Relationship Between Phosphodiesterase Inhibition Induced by Several Kampo Medicines and Smooth Muscle Relaxation of Gastrointestinal Tract Tissues of Rats

Yoshiki Saegusa, Atsushi Sugiyama*, Akira Takahara, Yoshinobu Nagasawa, and Keitaro Hashimoto

Department of Pharmacology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Tamaho-cho, Nakakoma-gun, Yamanashi 409-3898, Japan

Received May 6, 2003; Accepted June 30, 2003

Abstract. Given a lack of information, we assessed the effects of Kampo medicines: Dai-saiko-to, Tsu-do-san, San'o-shashin-to, and Sairei-to, which have been used for various gastrointestinal diseases, on the phosphodiesterase activity and smooth muscle tone of the gastrointestinal tract. Clinically relevant concentrations of each Kampo extract (0.1 – 1 mg/ml) decreased the phosphodiesterase activity as well as smooth muscle tone. The extent of phosphodiesterase inhibition as well as smooth muscle relaxation by these Kampo extracts was prominent for the lower gastrointestinal tract. Also, there was a good correlation between the extents of drug-induced phosphodiesterase inhibition and smooth muscle relaxation, indicating the presence of their causal link. These results may partially provide the basis for understanding the mechanism of the clinical utility of Kampo extracts in gastrointestinal tract diseases.

Keywords: phosphodiesterase, Kampo medicine, gastrointestinal tract, relaxation, cyclic AMP

Introduction

In the gastrointestinal tract, cyclic AMP-dependent pathways have been reported to play an important role in physiological as well as pathophysiological regulation (1 – 3). For example, phosphodiesterase (PDE) inhibitors like 3-isobutyl-1-methylxanthine (IBMX), which increase the cellular cyclic AMP level, have been shown to relax gastrointestinal smooth muscles (4 – 9) in addition to inhibiting their pacemaker activity of slow waves (10, 11).

Kampo medicines are now widely used as alternative or supplemental therapies to modern medicines (12 – 14). Indeed, some Kampo extracts have been used for the treatment of various gastrointestinal diseases including non-ulcer dyspepsia, functional bowel disease including spasmodic constipation for a “Jissho” (sthenic) person, acute gastroenteritis, and inflammatory bowel disease (12 – 14). Recently, we found that some Kampo medicines inhibit PDE activity at clinically relevant concentration (15). However, no information is available up to now regarding the relationship between the Kampo medicines-induced subcellular responses and functional consequences in the gastrointestinal tract.

In this study, we first assessed regional differences of cyclic AMP hydrolyzing PDE activity using the membrane preparations obtained from the stomach, small intestine, and colon of rats. Next, we examined the effects of Kampo extracts, which are clinically used for gastrointestinal diseases (12 – 14) as shown in Table 1, on the PDE activities in these three different regions. Finally, we assessed the relaxant effects of Kampo extracts on carbachol (CCh)-induced contraction in isolated stomach, ileum, and colon of rats to clarify the relationship between the drug-induced PDE inhibition and the smooth muscle relaxation. We used a typical PDE inhibitor IBMX as a reference compound for both biochemical assay and functional study (16).

Materials and Methods

All experimentation was performed in accordance with the rules and regulations of the Committee for Research at the University of Yamanashi. Animals were obtained through the Animal Laboratory for Research of
Effects of Kampo on Gastrointestinal Tissues

Table 1. Names of Kampo extracts and their clinical application used in this study

<table>
<thead>
<tr>
<th>Japanese</th>
<th>Chinese</th>
<th>Typical clinical application*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dai-saiko-to</td>
<td>Da-Chai-Hu-Tang</td>
<td>Spasmodic constipation, Nausea, Vomiting, Hypertension</td>
</tr>
<tr>
<td>Tsu-do-san</td>
<td>Tong-Dao-San</td>
<td>Spasmodic constipation, Lumbago</td>
</tr>
<tr>
<td>San’o-shashin-to</td>
<td>San-Huang-Xie-Xin-Tang</td>
<td>Spasmodic constipation, Nasal bleeding</td>
</tr>
<tr>
<td>Sairei-to</td>
<td>Chai-Ling-Tang</td>
<td>Diarrhea, Acute gastroenteritis, Edema</td>
</tr>
</tbody>
</table>

*Cited from drug information attached to commercially available Tsumura Kampo medicines and references (14, 24).

the University of Yamanashi.

