J. Pharm. Pharmacol. 1999, 51: 483–490
Received August 6, 1998
Accepted November 17, 1998

Effect of Saiboku-to, an Oriental Herbal Medicine, on Gastric Lesion Induced by Restraint Water-immersion Stress or by Ethanol Treatment

MITSUTOSHI YUZURIHARA, YASUSHI IKARASHI*, YOSHIO KASE, YASUTAKA TORIMARU, ATSUSHI ISHIGE AND YUJI MARUYAMA*

*Central Research Laboratories, Tsumura and Company, 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, and *Department of Neuropsychopharmacology (Tsumura), Gunma University, School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan*

Abstract

The effect of saiboku-to on gastric lesions induced by restraint water-immersion stress and ethanol has been examined in rats.

Thirty minutes after oral administration of saiboku-to, the rats were placed in restraint cages and immersed in water at 23°C for 7 h, or orally administered 99·5% ethanol (1 mL) and placed in normal cages for 1 h. The stress for 7 h or the ethanol treatment for 1 h induced erosion in the glandular area of the stomach. Histology showed that the surface epithelial cells were desquamated and part of the lamina propria mucosae was injured. The evaluation of lesion index, the cumulative length of the gastric lesion, on the gross appearance of the stomach, revealed that saiboku-to dose-dependently inhibited both the water-immersion stress-induced gastric erosion and ethanol-induced gastric erosion. To determine whether the anti-erosion effect of saiboku-to was because of a mild irritant effect, saiboku-to or 20% ethanol, which is known as a typical mild irritant, were given orally. After 30 min a strong irritant, 99·5% ethanol, was given orally. Histological examination was performed 30 min after administration of saiboku-to or the mild irritant, and 1 h after administration of the strong irritant. The mild irritant induced a reduction in surface epithelial cells 30 min after administration. Furthermore, the mild irritant protected the stomach against mucosal erosion produced by the strong irritant. Saiboku-to protected the strong irritant-induced erosion without producing mild irritation as observed in stomach treated with 20% ethanol. Pretreatment with saiboku-to also inhibited the decrease in the levels of hexosamine, gastric mucus glycoprotein, induced by the strong irritant. In pylorus-ligated rats, saiboku-to dose-dependently inhibited gastric acid secretion, a gastric aggressive factor.

These results suggest that the anti-erosion effect of saiboku-to which is not a mild irritant, involves both inhibition of aggressive factors, such as gastric acid secretion, and augmentation of defensive factors, such as gastric mucus cells.

Neurosis and psychosomatic disease are thought to be mainly caused by different physical stresses and mental anxieties (Adams & Victor 1993; Kudo & Kudo 1995). In particular, the stomach, duodenum and large intestine respond to stress more acutely, resulting in peptic ulcer (Varis 1987; Hernandez et al 1993; Piper & Tennant 1993). Diazepam, a benzodiazepine, the most clinically effective anxiolytic drug (File & Pearce 1981; Imperato et al 1994; Sieghart 1995), has been used to treat patients with acid-peptic diseases in a manner similar to the use of anti-ulcer drugs including histamine H_2 receptor antagonists, anticholinergics and proton-pump inhibitors (Isenberg et al 1991).

Saiboku-to, an oriental herbal medicine, is a traditional Chinese medicine called Kampo medicine in Japan. It is a specific mixture of dried plant materials and has been in practical use for thousands of years for the treatment of asthmatic and anxiety-related disorders such as anxiety neurosis (Umesato 1984; Nishiyori et al 1985; Homma et al

Correspondence: M. Yuzurihara, Central Research Laboratories, Tsumura and Company, 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan.

1993). Evidence from non-placebo controlled studies has confirmed that saiboku-to relieves anxiety and various nervous tensions (Narita 1990). Recently the anxiolytic effect of saiboku-to has been demonstrated by use of an improved elevated plus-maze device. The effect is enhanced by co-administration of diazepam (Kuribara & Maruyama 1996; Kuribara et al 1996), suggesting that it might be related to $GABA_A$-benzodiazepine receptors. These findings suggest that saiboku-to might, like diazepam, have an anti-ulcer effect. This possibility remains to be fully demonstrated.

The purpose of this study was to assess the effect of saiboku-to on gastric lesions induced by restraint water-immersion stress or by administration of ethanol in rats.

Materials and Methods

Animals

Male Sprague–Dawley rats, 210–250 g, were obtained from Charles River (Tokyo, Japan). The animals were housed individually and allowed free access to water and standard laboratory food (MF, Oriental Yeast, Tokyo, Japan). They were housed at $24 \pm 1^{\circ}C$, relative humidity of $55 \pm 5\%$, and under controlled lighting with lights on from 0700 to 1900 h daily.

Experimental protocols meet the ''Guidelines for Animal Experimentation'' approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society.

Drugs

Saiboku-to was obtained in the form of dried powder extract from Tsumura (Tokyo, Japan). The quality of the drug was assured by maintaining the prescribed range of index components. This drug was manufactured from a mixture of bupleuri radix (7·0 g), pinelliae tuber (5·0 g), hoelen (5·0 g), scutellariae radix (3·0 g), magnoliae cortex (3·0 g), zizyphi fructus (3·0 g), ginseng radix (3·0 g) glycyrrhizae radix (2·0 g), perillae herba (2·0 g) and zingiberis rhizoma (1·0 g). The yield was 14·7% for saiboku-to. The doses of the drug used in this study ($125–1000\ mg\ kg^{-1}$) correspond to 1·25–10 times the clinical dose.

