The Effects of Hange-shashin-to on Gastric Function in Comparison with Sho-saiko-to

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The effects of “Hange-shashin-to (TJ-14)” on gastric function were examined in comparison with “Sho-saiko-to (TJ-9)”. Oral treatment with TJ-14 (125–500 mg/kg) caused dose-dependent suppression of ethanol-induced gastric injury, while it did not suppress gastric lesions induced by water-immersion stress. TJ-9 (125–500 mg/kg, p.o.) suppressed both water-immersion stress-induced gastric lesions and ethanol-induced gastric injury in a dose-dependent manner. Intraduodenal administration of TJ-14 even at 500 mg/kg did not affect gastric juice secretion, while TJ-9 at 125 to 500 mg/kg dose-dependently suppressed gastric juice secretion. TJ-14 (125–500 mg/kg, p.o.) accelerated gastric emptying in normal rats and improved the delayed gastric emptying induced by BaCl2 in a dose-dependent manner, whereas such effect was not noted with TJ-9. Oral treatment with TJ-14 at 500 mg/kg significantly suppressed apomorphine-induced vomiting, but it did not affect copper sulfate-induced vomiting. These results suggest that TJ-14 exhibits an anti-ulcer action (probably based on its ability to protect the gastric mucosa), improvement of gastric emptying and an anti-emetic action. TJ-9 also showed anti-ulcer effects, probably based on its ability to suppress gastric secretion and to protect the gastric mucosa.

Thus, the present study demonstrated the effectiveness of TJ-14 and TJ-9 against gastric disease, and provided basic data which explain the differences in clinical application between these two kampo medicines.

Key words Hange-shashin-to; Sho-saiko-to; gastric ulcer; gastric emptying; emesis

Hange-shashin-to (TJ-14) is often used to treat acute and chronic gastrointestinal catarrh, fermentative diarrhea and acute gastroenteritis.1–4) Sho-saiko-to (TJ-9) is indicated for acute febrile disease, pneumonia, bronchitis, the common cold, hepatic dysfunction, and chronic gastroenteric disease. A variety of basic studies have been conducted concerning the effects of TJ-9 in treating hepatic dysfunction.5–8) Both kampo medicines are used clinically to treat gastric diseases. The herbal contents are approximately the same in both preparations. The only difference is that TJ-14 contains Coptidis Rhizoma while TJ-9 contains Bupleuri Radix instead of C. Rhizoma. Despite the similarities in composition, there are slight differences in their indications. TJ-14 is indicated for nausea, vomiting and neurogenic gastritis, while TJ-9 is indicated for stomach oppression, gastritis and gastric ulcers. Although a number of studies have examined the effects of TJ-14 and TJ-9 on the digestive system,9,10,11 the relationship between their indications and pharmacological profiles has not yet been studied in detail.

The present study was performed to characterize the impact of TJ-14 and TJ-9 by assessing their effects on gastric function.

MATERIALS AND METHODS

Animals Eight-week-old male Wistar rats (Japan SLC) and male Marshall ferrets (Marshall Farms, U.S.A.) weighing 1.6–2.8 kg were used. The animals were bred in quarters where the temperature and relative humidity were kept at 23 ± 2 ºC and 55 ± 10% with light on between 7:00 and 19:00. The animals were allowed free access to food and drinking water.

Drugs TJ-14 and TJ-9 were obtained as spray dried powder extracts from Tsunara Co., Ltd. The quality of both preparations was assured by keeping the amount of index components within a prescribed range. TJ-14 was manufactured from a mixture of Pinelliae Tuber (5.0), Scutellariae Radix (2.5), Glycyrrhizae Radix (2.5), Zizyphi Fructus (2.5), Ginseng Radix (2.5), Zingiberis sieciatum Rhizoma (2.5) and Coptidis Rhizoma (1.0), while TJ-9 was manufactured from a mixture of Bupleuri Radix (7.0), Pinelliae Tuber (5.0), Scutellariae Radix (3.0), Zizyphi Fructus (3.0), Ginseng Radix (3.0), Glycyrrhizae Radix (2.0) and Zingiberis Rhizoma (1.0). The yield of each extract was 24% for TJ-14 and 19% for TJ-9. Atropine sulfate, apomorphine hydrochloride, chlorpromazine hydrochloride, cimetidine and metoclopramide hydrochloride were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Copper sulfate and barium chloride were purchased from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). Cetaxate (Neuer, Daiichi Pharmaceutical Co., Tokyo, Japan) was purified by extraction at our company before use.

Effects on Ethanol-induced Gastric Lesions The experiment was carried out according to the method of Robert et al.12) Rats, fasted for 24 h, received an oral dose of the test drug. Thirty minutes later, 1 ml of 95.5% ethanol was orally administered to these rats. One hour after the ethanol administration, the animals were sacrificed, and the total length of injury observed in the stomach was measured to serve as the lesion index.

