Inhibition of Gastric Acid Secretion by Saiboku-to, an Oriental Herbal Medicine, in Rats

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Intraduodenal saiboku-to (250–1000 mg/kg) dose-dependently reduced gastric acid secretion and histamine output, without altering acetylcholine output in pylorus-ligated rats. Saiboku-to also inhibited subcutaneous bethanechol (1 mg/kg) and tetragastrin (0.3 mg/kg) -induced increases in gastric acid secretion in vagotomized pylorus-ligated rats; however, it did not inhibit subcutaneous histamine (20 mg/kg) -induced increase in acid secretion. These results, taken together, suggest a possibility that saiboku-to may inhibit histamine release. Thus, the effect of saiboku-to on histamine release was directly investigated by using anti-dinitrophenyl IgE-sensitized rat peritoneal mast cells. Antigen (dinitrophenyl)-induced histamine release from the mast cells was clearly dose-dependently inhibited by saiboku-to at concentrations of 0.1–1.0 mg/ml. These results suggest that the inhibited gastric acid secretion with saiboku-to is due to inhibited histamine release.

KEY WORDS: gastric acid; histamine; mast cells; pylorus-ligated rats; saiboku-to.

Saiboku-to, an Oriental herbal medicine, is a traditional Chinese medicine called Kampo medicine in Japan. It is a mixture of 10 medical herbs and has been used for treatment of bronchial asthma (1) and anxiety-related disorders such as neurosis (2). Recently, we demonstrated that saiboku-to inhibited gastric erosion induced by restraint water-immersion stress or by ethanol treatment (3). The antierosion effect involved the inhibition of gastric acid secretion, one of aggressive factors. However, acid secretion is regulated by many factors including anxietic effect in the central nervous system (CNS); vagal activity; cholinergic, histaminergic, and gastrinergic neurotransmissions; and the activities of various postsynaptic receptors such as muscarine, histamine H2 and gastrin receptors; and proton pump (4). On the other hand, Nishiyori et al (5) and Toda et al (6) have suggested that saiboku-to inhibits type I hypersensitivity reaction through the suppression of histamine release. We, thus, hypothesized in a previous paper (3) that the inhibition of gastric acid secretion might be also related to the release of gastric histamine. However, the specifics of the inhibitory mechanism of saiboku-to on gastric acid remain to be fully demonstrated.

The purpose of the present study is to clarify the effect of the inhibitory mechanism of saiboku-to on gastric acid secretion.

MATERIALS AND METHODS

Animals. Male Wistar rats purchased from SLC Japan (Hamamatsu, Japan) were housed in a facility at a temperature of 24 ± 1°C, relative humidity of 55 ± 5%, and controlled lighting with lights on from 07:00 to 19:00 hr
Acid, Histamine and ACh Levels in Gastric Juice.

The rats saiboku-to were prepared in distilled water (10 ml) and collected and centrifuged at 17,300 g under the same halothane anesthesia. Gastric juice wasachs of the rats were removed after the cardia was ligatedened rats. Four hours following pylorus ligation, the stom-

for determination of ACh level in gastric juice, 1 ml of 1

min after the surgical anesthesia stopped. In the experiment immediately after pylorus-ligation. The rats awaked within 5

were intraduodenally or subcutaneously administered im-

posterior) gastric branches of the celiac vagus (7). Drugs

anesthesia performed using a small animal anesthetizer

weighing 240 –260 g after a 24-hr fast were pylorus-ligated

resultant decoction freeze-dried to obtain saiboku-to ex-

gently in 500 ml of water for 60 min, filtered, and the

rhizoma (1 g). The 10 dried medical herbs were boiled

glycyrrhizae radix (2 g), perillae herba (2 g) and zingiberis
cortex (3 g), zizyphi fructus (3 g), ginseng radix (3 g),

and iodoethane purchased from Sigma.

iodide (EHC) was synthesized from 3-diamino-1-propanol

injection (Green Cross, Osaka, Japan). Ethylhomocholine
icals, Ann Arbor, Michigan, USA), and heparin sodium

standards laboratory food (MF, Oriental Yeast, Tokyo, Ja-

daily. The animals were allowed free access to water and

and expressed as milliequivalents per liter. Acid output was

calculated as follows: Acid output (μeq/hr) = volume (ml/4 hr) × acidity (meq/liter/sampling time (4 hr).

Determination of Histamine Level in Gastric Juice. One hundred microliters of 1.0 mg/ml spermidine as an internal standard of histamine, 120 μl of 5M NaOH, 400 mg of NaCl, and 3.5 ml of n-butanol were added to 1.0 ml of gastric juice. The mixture was centrifuged at 1300g at 4°C for 5 min. After the lower layer was removed, an upper

layer was washed twice with 2 ml of NaCl-saturated 0.1 M NaOH. Three milliliters of the butanol layer (an upper layer) was transferred to the other test tube, and 4 ml of n-heptane as well as 2.5 ml of 0.1 M HCl were added to it and mixed. After 5 min, the upper layer was removed by aspiration. An aliquot (100 μl) of the lower layer was injected into a high-performance liquid chromatography (HPLC) system for the determination of histamine level.

The HPLC system is equipped with fluorescent detection (LC-10AD, Shimazu, Kyoto, Japan) as well as postcolumn derivatization with OPA (8). A STR ODS-II reversed-phase column (4.6 mm ID × 150 mm length, Shimazu) was used for separation of histamine and spermidine. The mobile phase consisted of a mixture of solution A (100 mM sodium tartaric acid buffer, pH 4.4, containing 10 mM sodium 1-octanesulfonate) and solution B (99.7% methanol for HPLC) (A/B 2:1). The flow-rate was maintained at 1.0 ml/min. The effluent from the column was mixed with a postcolumn solution (400 mM sodium borate buffer, pH 9.2, and 10 mM OPA in methanol; 2:1) at a flow rate of 0.5 ml/min. The mixture of the effluent and the postcolumn solution flowed to a reaction coil of stainless steel tubing (0.5 mm ID × 4.0 m length) at a final flow rate of 1.5 ml/min and passed through a fluorescent detector where the OPA derivatives of histamine and spermidine were detected at excitation of 360 nm and emission of 440 nm. Temperatures of the separation column and the reaction coil were maintained at 50°C. A Shimazu LC-10A workstation was employed for data collection and processing. The retention times were 4.40 min for histamine and 7.89 min for spermidine. The detection limit of histamine in the injected sample was 10 ng.

Histamine output was calculated as follows: Histamine output (ng/hr) = volume (ml/4 hr) × histamine level (ng/ml)/sampling time (4 hr).

Determination of ACh Level in Gastric Juice. One milliliter of the gastric juice containing eserine was neutralized at pH 7.4 by 1 M NaOH. EHC (1 μmol/20 μl), an internal standard of ACh, was added to it. The mixture was centrifuged at 17,300g at 4°C for 15 min. The supernatant was purified by passage through a 0.45-μm millipore filter. An aliquot, typically 10 μl of the filtrate, was injected into HPLC system for determination of the ACh level.

The HPLC system (Nanospec, Shiseido, Tokyo, Japan) is equipped with electrochemical detection (9). An ACh Separation analytical column [3 μm, 4 × 60 mm, polymeric styrene-based packing materials, Bioanalytical Systems (BAS) Tokyo, Japan] was used for separation of EHC and ACh. An immobilized column (5 × 4 mm, BAS) containing acetylcholinesterase and choline oxidase was used for postcolumn reaction. A glassy carbon precolumn (4 × 10 mm, Irica, Kyoto, Japan) was set up between the injector and the

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