Diazepam (Cercine injection, $5\ mg\ mL^{-1}$ per ampoule) was purchased from Takeda Pharmaceutical (Tokyo, Japan). It was diluted to $0{\cdot}5\ mg\ mL^{-1}$ with saline before use. Atropine sulphate was purchased from Sigma (St Louis, MO). Cetraxate purchased from Dai-Ichi Pharmaceutical (Tokyo Japan) was used after purification. Doses ($mg\ kg^{-1}$) of these compounds were prepared in distilled water (10 mL).

Restraint water-immersion stress-induced gastric lesion

Restraint water-immersion stress-induced gastric lesion was produced by the method described by Takagi & Okabe (1968). Thirty minutes after oral administration of saiboku-to, animals were placed in wire-restraint cages and immersed in a water bath at a temperature of 23°C. The rats were killed 7 h later by decapitation and the stomachs were immediately removed, inflated by injection of formalin (2%, 10 mL), then immersed in the same concentration of formalin for 30 min. The stomachs were subsequently incised along the greater curvature and examined for lesions developed in the glandular area. The extent of the lesions was expressed as the lesion index calculated as the cumulative length (mm) of gastric lesions. Histological examination was conducted on some stomachs in each group and the results were compared with those obtained from the non-stressed rats.

Ethanol-induced gastric lesion

The experiment was conducted by the method of Robert et al (1979). All test drugs except diazepam (administered subcutaneously) were administered orally to rats after a 24-h fast. Thirty minutes later 99·5% ethanol (1 mL) was administered orally. The animals were killed 1 h after ethanol treatment. Stomach processing for evaluation of the lesion index was performed by the methods described for water-immersion stress-induced gastric lesion. Histological examination was conducted in a separate set of experiments on a mild irritant.

In another set of experiments, rats were killed 30 min after oral injection of saiboku-to (500 and $1000\ mg/10\ mL\ kg^{-1}$) or water ($10\ mL\ kg^{-1}$) as control. The stomachs were immediately removed and the volume of the gastric contents was measured.

Gastric secretion in pylorus-ligated rats

The effects of the test drugs on gastric secretion were examined by the method of Yamamoto et al (1986). After a 24-h fast rats were anaesthetized with ether and then pylorus-ligated. Drugs except for diazepam (administered subcutaneously) were administered intraduodenally immediately after pylorus-ligation. The stomachs were removed 4 h later, after ligation of the cardia. Collected gastric juice was centrifuged at $3000\ rev\ min^{-1}$ and 4°C for 10 min and analysed for volume, pH and acidity. The acidity was measured by titration with 0·10 M NaOH, by means of an automatic titrator (AUT-1, TOA Electronics Japan, Tokyo, Japan) and expressed as $mEq\ L^{-1}$. Acid output was expressed as $\mu Eq\ h^{-1}$.

Determination of whether saiboku-to is a mild irritant to gastric mucosa

To clarify whether saiboku-to is a mild irritant to gastric mucosa, saiboku-to (1 g kg^{-1}, n = 14), 20% ethanol (mild irritant, n = 14) or water (vehicle control, n = 14) was administered orally in a volume of 10 mL kg^{-1} to rats after a 24-h fast. Three animals in each group were killed 30 min after oral administration and the stomachs were histologically examined. The remaining animals (n = 11) in each group were orally administered 99·5% ethanol (1 mL). The animals were killed 1 h later and the stomachs were histologically examined (n = 3). Gastric mucus glycoprotein, hexosamine (n = 8) was then measured. Normal rats, fasted and non-treated rats (n = 8) were used for determination of normal levels of hexosamine.

Histology

The stomachs were fixed in 15% buffered formalin, dehydrated with successively graded concentrations of ethanol, and embedded in paraffin. Paraffin sections were stained with alcian blue and periodic acid Schiff (AB-PAS) for staining of mucus glycoprotein in gastric mucosa.

Determination of gastric mucus glycoprotein, hexosamine

Gastric hexosamine was determined colorimetrically by the method described by Boas (1953). The stomach was quickly removed and a part of the fundus portion was detached and used for the determination. The tissue was dried at 85°C for 18 h and weighed. The dried tissue was hydrolysed in 4 M HCl in a sealed ampoule at 100°C for 4 h. After cooling, the hydrolysate was neutralized with 4 M NaOH. The solution was then diluted to 20 mL with distilled water and filtered. Filtrate (1 mL) was heated for 20 min with an equal volume of acetylacetone reagent—0·75 M Na_2CO_3 and 0·25 M $NaHNO_3$ (50 mL) containing acetylacetone (1 mL). After cooling, isoamyl alcohol (2 mL) was added to the reaction mixture. The mixture was shaken and centrifuged at 3000 rev min^{-1} for 10 min and the organic phase (1 mL) was placed in a tube and mixed with Ehrlich reagent (equal volumes (30 mL) of conc. HCl and isoamyl alcohol containing *p*-dimethylaminobenzaldehyde (0·8 g); 0·5 mL). The absorbance was measured 15 min later at a wavelength of 530 nm. Glucosamine was used as the standard. The hexosamine level was expressed as μg mg^{-1} of tissue dry weight.

Statistics

All values are shown as the means ± standard errors of the means (s.e.m.). Statistical significance was determined by a one-way analysis of variance then Fisher's least significant difference procedure.