Effects on Water-immersion Stress Induced Gastric Lesions The method reported by Takagi and Okabe was employed.13) Thirty minutes after an oral dose of the test drug, the animals were placed in restraint cages and were then immersed in water (23 ºC) for 7 h. At the end of this stress, the animals were sacrificed and the total length of injury observed in the glandular area of their stomachs was measured to serve as the lesion index.

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Effects on Gastric Juice Secretion The effects of test drugs on gastric juice secretion were examined using the method of Shay et al.14) Rats were derived of food but allowed free access to water for 24 h. Under ether anesthesia, the abdomen of each rat was incised and the pylorus was ligated. The abdomen was then closed. Four hours later, celiotomy was conducted again to ligate the cardia and remove the stomach. Gastric juice was collected, and then centrifuged at 3000 rpm and 4°C for 10 min. The supernatant was used to measure volume, pH, total acidity and pepsin activity. The acidity of gastric juice was determined by titration against 0.1N NaOH using an automatic titrator (AUT-1, TOA, Japan). Pepsin activity was measured according to the method of Anson.15) The amounts of acid and pepsin were expressed as μE/q h and mg/h, respectively. Test drug or distilled water was administered to the duodenum immediately after pylorus ligation.

Effects on Gastric Emptying Rats, fasted for 24 h, received an oral dose of the test drug. Thirty minutes later, phenol red at 100 μg/ml was orally administered, and fifteen minutes thereafter, the animals were sacrificed and the amount of phenol red retained in their stomachs was measured using the method of Yokochi et al.16) Barium chloride at 3.5 mg/kg was intraperitoneally injected to the rats five minutes prior to the phenol red dose in order to reduce gastric emptying. The effects of test drugs on barium chloride-induced reduction of gastric emptying were also assessed.

Effects on Vomiting Induced by Apomorphine or Copper Sulfate The method reported by Costall et al. was used.17) Both TJ-14 (125—500 mg/kg) and chlorpromazine (5 mg/kg) were orally administered to the ferrets. One hour later, vomiting was induced in these animals by either a subcutaneous injection of apomorphine (0.25 mg/kg) or an oral dose of copper sulfate (50 mg/kg). Both the incidence and the frequency of vomiting were measured during the one-hour period following its administration.

Statistical Analysis All values are expressed as means ± S.E. The data were evaluated by one-way analysis of variance (ANOVA) followed by Fishers least significant difference procedure.

RESULTS

Effects on Ethanol-induced Gastric Lesions Both TJ-14 and T9 suppressed ethanol-induced gastric lesions in a dose-dependent manner at doses between 125 and 500 mg/kg. Oral treatment of cetraxate at 100 mg/kg markedly suppressed these lesions (Fig. 1).

Effects on Water-immersion Stress Induced Gastric Lesions TJ-14 did not markedly suppress gastric lesions induced by water-immersion stress at 500 mg/kg, while T9 at 500 mg/kg significantly suppressed lesions induced in this way. Significant suppression was noted by atropine at 3 mg/kg (Fig. 2).

Effects on Gastric Juice Secretion TJ-14 did not affect gastric juice secretion even at 500 mg/kg, while T9 reduced the volume of gastric juice, acid output and pepsin output in a dose-dependent manner at doses between 125 and 500 mg/kg. Cimetidine markedly suppressed gastric juice secretion at 50 mg/kg (Tables 1 and 2).

Effects on Gastric Emptying TJ-14 dose-dependently

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Table 1. Effects of TJ-14 and Cimetidine on Gastric Secretion in Pylorus-Ligated Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>pH</th>
<th>Volume (ml)</th>
<th>Acidity (mEq/l)</th>
<th>Acid output (μEq/h)</th>
<th>Pepsin output (mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>10</td>
<td>1.30 ± 0.02</td>
<td>5.2 ± 0.3</td>
<td>102.4 ± 3.9</td>
<td>135.5 ± 11.6</td>
<td>3.70 ± 0.29</td>
</tr>
<tr>
<td>TJ-14</td>
<td>125</td>
<td>10</td>
<td>1.33 ± 0.04</td>
<td>5.3 ± 0.4</td>
<td>94.9 ± 5.0</td>
<td>126.5 ± 13.5</td>
<td>3.78 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>10</td>
<td>1.37 ± 0.05</td>
<td>5.0 ± 0.7</td>
<td>88.7 ± 5.9</td>
<td>117.5 ± 23.2</td>
<td>3.61 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>10</td>
<td>1.36 ± 0.04</td>
<td>4.3 ± 0.5</td>
<td>91.2 ± 5.8</td>
<td>102.9 ± 17.8</td>
<td>3.33 ± 0.35</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>50</td>
<td>10</td>
<td>1.88 ± 0.15***</td>
<td>2.7 ± 0.4***</td>
<td>59.7 ± 4.7***</td>
<td>41.6 ± 6.9***</td>
<td>2.00 ± 0.27***</td>
</tr>
</tbody>
</table>