Drugs

The following Kampo extracts: Dai-saiko-to (Formula bupleuri major, TJ-8), Tsu-do-san (Pulvis purgitionis sanguinolentiae, TJ-105), San’o-shashin-to (Formula dispellendi cordis tres-flavorum, TJ-113) and Sairei-to (Formula hoelen et bupleuri, TJ-114) were generously provided by Tsumura Co., Ltd. (Tokyo) as a freeze-dried powder made of boiled water-extracts of natural products. One gram of each powder was dissolved or suspended in 40 ml of distilled water, mixed for 2 h at room temperature, and centrifuged for 5 min at 10,000 × g as described previously (17, 18). The top clear part of the fluid was passed through a filter with a pore size of 0.22 µm to get the desired solution of 25 mg/ml. All enzymes and substrates including IBMX and CCh were obtained from Sigma Chemical Company (St. Louis, MO, USA).

Effects of the drugs on the PDE activity of the isolated stomach, ileum, and colon

Production of the membrane preparation: The female Sprague-Dawley rats weighting 200 – 300 g (n = 6) were fasted 24 h and sacrificed by a blow to the head. Then, the animals were exsanguinated and portions of stomach, small intestine, and colon were stripped. After removal of fatty tissue from these segments, they were placed in ice-cold SET buffer (0.25 mol/l sucrose; 0.1 mmol/l EDTA; and 5 mmol/l Tris-acetate, pH 7.4) and homogenized. The homogenates were filtered (Nitex filter; Tetko, Los Angeles, CA, USA) and centrifuged at 10,000 × g for 5 min at 4°C. The pellets were resuspended in the SET buffer and the mixtures were centrifuged under the same setting. After this procedure was repeated twice, the pellets were finally suspended in SET buffer. Protein analysis was performed using a commercially available protein assay reagent (Pierce, Rockford, IL, USA). The membrane suspensions were diluted with SET buffer to a concentration of 5 – 5 mg protein/ml, and they were stored at −80°C until their PDE activities were measured.

Determination of PDE activity: The assay consists of two parts: hydrolysis of externally added cyclic AMP by PDE in the membrane preparation and measurement of residual cyclic AMP concentration (19). Fifty microliters of reaction mix (100 mmol/l Tris-acetate, pH 7.4; 10 mmol/l MgCl₂; 0.4 mg/ml bovine serum albumin; and 20 µmol/l cyclic AMP) was added to each micro-centrifugation tube in duplicate with or without either of IBMX (2, 20, and 200 µmol/l) or TJ-8, TJ-105, TJ-113, and TJ-114 (0.2 and 2 mg/ml). These concentrations of drugs were determined based on our recent study, which could reflect their clinical plasma concentrations (15, 17, 18). Next, the membrane suspension in a volume of 50 µl was added to each tube on ice. The reaction was initiated by placing the tubes in a water bath maintained at 37°C. After 20 min, the reaction was terminated by heating at 95°C for 5 min. The mixture was vortexed 3 times and centrifuged at 10,000 × g for 5 min. A 2-µl aliquot of the supernatant was transferred to a 10 × 75 mm disposable assay tube (Iwaki Lab Ware, Tokyo) in triplicate. For the cyclic AMP standard, 2 µl of a known amount of cyclic AMP was added to the tubes. The cyclic AMP concentrations were assayed using the enzymatic fluorometric technique as described before (20).

Relaxant effect of drugs on the isolated stomach, ileum, and colon

Tissue preparation: The female Sprague-Dawley rats weighing 300 – 450 g (n = 5) were killed by stunning and exsanguinations. The stomach, ileum and proximal colon were isolated and placed in oxygenated (95% O₂ and 5% CO₂) Tyrode’s solution (136.8 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 0.4 mM NaH₂PO₄, 11.9 mM NaHCO₃, and 5.6 mM glucose) at 31°C. Gastric fundus, ileum, and colon (length of 10 – 15 mm, each) were cut out and suspended in an organ bath filled with Tyrode’s solution at 31°C. One end of each strip was attached to a stainless steel tissue holder and the other to an isotonic transducer (TD-112S; Nihon Kohden, Tokyo). One gram of tension was applied to each strip that was equilibrated for >30 min.