Results

Effects of saiboku-to on gastric lesions induced by the restraint water-immersion stress

Figure 1 shows a typical gross appearance and the corresponding histology of the stomachs of non-stressed rats, stressed rats and rats pretreated with saiboku-to before being stressed by restraint water-immersion. Obvious linear haemorrhagic lesions were observed in the glandular area of the stomachs after exposure to stress for 7 h (Figure 1B1) compared with non-stressed rats (Figure 1A1). Histological findings showed that desquamation of surface epithelial cells and injury to the lamina propria mucosae was induced by the stress (Figure 1B2); it was confirmed as erosion. Few mucus cells were stained by AB-PAS. It was apparent from the gross appearance that pretreatment with saiboku-to prevented the development of the lesions (Figure 1C1). Histological findings also showed that saiboku-to inhibited stress-induced gastric mucosal injury (Figure 2B). No significant changes were

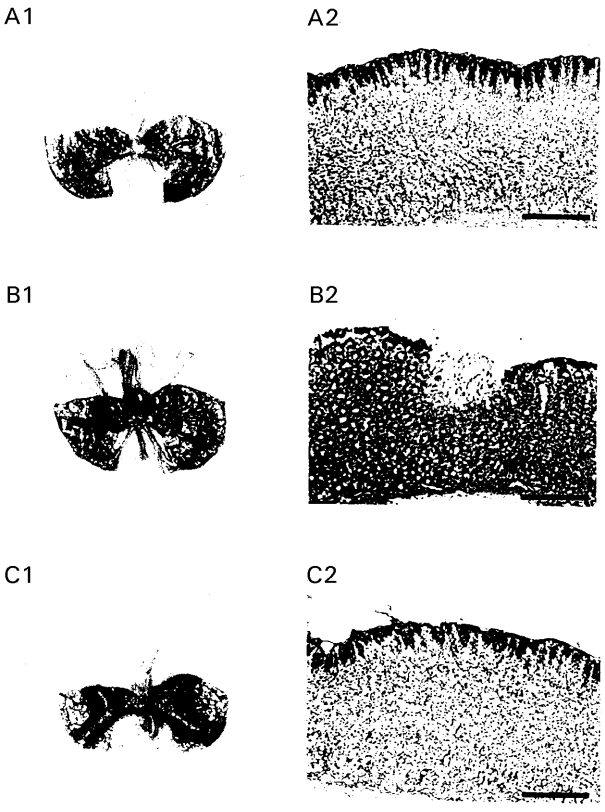

Figure 1. Gross appearance and corresponding histology (AB-PAS stain; magnification 100×, bar = 100 μm) of the stomachs of non-stressed rat (A1 and A2), stressed rat (B1 and B2) and rat treated with saiboku-to (C1 and C2).

observed in comparison with gastric mucosa in non-stressed rats (Figure 1A2).

The effects of oral saiboku-to (250, 500 and 1000 mg kg^{-1}), subcutaneous diazepam (1·0 mg kg^{-1}), oral atropine (1·0 mg kg^{-1}), oral cetraxate (100 mg kg^{-1}) and oral vehicle (10 mL kg^{-1} water) on gastric lesion (lesion index) induced by the stress are shown in Figure 2. Saiboku-to dose-dependently reduced the stress-induced gastric lesions. Significant inhibition was observed at 500 mg kg^{-1} ($P < 0·05$) and higher doses ($P < 0·01$). Atropine ($P < 0·01$) and diazepam ($P < 0·05$), but not cetraxate, significantly inhibited gastric lesions induced by the stress.

Effects of saiboku-to on gastric lesion induced by ethanol

The effects of oral saiboku-to (125, 250 and 500 mg kg^{-1}), subcutaneous diazepam (1·0 mg kg^{-1}), oral atropine (1·0 mg kg^{-1}), oral cetraxate (100 mg kg^{-1}), and oral vehicle (10 mL kg^{-1} water) on gastric lesion (lesion index) induced by 99·5% ethanol are shown in Figure 3. Saiboku-to dose-dependently inhibited the formation of gastric lesions induced by ethanol. Significant inhibition was observed at 250 mg kg^{-1} ($P < 0·05$) and higher doses ($P < 0·01$). Diazepam and atropine did not prevent the formation of gastric lesions at the dose which caused significant inhibition in the stress-induced gastric lesions. Cetraxate resulted in significant inhibition ($P < 0·01$) at the dose which did not inhibit stress-induced gastric lesions. Histological findings are described below.

The volume of the gastric contents of rats treated with saiboku-to or water (as control) are shown in Table 1. Until 30 min after oral administration, the residual volumes in the stomachs were 0·31 mL (87·4% excretion) and 0·44 mL (82·58% excretion) for the 500 and 1000 mg kg^{-1} saiboku-to-treated groups, respectively. There were no significant differences between these volumes and that of the control group (0·33 mL, 86·4% excretion).

Effects of saiboku-to on gastric secretion in pylorus-ligated rats

The effects of intraduodenal saiboku-to (250, 500 and 1000 mg kg^{-1}), intraduodenal atropine (1 mg kg^{-1}), subcutaneous diazepam (1 mg kg^{-1}), intraduodenal cetraxate (100 mg kg^{-1}) and intraduodenal water (control; 10 mL kg^{-1}) on the volume, pH, acidity and acid output in the gastric juice of pylorus-ligated rats are shown in Table 2. Saiboku-to dose-dependently reduced the volume, acidity and acid output and elevated the pH of the juice. Significant changes were observed at 500 mg kg^{-1} ($P < 0·05$) and higher doses ($P < 0·01$). Similar significant changes in gastric secretion were observed in atropine- or diazepam-treated rats. No significant changes were observed in cetraxate-treated rats.