Animals were sacrificed 4 h after pylorus ligation. Each drug was given intraduodenally immediately after pylorus ligation. Each value represents the mean ± S.E. ** and ***: Significantly different from the control at p < 0.01 and p < 0.001, respectively.
Table 2. Effects of TJ-9 and Cimetidine on Gastric Secretion in Pylorus-Ligated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>pH</th>
<th>Volume (ml)</th>
<th>Acidity (mEq/l)</th>
<th>Acid output (µEq/h)</th>
<th>Pepsin output (µg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.27 ± 0.02</td>
<td>10</td>
<td>5.8 ± 0.3</td>
<td>102.9 ± 3.6</td>
<td>151.6 ± 11.8</td>
<td>4.14 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>TJ-9</td>
<td>1.31 ± 0.03</td>
<td>10</td>
<td>5.5 ± 0.4</td>
<td>96.5 ± 3.2</td>
<td>135.2 ± 13.5</td>
<td>3.75 ± 0.31</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>1.34 ± 0.03</td>
<td>10</td>
<td>5.0 ± 0.6</td>
<td>93.3 ± 3.9</td>
<td>119.4 ± 16.6</td>
<td>3.47 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>1.44 ± 0.05**</td>
<td>10</td>
<td>4.2 ± 0.1**</td>
<td>85.3 ± 4.4**</td>
<td>90.9 ± 7.2**</td>
<td>2.65 ± 0.25**</td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>1.88 ± 0.08***</td>
<td>10</td>
<td>2.6 ± 0.3***</td>
<td>53.6 ± 2.8***</td>
<td>34.8 ± 3.8***</td>
<td>1.77 ± 0.16***</td>
<td></td>
</tr>
</tbody>
</table>

Animals were sacrificed 4 h after pylorus ligation. Each drug was given intraduodenally immediately after pylorus ligation. Each value represents the mean ± S.E. * ** and ***: Significantly different from the control at p < 0.05, p < 0.01 and p < 0.001, respectively.

Table 3. Effects of TJ-14 and Chlorpromazine on Apomorphine-Induced Emesis in the Ferret

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of animals Vomited (%)</th>
<th>Inhibition (%)</th>
<th>Frequency of vomiting mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>9/9</td>
<td>3.3 ± 0.6</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>TJ-14</td>
<td>125</td>
<td>8/8</td>
<td>0.0</td>
<td>1.0 ± 0.3**</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>500</td>
<td>5/8</td>
<td>37.5</td>
<td>0.1 ± 0.1**</td>
</tr>
</tbody>
</table>

Apomorphine at 0.25 mg/kg was injected subcutaneously to the ferrets. Each drug was given orally 60 min before the subcutaneous injection of apomorphine. **: Significantly different from the control at p < 0.001.

Table 4. Effects of TJ-14 and Chlorpromazine on Copper Sulfate-Induced Emesis in the Ferret

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. of animals Vomited (%)</th>
<th>Inhibition (%)</th>
<th>Frequency of vomiting mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>5/5</td>
<td>12.6 ± 1.4</td>
<td>9.4 ± 2.2</td>
</tr>
<tr>
<td>TJ-14</td>
<td>500</td>
<td>5/5</td>
<td>0.0</td>
<td>5.7 ± 1.1**</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>500</td>
<td>6/6</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Copper sulfate at 50 mg/kg was administered orally to the ferrets. Each drug was given orally 60 min before the oral administration of copper sulfate. **: Significantly different from the control at p < 0.01.