Effects of IBMX and Kampo extracts: After confirming the reproducibility of the responses of the tissues...
to 1 μM of CCh, the tissues were contracted with 1 μM of CCh again. When the contraction reached a steady state level, which was usually observed about 10 min after the administration of CCh, IBMX (0.1 – 300 μM) or each Kampo extract (0.1 – 1.0 mg/ml) was administered in a cumulative manner every 3 – 5 min.

Analyses of data and statistics
The data are presented as the mean ± S.E.M. Relaxation was expressed as percent reduction from the CCh-induced contraction. Statistical comparisons of mean values within a group were performed by one-way repeated-measures ANOVA followed by the contrast, while those between the groups were assessed by one-way Factorial ANOVA with multiple comparison tests. A correlation between PDE inhibition and smooth muscle relaxation by the drugs was assessed using Pearson’s correlation coefficient. A P-value of less than 0.05 was considered significant.

Results
Effect of the drugs on the PDE activity in the isolated stomach, ileum, and colon
Regional difference of the PDE activity: The effects of IBMX on the PDE activity in the membrane preparations from each region are summarized in Fig. 1 (n = 6). The basal PDE activities (pmol·min⁻¹·mg protein⁻¹) were 198 ± 14 in the stomach, 116 ± 4 in the small intestine, and 184 ± 9 in the colon. IBMX inhibited the PDE activities of each region in a concentration-related manner. It should be noted that the absolute PDE activities at the basal state as well as in the presence of IBMX were significantly smaller in the small intestine than those in the stomach or colon.

Effect of Kampo extracts on the PDE activity: The effects of the Kampo extracts on the PDE activities in the membrane preparations from each region are summarized in Fig. 2 (n = 6). Percent changes of the PDE activities induced by IBMX were calculated using the data shown in Fig. 1 for comparison, which were also depicted in Fig. 2. Each Kampo extract inhibited the PDE activity of each region in a concentration-related manner, except that TJ-114 did not induce any statistically significant change in the stomach. The percent decreases of the activity by each Kampo extract in the small intestine and colon were significantly greater than that in the stomach, whereas such decreases of the activity by IBMX in the colon were significantly smaller than that in the stomach or small intestine. It should be also noted that the percent decreases of the activity in the small intestine and colon by 1 mg/ml of TJ-8, TJ-105, and TJ-113 were comparable to those by 100 μM of IBMX.

Relaxant effect of drugs on the isolated stomach, ileum, and colon
Effect of IBMX: The effect of IBMX on CCh-induced contraction are summarized in Fig. 3 (n = 5). IBMX significantly relaxed each region in a concentration-related manner. Significant changes were detected at ≥30 μM in the stomach and ≥10 μM in both ileum and colon. The extent of relaxation of the stomach was significantly smaller than that of the ileum or colon.

Effects of Kampo extracts: The effects of the Kampo extracts on CCh-induced contraction are summarized in Fig. 4 (n = 5) and typical tracings showing the relaxant effects of TJ-113 are depicted in Fig. 5. Each Kampo extract relaxed the ileum and colon in a concentration-related manner. On the other hand, TJ-105 and TJ-113 relaxed the stomach, but TJ-8 and TJ-114 hardly affected it. The extent of the relaxant effects of each Kampo extract on the ileum and colon were significantly greater than that on the stomach. The potency of relax-
Effects of Kampo on Gastrointestinal Tissues 65

ation produced by TJ-113 was the greatest in each region. It should also be noted that the extent of relaxation by 1 mg/ml of TJ-113 in the ileum and colon was comparable to that by 30 – 100 µM of IBMX.

Relationship between the drug-induced PDE inhibition and smooth muscle relaxation

The relationship between the extent of IBMX or Kampo extracts-induced PDE inhibition and smooth muscle relaxation is summarized in Fig. 6. There was a good correlation between the extents of PDE inhibition and smooth muscle relaxation by the treatment of the four Kampo extracts ($P<0.001$, $n=36$) as well as IBMX ($P<0.001$, $n=12$).