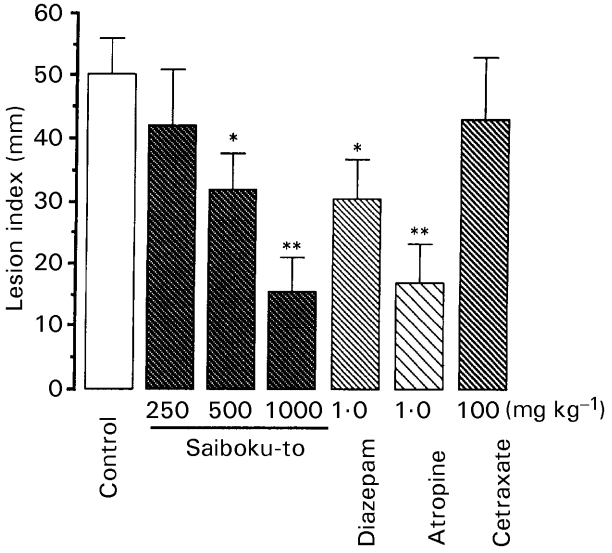

Figure 2. Effects of distilled water (10 mL kg^{-1}, as vehicle control), oral saiboku-to (250, 500 and 1000 mg kg^{-1}), subcutaneous diazepam (1·0 mg kg^{-1}), atropine (1·0 mg kg^{-1}, p.o.), and cetraxate (100 mg kg^{-1}, p.o.) on gastric lesions induced in rats by restraint water-immersion stress. Mean ± s.e.m. n = 8. *$P < 0·05$, **$P < 0·01$ compared with control.

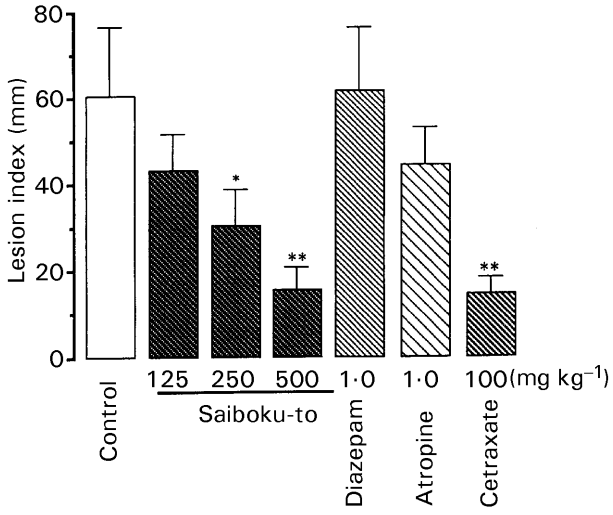

Figure 3. Effects of distilled water (10 mL kg^{-1}, as vehicle control) oral saiboku-to (125, 250 and 500 mg kg^{-1}), subcutaneous diazepam (1·0 mg kg^{-1}), atropine (1·0 mg kg^{-1}, p.o.), and cetraxate (100 mg kg^{-1}, p.o.) on gastric lesions induced in rats by 99·5% ethanol treatment. Mean ± s.e.m. n = 8. *$P < 0·05$, **$P < 0·01$ compared with control.

Table 1. The volumes of the gastric contents of rats 30 min after treatment with saiboku-to.

Group	Injection volume (mL/animal)	Volume (mL) of gastric contents	Excretion rate (%)
Control (10 mL kg^{-1} water)	2·44 ± 0·03	0·33 ± 0·04	86·4 ± 1·7
Saiboku-to 500 mg/10 mL kg^{-1}	2·43 ± 0·05	0·31 ± 0·10	87·4 ± 4·0
Saiboku-to 1000 mg/10 mL kg^{-1}	2·48 ± 0·12	0·44 ± 0·09	82·8 ± 3·5

Excretion rate = [1−(volume of gastric content)/(injection volume)] × 100. Mean ± s.e.m. n = 4.

Table 2. Effects of saiboku-to, diazepam, atropine and cetraxate on gastric secretion in pylorus-ligated rats.

Group	Dose (mg kg^{-1})	pH	Volume (mL/rat)	Acidity (mEq L^{-1})	Acid output (μEq h^{-1})
Control	–	1·22 ± 0·04	7·2 ± 0·30	106·7 ± 4·1	192·8 ± 14·3
Saiboku-to	250	1·25 ± 0·03	6·7 ± 0·84	95·9 ± 3·7	164·1 ± 25·9
Saiboku-to	500	1·38 ± 0·05*	6·0 ± 1·01	79·5 ± 6·5**	127·3 ± 33·6*
Saiboku-to	1000	1·49 ± 0·07**	4·1 ± 0·40**	69·2 ± 7·4***	71·8 ± 11·2***
Diazepam	1	1·37 ± 0·21*	4·2 ± 0·37**	79·0 ± 2·7**	84·0 ± 9·1**
Atropine	1	1·36 ± 0·06*	4·7 ± 0·78*	88·8 ± 7·1*	109·9 ± 23·6*
Cetraxate	100	1·24 ± 0·03	8·5 ± 0·45	102·8 ± 4·6	217·6 ± 13·6

Animals were killed 4 h after pylorus ligation. Drugs (or 10 mL kg^{-1} water in control group) except for diazepam (subcutaneous) were given intraduodenally immediately after pylorus ligation. Mean ± s.e.m. (n = 7). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control.

