, thymus weight, or the ratio of the two (mg%) in any groups (Table 1). Adrenal weight was increased by WIR, and the ratio of adrenal to body weight (mg%) increased with the increase in adrenal weight. These
Fig. 2. Effect of SE on gastric lesion indices induced by WIR of rats. Each column represents the mean±S.D. of 5 to 6 rats. (a) number of mucosal hemorrhagic cicatrices, (b) length of mucosal hemorrhagic cicatrices, (c) thickness of mucosa (area (cm²) of mucosal layer per cross length (cm) of regio corpus ventriculi in pathological specimens. *, ** and ***: Significantly different from the stress (non-treated) group (p<0.05, p<0.01 and p<0.001), •: Significantly different from the control (<0.05).

Fig. 3. Light micrographs of the regio corpus ventriculi in WIR rats. A: control, B: WIR, C: 5 ml/kg SE p.o., D: 10 ml/kg SE p.o. Hematoxylin-eosin stain.
Table 1. Effect of SE on body weight and organ weight in water-immersion and restraint stressed rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Water (ml/kg)</th>
<th>SE (ml/kg)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>208 ± 19</td>
<td>217 ± 8</td>
<td>212 ± 12</td>
</tr>
<tr>
<td>Thymus (mg)</td>
<td>515 ± 49</td>
<td>517 ± 38</td>
<td>491 ± 52</td>
</tr>
<tr>
<td>(mg%)</td>
<td>248 ± 10</td>
<td>238 ± 23</td>
<td>230 ± 13</td>
</tr>
<tr>
<td>Adrenals (mg)</td>
<td>35 ± 6</td>
<td>52 ± 4*</td>
<td>43 ± 5**</td>
</tr>
<tr>
<td>(mg%)</td>
<td>17 ± 1</td>
<td>23 ± 1**</td>
<td>20 ± 2*</td>
</tr>
<tr>
<td>Spleen (mg)</td>
<td>489 ± 26</td>
<td>316 ± 22**</td>
<td>316 ± 34**</td>
</tr>
<tr>
<td>(mg%)</td>
<td>236 ± 20</td>
<td>145 ± 7**</td>
<td>148 ± 13**</td>
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</table>

Each value represents the mean±S.D. of 5 to 7 rats.

* and **: Significantly different from the water-administered group (p<0.05 and p<0.01), * and **: Significantly different from the control group (p<0.05 and p<0.01).

Fig. 4. Effect of SE on gastric lesion induced by ethanol in rats.

SE was given orally 24, 6 and 1 hr before ethanol administration. The stomach was removed 1 hr after ethanol administration. A: ethanol-administered control, B: 1 ml/kg SE p.o., C: 5 ml/kg SE p.o., D: 10 ml/kg SE p.o.

Increases were inhibited by the administration of SE. Spleen weight and the ratio to body weight (mg%) was decreased by WIR. SE at 10 ml/kg inhibited the decrease in spleen weight, and SE at 5 ml/kg inhibited the decrease in spleen weight to body weight ratio (mg%), but spleen weight relative to body was significantly lower than in the control (non-stress) group (Table 1).
Fig. 5. Effect of SE on gastric lesion indices induced by ethanol in rats.

* , ** and ***: Significantly different from the ethanol-administered group (p<0.05, p<0.01 and p<0.001), •: Significantly different from the control (p<0.01). See explanation in Fig. 2.

Fig. 6. Light micrographs of the regio corpus ventriculi in ethanol-administered rats.
A: ethanol, B: 5 ml/kg SE p.o., C: 10 ml/kg SE p.o. Hematoxylin-eosin stain.
2. Effect of SE on ethanol-induced gastric lesions

Stomach specimens from ethanol-administered rats are shown in Fig. 4. The erosion and hemorrhagic cicatrices in the regio corpus ventriculi were caused by the administration of ethanol (Fig. 4A). SE at 1 ml/kg did not prevent these ethanol-induced mucosal lesions (Fig. 4B), but SE at 5 and 10 ml/kg did (Figs. 4C and 4D). Figure 5 shows the reduction in the number and length of mucosal hemorrhagic cicatrices produced by ethanol after the administration of SE at 5 and 10 ml/kg; 1 ml/kg SE did not produce significant inhibition. The thickness of mucosa was increased by the administration of ethanol. Although this increase was inhibited by 10 ml/kg SE, the thickness of mucosa in SE-administered rats was still significantly higher than that in the control group (Fig. 5).