Discussion

Given a lack of information, we assessed effects of Kampo extracts, which have been used for gastrointestinal diseases (12 – 14), on the PDE activity and smooth muscle tone of the gastrointestinal tract. As clearly shown in the results, we found that clinically relevant concentrations of Kampo extracts decreased PDE activity as well as smooth muscle tone of the gastrointestinal tract. More importantly, there is a good correlation between the extents of drug-induced PDE inhibition and smooth muscle relaxation, indicating the presence of their causal link. This observation confirms the previous findings that accumulation of cyclic AMP by adenylate cyclase stimulation or PDE inhibition may relax the gastrointestinal smooth muscles (4 – 9).

The constitutive PDE activities were significantly smaller in the small intestine than in other portions as shown in Fig. 1, reflecting the regional variability of the cyclic AMP-dependent pathway in the physiological regulation of the gastrointestinal tract. Moreover, per-
cent decreases of the activity by each Kampo extract in the small intestine and colon were significantly greater than that in the stomach, whereas the inhibitory effect of IBMX was the smallest in the colon. The difference of specificity towards the PDE isoenzymes between Kampo extracts and IBMX may explain this observation (4 – 9), which needs to be clarified by further studies.

Some components of currently used Kampo extracts have been shown to inhibit the PDE activity (14, 21 – 23). For example, Glycyrrhizae radix (Kanzo) in TJ-105 and TJ-114, Bupleuri radix (Saiko) in TJ-8 and TJ-114, and Aurantii nobilis pericarpium (Chinpi) in TJ-105 inhibit the PDE activity, which may provide a rationale for the present results. Rhei Rhizoma (Daio) in TJ-113 has been shown to stimulate cyclic AMP production (17) possibly by stimulating adenylate cyclase.

Fig. 4. Effects of Kampo extracts (TJ-8, TJ-105, TJ-113, and TJ-114) on carbachol-induced contraction of isolated stomach, ileum, and colon. Data are expressed as the mean ± S.E.M. (n = 5). *P<0.05 vs control, †P<0.05 between stomach and colon, ‡P<0.05 between stomach and ileum, and §P<0.05 between ileum and colon.
activity via \( \beta \)-adrenoceptors (14); however up to now, no component of TJ-113 has been shown to exert PDE inhibition. Thus, the present results suggest that some of the components of TJ-113, namely, *Scutellariae Radix* (Ogon), *Coptidis Rhizoma* (Oren), and/or *Rhei Rhizoma* (Daio), inhibit the PDE activity. It will be also important to determine which components of the crude drugs of *Kampo* medicines used in this study are essential for the current observation.

*Kampo* extracts are prescribed for specific conditions of each patient according to a unique empiric system peculiar to *Kampo* diagnosis (24). In the clinical practice, TJ-8, TJ-105, and TJ-113 have been used for the treatment of constipation, whereas TJ-114 is administered to patients with diarrhea (12–14) as summarized in Table 1. In this study, we found that extents of PDE inhibition as well as smooth muscle relaxation by these *Kampo* extracts were more prominent for the lower gastrointestinal tract. Also, the extents of the biochemical and functional actions by TJ-114 were the smallest. These observations might at least in part reflect the mechanisms of the *Kampo* medicines for the constipation and diarrhea in clinical practice. Further studies would be required regarding their influences on water and electrolytes transport across the gut as well as the effects on the intestinal pacemaker activity to fully understand the causal link between the present in vitro observation and clinical utility.

In summary, PDE activity differs in the discrete portions of the gastrointestinal tract, and clinically relevant concentrations of TJ-8, TJ-105, TJ-113, and TJ-114 decrease PDE activity as well as smooth muscle tone, which is prominent for the lower gastrointestinal tract. Thus, the present study will partially provide the basis for understanding the mechanism of the clinical utility of *Kampo* extracts in the gastrointestinal tract diseases.

**Acknowledgments**

The authors thank Mr. Y. Nakamura for his technical assistance. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (#15590222) and a Grant-in-Aid from Yamanashi Research Center of Clinical Pharmacology.

**References**


2 Kunzelmann K, Mall M. Electrolyte transport in the mammalian colon: mechanisms and implications for disease. Physiol Rev.