Examination of mild irritant

Histological examination conducted 30 min after oral administration of water (control), 20% ethanol (mild irritant) or saiboku-to revealed no significant morphological changes in the gastric mucosa of either saiboku-to-treated (Figure 4C1) or control rats (Figure 4A1). Abundant mucus cells, well-stained with AB-PAS were observed for both groups. Mild irritant, 20% ethanol, reduced the surface epithelial or mucus cells stained with AB-PAS (Figure 4B2).

Histological examination conducted 1 h after oral administration of 99·5% ethanol (necrotizing agent) revealed erosion in the water-pretreated control group; surface epithelial cells or mucus cells stained with AB-PAS were desquamated and lamina propria mucosae was slightly injured (Figure 4A2). Pretreatment with mild irritant (Figure 4B2) prevented the erosion induced by 99·5% ethanol; mucus cells stained with AB-PAS decreased but not desquamated, similar to the results obtained 30 min after 20% ethanol treatment. Saiboku-to (Figure 4C2) prevented the development of the mucosal lesions induced by 99·5% ethanol; mucus cells were well-stained with AB-PAS, as was the stomach not exposed to 99·5% ethanol (Figure 4A2).

The normal level of gastric mucosal hexosamine in the rats not exposed to 99·5% ethanol was 1573·9 ± 55·6 μg mg^{-1}. In the 99·5%-ethanol group, a decrease from the normal level of

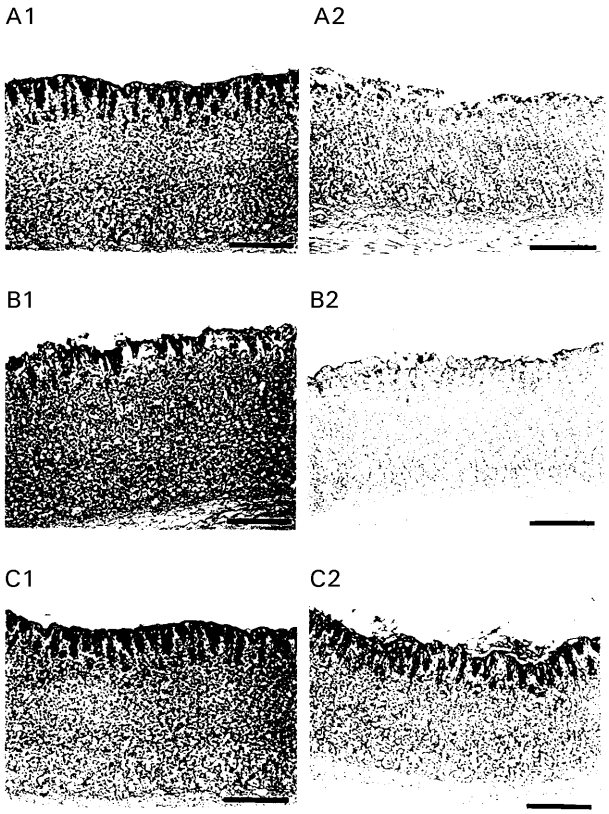

Figure 4. Histological findings (AB-PAS stain; magnification 100×, bar = 100 μm) 30 min after oral administration of water (10 mL kg^{-1}; A1), 20% ethanol (10 mL kg^{-1}; B1) or saiboku-to (500 mg kg^{-1}; C1) and 1 h after oral administration of 99·5% ethanol (A2, B2 and C2, respectively). AB-PAS stain was used for detecting mucus cells.

(approx.) 33% was observed ($1055{\cdot}3 \pm 66{\cdot}7$ $\mu g\,mg^{-1}$, $P < 0{\cdot}01$). Pretreatment with the mild irritant 20% ethanol ($1302{\cdot}0 \pm 61{\cdot}0\,\mu g\,mg^{-1}$, $P < 0{\cdot}05$) or saiboku-to ($1237{\cdot}3 \pm 40{\cdot}0\,\mu g\,mg^{-1}$, $P < 0{\cdot}05$) inhibited the decrease of hexosamine induced by 99·5% ethanol.

Discussion

In this study two types of experimental gastric lesion model were used to examine the possibility of saiboku-to having an anti-ulcer effect. Gross and histological findings confirmed that gastric lesions induced by restraint water-immersion stress or treatment with 99·5% ethanol was the result of erosion; the lesion index was almost the same as those reported in previous studies (Yamamoto et al 1986; Takase et al 1988; Matsuta et al 1996). The current work demonstrated that saiboku-to inhibited the development of gastric erosion induced by restraint water-immersion stress or by ethanol treatment.

In the experiment on ethanol-induced gastric lesions, 99·5% ethanol was administered orally to induce gastric lesion 30 min after the oral administration of saiboku-to. If a large amount of the injected saiboku-to remains in the stomach 30 min after the administration, the gastric lesions induced by subsequent administration of 99·5% ethanol might be inhibited by reduction of the concentration of ethanol as a result of dilution. We therefore examined the volume of the gastric contents of rats treated with saiboku-to 30 min after the oral administration. As shown in Table 1, the residual volumes in the stomachs of saiboku-to-treated and control animals was small, suggesting that the anti-gastric lesion effect of saiboku-to is not a consequence of ethanol dilution.