DISCUSSION

There are several types of experimental models for evaluating anti-ulcer drugs. Ethanol-induced gastric lesions are thought to represent direct injury of gastric mucosal cells and to involve free radicals and lipid peroxidation.\textsuperscript{18,19}\textsuperscript{18,19} Robert et al. reported that cytoprotective drugs such as PGE\textsubscript{2} are effective against this type of gastric lesion.\textsuperscript{12} Therefore, ethanol-induced gastric lesions are considered as an experimental model useful for the evaluation of gastric mucosal protective actions of drugs. In the present study, both TJ-14 and TJ-9 suppressed ethanol-induced gastric lesions in a dose-dependent manner at doses between 125 and 500 mg/kg. Both were also reported to eliminate oxygen radicals.\textsuperscript{2,20} Ogata et al. reported that the effect of TJ-14 in suppressing the onset of ethanol-induced gastric lesions is due to its ability to enhance the secretion of mucin, a glycoprotein contained in mucus.\textsuperscript{20} Li et al. also showed that Hange-shashin-to suppressed gastric lesions through its ability to repair tissue damage (an action which primarily involves stimulation of mucin secretion).\textsuperscript{10} TJ-14 did not affect gastric lesions.
induced by water-immersion stress, while TJ-9 suppressed this type of gastric lesion and gastric juice secretion in a dose-dependent manner. Toda et al. reported that TJ-9 suppressed the release of histamine from intraperitoneal mast cells and degranulation induced by compound 48/80. Since histamine enhances gastric acid secretion and plays an important role in the onset of gastric ulcers, H₂-blockers are useful in treating these ulcers. These findings from the present and previous studies indicate that TJ-14 is effective against gastric diseases due to its action to protect gastric mucosa, and that TJ-9 exerts anti-ulcer effects by protecting the gastric mucosa and suppressing gastric acid secretion. The herbal contents are approximately the same in both preparations, the only difference lies in C. Rhizoma being substituted for B. Radix in TJ-14. B. Radix, a major component of TJ-9, contains saponins which have been reported to manifest anti-ulcer action and to suppress gastric acid secretion. A polysaccharide fraction isolated from B. Radix also showed the ability to protect gastric mucosa. The observed differences in the effects on gastric lesions between TJ-14 and TJ-9 may be associated with B. Radix contained in TJ-9.

We also assessed the effects of these two kampo medicines on gastric emptying. TJ-9 did not affect gastric emptying even at a dose level of 500 mg/kg at which the drug showed an anti-ulcer action. This suggests that TJ-9 is probably ineffective against stomach oppression caused by suppressed gastric emptying. TJ-14, however, accelerated gastric emptying in normal rats and improved the delayed gastric emptying induced by BaCl₂ in a dose-dependent manner. The effect of barium chloride in suppressing gastric emptying is due to the contraction of the gastric pylorus. We previously reported that TJ-14 did not affect intestinal contraction induced by barium chloride. Therefore, it seems unlikely that the effect of TJ-14 in improving gastric emptying involves relaxation of the smooth muscles of the gastric pylorus. Gastrokinetic drugs such as domperidone, metoclopramide and cisapride reportedly are useful in the treatment of various gastric dysmotility disorders. TJ-14 seems to enhance gastric emptying although its effect is weaker than those of existing gastrokinetic drugs. The composition is almost the same for these two herbal preparations; the only difference is that TJ-14 contains C. Rhizoma while TJ-9 contains B. Radix in its place. B. Radix, a major component of TJ-9, contains saponins which have been reported to suppress the central nervous system (CNS).

C. Rhizoma, a component of TJ-14, is known to enhance gastric motility. Although the exact reason for the difference of the effects on gastric emptying between the two preparations remains unknown, the effect of TJ-9 may reflect the CNS suppressive action of B. Radix.

As stated, TJ-14 enhanced gastric emptying while TJ-9 had no marked effect on it. In view of the fact that TJ-14 is used to control nausea and vomiting, it is possible that its effect in enhancing gastric emptying is related with its anti-emetic action. We therefore assessed the effects of TJ-14 on emesis in the ferret and found that it suppressed apomorphine-induced vomiting but was ineffective against copper sulfate-induced vomiting. This result may be associated with P. Tuber (a major component of TJ-14) which has been reported to have an anti-emetic action. Apomorphine is known to induce emesis by stimulation of the chemoreceptor trigger zone (CTZ), which is situated in the area postrema. Chlorpromazine suppresses apomorphine-induced vomiting almost completely, but this drug often induces catalepsy in laboratory rodents. TJ-14, however, did not show such symptoms in this study (data not shown). It has been reported that domperidone, a dopamine antagonist, is effective against apomorphine-induced vomiting but is less effective against vomiting induced by copper sulfate. The anti-emetic action of domperidone is thought to involve blockade of the dopamine receptors in CTZ located outside of the blood brain barrier. Dopamine was also reported to regulate the release of acetylcholine from postganglionic cholinergic neurons in the stomach. In contrast, domperidone is thought to stimulate gastric contraction by antagonizing the inhibitory effects of dopamine in the myenteric plexus. Nijima et al. reported that the suppressive effect on vagal gastric activity due to apomorphine was antagonized by the extract of P. Tuber (a major component of TJ-14). Although a more detailed study is needed, these findings from the present and previous studies suggested that the effect of TJ-14 in suppressing emesis might be the outcome of its antagonistic impact on peripheral dopaminergic neurons. We can't rule out, however, that some other mechanisms may be involved in the anti-emetic effect of TJ-14.

In conclusion, TJ-14 is characterized by its anti-ulcer action (probably based on its ability to protect gastric mucosa), improvement of gastric emptying and an anti-emetic action. The findings also suggest the effectiveness of TJ-9 against gastric disorders. This study thus provided basic data to explain the differences in clinical application between these two kampo medicines.

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