Pathological observation of the regio corpus ventriculi, revealed wide erosion of surface epithelium and necrotic lesions of mucosa in the ethanol-administered rats (Fig. 6A). The degeneration of gastric mucosa caused by the administration of ethanol was suppressed by 5 and 10 ml/kg SE; in particular, erosion of the surface epithelium was markedly inhibited (Figs. 6B and 6C).

3. Effect of SE on gastrointestinal transport of carbon powder

The gastrointestinal transport distance of carbon powder was not significantly changed by the administration of SE at 1 and 5 ml/kg, but 10 ml/kg increased the distance significantly more than that of the control group (Table 2).

**Discussion**

The stress as a physiological response to various stressors is the main risk factor causing gastric ulcer. Gastric ulcer in rats can be produced by WIR. The suggested mechanisms of production of gastric ulcer induced by WIR are as follows: increase in gastric acid secretion and gastric motility due to stimulation of the cerebral limbic system and hypothalamic-mediated vagus nerves of the medulla oblongata; oligotrophy of regio corpus ventriculi by circulatory failure of gastric blood flow resulting from blood vessel contraction induced by stimulation of the splanchnic nerve of the medulla spinals; and reduction of mucosal resistance and increase in gastric acid secretion by stimulation of the hypophysis-adrenal system.

In the present study, SE inhibited ulcer indices such as the number and length of gastric lesions caused by WIR. SE also suppressed the deep hemorrhagic necrosis and damage of the mucosal layer in the regio corpus ventriculi caused by WIR. The apparent inhibition of mucosal lesions in WIR rats suggests a preventive effect of SE on the mucosal lesions due to enhancement of mucosal resistance and protection.
Kuboyama et al. reported that bamboo leaf extract (BLE) increases gastric acid secretion and gastric motility by direct stimulation of gastric mucosa in rats. On the other hand, 1% BLE solution ad libitum or 70 mg/kg of freeze-dried BLE prevented the gastric lesion formation caused by WIR, but high doses of BLE (10% solution or 700 mg/kg of freeze-dried BLE) exacerbated the gastric lesion formation because of the increase in gastric acid secretion by BLE. Shibata et al. also reported that folin, Sasa albomarginate Makino et Shibata extract, has a protective effect on gastric ulcer induced by WIR, and pylorus ligation combined with aspirin administration in rats. Cu-chlorophyllin sodium, a major component in folin, inhibits the gastric ulcer induced by WIR and acetic acid, and the anti-ulcer mechanism of this compound is considered to be protection of gastric mucosa and enhancement of regeneration of gastric tissue due to hyperplasia of granulation tissue. Those reports confirm that the protective mechanism of SE on gastric lesions in WIR rat is due to enhancement of mucosal resistance and protection.

In the experimental model of ethanol-induced gastric lesion which is widely used for examination of the protective potency on gastric mucosa, the gastric lesions induced by ethanol are produced by direct destruction of the gastric mucosal barrier. It is accepted that gastric acid does not affect gastric lesions induced by ethanol, since ethanol damage was not inhibited by treatment with a H₂-receptor antagonist. SE inhibited the gastric lesion induced by ethanol; especially, erosion of the surface epithelium. This result also indicates that the preventive effect of SE on gastric lesions is due to mucosal protection. It is considered that the increase of thickness of gastric mucosa by WIR or ethanol administration may be a physiological response to maintain homeostasis of the gastric mucosa. The inhibitory effect of 10 ml/kg SE on the increase of mucosal thickness by ethanol may be associated with the prevention of mucosal lesions.

Ohizumi et al. reported that SE, composed of polysaccharide, lignin and chlorophyll, remarkably inhibits hypotonic hemolysis and enzyme leakage from cultured hepatocytes, and has a potent membrane protective effect. The prevention of gastric mucosa by SE appears to be related to the membrane protective effect.

Atrophy of the thymus and spleen, and hypertrophy of the adrenals were caused by the stimulation with various stressors. In the present study, an increase in the real and relative weight of the adrenals and a decrease in the real and relative weight of the spleen were observed after WIR. SE inhibited these changes so it is suggested that SE may be effective in removing stress. In addition, the effectiveness of SE in increasing intestinal motility was demonstrated by the enhancement of carbon powder transport in the mouse intestine.

In conclusion, the present study suggested that the protection by SE against gastric lesions induced by WIR or ethanol was due to the enhancement of mucosal resistance and protection.

References


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