Robert et al (1983) have reported the occurrence of protection by mild irritant, denoted adaptive cytoprotection—pretreatment with several mild irritants, for example 10–20% ethanol, 0·2–0·35 M HCl, 0·05–0·07 M NaOH, 2–4% NaCl or water at 70°C, inhibits the necrotic lesion induced by subsequent oral administration of necrotizing agents, for example 100% ethanol, 0·6 M HCl, 0·2 M NaOH, 25% NaCl solution or boiling water. Therefore, it is important to determine whether saiboku-to is indeed a mild irritant. The mild irritant (20% ethanol) used in this study induced a decrease in the number of surface epithelial cells 30 min after administration and protected the stomach against mucosal erosion caused by the strong irritant. However, saiboku-to protected the strong irritant-induced erosion without causing the mild irritation observed for stomachs treated with 20% ethanol. A general pharmacological study has demonstrated that orally administered saiboku-to, at 500, 1000 or 2000 $mg\,kg^{-1}$, had no effect on gastric mucosa and intestinal mucosa in rats (Matsumoto et al 1994). Taken together with current results this clearly suggests that saiboku-to is not a mild irritant, and that the anti-erosion effect of saiboku-to is different from the protection afforded by mild irritants.

Ethanol-induced gastric lesions are thought to arise as a result of direct damage of gastric mucosal cells, resulting in the development of free radicals and hyperoxidation of lipid (Puurunen et al 1980; Pihan et al 1987). It is reported that ethanol-induced gastric lesions are not inhibited by an anti-gastric acid secretory agent, cimetidine (Szabo 1987), but are inhibited by agents which enhance mucosal defensive factors such as sucralfate (Matsuta et al 1996) and cetraxate (Hashizume et al 1976; Suzuki et al 1979; Chouno et al 1982; Kurebayashi et al 1988). Similar results were obtained in the current study—ethanol-induced erosion was not inhibited by diazepam, the central anxiolytic drug, and atropine, an anti-cholinergic, at the $1{\cdot}0\,mg\,kg^{-1}$ dose which caused significant inhibition of gastric acid secretion, whereas cetraxate prevented the erosion at the $100\,mg\,kg^{-1}$ dose which did not affect the acid secretion. The anti-erosion effect of saiboku-to in the ethanol-induced lesion, also observed with the $250\,mg\,kg^{-1}$ dose, did not affect acid secretion, suggesting that the protection involves an acid-independent mechanism. Pretreatment with saiboku-to prevented disruption of the surface epithelial cells stained with AB-PAS and the decrease of the level of mucus glycoprotein, hexosamine, as quantitative index of gastric mucus damage. It is well known that gastric mucus cells form a mucous barrier to protect gastric mucosa from the aggression of gastric acid and pepsin (Isenberg et al 1991). Therefore, the current results suggest that saiboku-to might enhance gastric mucosal defensive factors.

With regard to the restraint water-immersion stress model, it has been reported that stress-induced gastric lesions develop as a result of stimulation of the vagal nerve which increases gastric secretion (Kitagawa et al 1979), gastric motility (Garrick et al 1986), the diminution of gastric mucus (Hakkinen et al 1966), and the alteration in the microcirculation of gastric mucosa (Guth & Hall 1966). In the current study the anti-cholinergic agent atropine and the anxiolytic drug diazepam inhibited the production of stress-induced gastric acid, whereas cetraxate did not. This implies that restraint water-immersion stress-induced gastric lesions arise primarily as a result of the secretion of gastric acid, an aggressive factor affecting gastric ulcer. Saiboku-to inhibited the volume, the acid

output and the acidity of gastric juice (confirmed by the increase in pH). In this gastric secretion study saiboku-to was injected into the duodenum after pylorus ligation and so the inhibition of gastric secretion induced by saiboku-to was thought to be because of absorption from the duodenum. Gastric acid secretion is regulated by many factors including anxietic effect in the CNS, vagal activity, cholinergic, histaminergic and gastrinergic neurotransmissions, the activities of various post-synaptic receptors such as histamine H_2, muscarine and gastrine receptors, and the proton pump (Isenberg et al 1991). It is therefore difficult to elucidate the relationship between the mechanism of inhibition of gastric acid and saiboku-to. However, Nishiyori et al (1985) have reported the effect of saiboku-to used for treatment of bronchial asthma on Type I hypersensitivity reactions, and have suggested that saiboku-to inhibits Type I hypersensitivity reaction by suppression of histamine release. Toda et al (1988) have also found that saiboku-to inhibits histamine release from mouse peritoneal mast cells and the degranulation of these cells induced by compound 48/80. These findings suggest that inhibition of gastric acid secretion by saiboku-to reported in this paper might be related to the release of gastric histamine. Although further investigation is necessary to confirm this hypothesis, our current data clearly demonstrate that, at the very least, saiboku-to dose-dependently inhibits the aggressive factor, gastric acid secretion.

Compared with the anti-erosion effect of saiboku-to on stress-induced gastric lesions, the effect on the ethanol model was observed at a lower concentration ($250\,mg\,kg^{-1}$ compared with $500\,mg\,kg^{-1}$ for the stress model). These results suggest that the augmentative effect of gastric mucosal defensive factor of saiboku-to might be greater than the inhibitory effect on the aggressive factor, gastric acid secretion.

Identification of active compounds in saiboku-to was not performed in this study. As described above, saiboku-to consists of 10 herbal medicines, bupleuri radix, pinelliae tuber, hoelen, scutellariae radix, magnoliae cortex, zizyphi fructus, ginseng radix, glycyrrhizae radix, perillae herba and zingiberis rhizoma. After pharmacological investigations of some herbal medicines and several components of saiboku-to, it has been reported that magnoliae cortex, which contains magnolol and honokiol, inhibits the CNS and vagal activity (Watanabe et al 1973, 1983). Furthermore, magnolol, but not honokiol, has been reported to inhibit stress-induced lesions and gastric secretion (Yano 1997). More recently, we concluded that both honokiol and magnolol have an anxiolytic effect (Maruyama et al 1998). Polysaccharides prepared from bupleuri radix have been reported to enhance factors protecting the gastric mucosa (Shibata et al 1973, 1976; Yamada et al 1991). Pinelliae tuber has been reported to suppress the vagal-gastric activity induced by apomorphine and copper sulphate (Niijima et al 1993). It is also known that glycyrrhizin in glycyrrhizae radix and the derivative, carbenoxolone, protect restraint water-immersion stress-induced ulcer (Yano 1997). Taken together, it is speculated that the anti-ulcer effects of saiboku-to might be because of the synergism (addition or potentiation) produced by these active components, though further studies are necessary to confirm this hypothesis.

In conclusion, it is suggested that the anti-erosion effect of saiboku-to, which is not a mild irritant, involves both the inhibition of the aggressive factor and the augmentation of the defensive factor.

Acknowledgements

The authors acknowledge valuable technical assistance of Takemi Shiobara of the Department of Neuropsychopharmacology (Tsumura) of Gunma University.

References

Adams, R. D., Victor, M. (1993) Psychiatric disorders. In: Adams, R. D., Victor, M. (eds) Principles of Neurology. 5th edn, McGraw-Hill, New York, pp 1285–1310

Boas, N. F. (1953) Method of the determination of hexosamine in tissue. J. Biol. Chem. 204: 553–563

Chouno, S., Arakawa, H., Yamada, H., Nakamura, H., Kobayashi, K. (1982) Effect of cetraxate on prostaglandins of rat gastric mucosa. Prog. Med. 2: 2065–2068

File, S. F., Pearce, J. B. (1981) Benzodiazepines reduced gastric ulcers induced in rat by stress. Br. J. Pharmacol. 74: 593–599

Garrick, T., Leung, F. W., Buack, S., Hirabayashi, K., Guth, P. H. (1986) Gastric motility is stimulated but overall blood flow is unaffected during cold restraint in the rat. Gastroenterology 91: 141–149

Guth, P. H., Hall, P. (1966) Microcirculatory and mast cell changes in restraint induced gastric ulcer. Gastroenterology 50: 562–570

Hakkinen, I., Hartiala, K., Lang, H. (1966) The effect of restraint on the content of acid polysaccharides of glandular gastric wall in rat. Acta Physiol. Scand. 66: 333–336

Hashizume, T., Asano, M., Aibara, S., Irie, K., Hirokawa, K., Fujita, H., Kasahara, A. (1976) Antiulcer effect and pharmacological properties of 4′-(2-carboxyethyl)phenyl *trans*-4-aminomethylcyclohexanecarboxylate hydrochloride (DV-1006). Pharmacometrics 12: 691–702

Hernandez, D. E., Arandia, D., Dehesa, M. (1993) Role of psychosomatic factors in peptic ulcer disease. J. Physiol. Paris 87: 223–227

Homma, M., Oka, K., Kobayashi, H., Niitsuma, T., Yamamoto, S., Itoh, H., Takahashi, N. (1993) Impact of free magnolol excretions in asthmatic patients who responded well to saiboku-to, a Chinese herbal medicine. J. Pharm. Pharmacol. 45: 844–846

Imperato, A., Dazzi, L., Obinu, M. C., Gessa, G. L., Biggio, G. (1994) The benzodiazepine receptor antagonist flumazenil increases acetylcholine release in rat hippocampus. Brain Res. 647: 167–171

Isenberg, J. I., McQuaid, K. R., Laine, L., Rubin, W. (1991) Acid-peptic disorders. In: Yamada, T., Alpers, D. H., Owyang, C., Powell, D. W., Silverstein, F. E. (eds) Textbook of Gastroenterology. J. B. Lippincott, Philadelphia, pp 1241–1339

Kitagawa, H., Fujiwara, M., Osumi, Y. (1979) Effects of water-immersion stress on gastric secretion and mucosal blood flow in rats. Gastroenterology 77: 298–302

Kudo, Y., Kudo, T. (1995) Recent progress in development of psychotropic drug: (1) anti-anxiety drugs. Jpn. J. Neuropsychopharmacol. 15: 75–86

Kurebayashi, Y., Ikeda, T., Osada, Y. (1988) Cytoprotective action of cetraxate against HCl.ethanol-induced gastric lesion. Jpn. J. Pharmacol. 46: 17–25

Kuribara, H., Maruyama, Y. (1996) The anxiolytic effect of oriental herbal medicines by an improved plus-maze test in mice: involvement of benzodiazepine receptors. Jpn. J. Neuropsychopharmacol. 18: 179–190

Kuribara, H., Morita, M., Ishige, A., Hayashi, K., Maruyama, Y. (1996) Investigation of anxiolytic effect of the extracts derived from saiboku-to, an oriental herbal medicine, by an improved plus-maze test in mice. Jpn. J. Neuropsychopharmacol. 18: 643–653

Maruyama, Y., Kuribara, H., Morita, M., Yuzurihara, M., Weintraub, S. T. (1998) Identification of magnolol and honokiol as anxiolytic agents in extracts of saiboku-to, an oriental herbal medicine. J. Nat. Prod. 61: 135–138

Matsuta, M., Kanita, R., Tsutsui, F., Yamashita, A. (1996) Antiulcer properties of shosaiko-to. Folia Pharmacol. Jpn 108: 217–225

Matsumoto, A., Iwasaki, S., Yamaguchi, K., Kyuki, K., Iwahashi, K. (1994) General pharmacology of saiboku-to extract. Pharmacometrics 48: 383–399

Narita, H. (1990) Use of Kampo medicine in psychiatry. Jpn J. Neuropsychopharmacol. 12: 165–172

Niijima, A., Okui, Y., Kubo, M., Higuchi, M., Taguchi, H., Mitsuhashi, H., Maruno, M. (1993) Effect of *Pinellia ternata* tuber on the efferent activity of the gastric vagus nerve in the rat. Brain Res. Bull. 32: 103–106

Nishiyori, T., Tsuchiya, H., Inagaki, N., Nagai, H., Koda, A. (1985) Effect of saiboku-to, a blended Chinese traditional medicine, on type 1 hypersensitivity reactions, particularly on experimentally-caused asthma. Folia Pharmacol. Jpn 85: 7–16

Pihan, G., Regillo, C., Szabo, S. (1987) Free radical and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. Dig. Dis. Sci. 32: 1395–1401

Piper, D. W., Tennant, C. (1993) Stress and personality in patients with chronic peptic ulcer. J. Clin. Gastroenterol. 16: 211–214

Puurunen, J., Huttunen, P., Hirvonen, J. (1980) Is ethanol-induced damage of the gastric mucosa a hyperosmotic effect? Comparative studies on the effects of ethanol, some other hyperosmotic solutions and acetylsalicylic acid on rat gastric mucosa. Acta Pharmacol. Toxicol. 47: 321–327

Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J. (1979) Cytoprotection by 4 prostaglandins in rats: prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology 77: 433–443

Robert, A., Nezamis, J. E., Lancaster, C., Davis, J. P., Field, S. O., Hanchar, A. J. (1983) Mild irritants prevent gastric necrosis through ''adaptive cytoprotection'' mediated by prostaglandins. Am. J. Physiol. 245: 113–121

Shibata, M., Yoshida, R., Motohashi, S., Fukushima, M. (1973) Pharmacological studies on *Bupleurum falcatum* L. IV. Some pharmacological effects of crude saikosides, saikogenin A and syrupy residue. Yakugaku Zasshi 93: 1660–1667

Shibata, M., Isomura, A., Inoue, T., Nagai, M. (1976) Some pharmacological studies on the crude drugs possessing anti-inflammatory properties: on the root of bupleurum and the leaves of fig. Syoyakugaku Zasshi 30: 62–66

Sieghart, W. (1995) Structure and pharmacology of gamma-aminobutyric acid A receptor subtype. Pharmacol. Rev. 47: 181–234

Suzuki, Y., Ito, M., Sudo, Y. (1979) Studies on the mechanism of action of cetraxate [4′-(2-carboxyethyl)phenyl *trans*-4-aminomethylcyclohexanecarboxylate hydrochloride], a new anti-ulcer agent. Jpn. J. Pharmacol. 29: 829–838

Szabo, S. (1987) Mechanisms of mucosal injury in the stomach and duodenum: time-sequence analysis of morphologic, functional, biochemical and histochemical studies. Scand. J. Gastroenterol. 22: 21–28

Takagi, K., Okabe, S. (1968) The effects of drugs on the production and recovery processes of the stress ulcer. Jpn. J. Pharmacol. 18: 9–18

Takase, H., Imanishi, K., Miura, O., Yumioka, E., Watanabe, H. (1988) Pharmacological studies on the effects of some traditional Chinese medicines on gastric functions. 3. The effects of oren-gedoku-to (OGT), san'o-syasin-to (SST), antyu-san (AS) and dai-saiko-to (DST) on ethanol-and aspirin-induced gastric lesions in rats. Folia Pharmacol. Jpn 91: 319–324

Toda, S., Kimura, M., Ohnishi, M., Nakashima, K. (1988) Effects of the Chinese herbal medicine ''Saiboku-to'' on histamine release from and the degranulation of mouse peritoneal mast cells induced by compound 48/80. J. Ethnopharmacol. 24: 303–309

Umesato, Y. (1984) Asthmatic children and Chinese medicine. Allergy 33: 1047–1053

Varis, K. (1987) Psychosomatic factors in gastrointestinal disorders. Ann. Clin. Res. 19: 135–142

Watanabe, K., Goto, Y., Yoshitomi, K. (1973) Central depressant effects of the extracts of Magnolia Cortex. Chem. Pharm. Bull. 21: 1700–1708

Watanabe, K., Watanabe, H., Goto, Y., Yamaguchi, M., Yamamoto, N., Hagino, K. (1983) Pharmacological properties of magnolol and honokiol extracted from *Magnolia officinalis*: central depressant effects. Planta Med. 49: 103–108

Yamada, H., Sun, X. B., Matsumato, T., Ra, K. S., Hirano, M., Kiyohara, H. (1991) Purification of anti-ulcer polysaccharides from the roots of *Bupleurum falcatum*. Planta Med. 57: 555–559

Yamamoto, H., Nakamura, Y., Kunoh, Y., Ichihara, K., Nagasaka, M., Asai, H. (1986) Antiulcer effect of (−)-*cis*-2,3-dihydro-3-(4-methylpiperazinylmethyl)-2-phenyl-1,5-benzothiazepin-4-(5*H*)-one hydrochloride (BTM-1086) in experimental animals. Jpn. J. Pharmacol. 41: 283–292

Yano, S. (1997) Effect of kampo medicine on stress-induced ulceration. Prog. Med. 17: 887–893