Effects of Clinically Available Blended Herbal Medicines (Kampo Extracts) on the Phosphodiesterase Activity in Rat Hearts

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Abstract: We examined the effects of clinically available Kampo medicines; Kakkon-to (Formula puerariae), Boi-ogi-to (Formula astragali et sinomenii), Chorei-to (Formula polypori) and Tsu-do-san (Pulvis purgitionis sanguinolentiae), on phosphodiesterase (PDE) activity in the rat heart. PDE activity was estimated by measuring the cyclic AMP hydrolyzing speed using the enzymatic fluorometric assay technique. Kakkon-to, Boi-ogi-to and Tsu-do-san decreased PDE activity in a concentration-related manner, while Chorei-to hardly affected it. These results suggest that recently demonstrated cardiotonic effects of Kakkon-to, Boi-ogi-to and Tsu-do-san may be exerted in part via their PDE inhibitory action.

Key words: phosphodiesterase, herbal medicines, Kampo, cardiotonic effects

INTRODUCTION

Blended herbal medicines made from numerous crude components of natural origin (Kampo medicines) are currently widely used as alternative or supplemental therapies to modern medicines1,2. In our recent study, we assessed the cardiac effects of clinically available Kampo medicines in their originally prescribed forms, and found that some Kampo medicines, including Kakkon-to (TJ-1), Boi-ogi-to (TJ-20) and Tsu-do-san (TJ-105), at clinically relevant doses could exert β-adrenoceptor-dependent cardiotonic effects3. However, these actions were not totally suppressed by an effective dose of β-blocker propranolol1. More importantly, the potency of Kampo medicines to increase the adenylate cyclase activity was much smaller than that by an equivalent concentration of isoproterenol3 to show cardiotonic effects, suggesting the presence of alternative cardiotstimulatory mechanisms other than β-adrenoceptor stimulation. The purpose of this study was to analyze the effects of Kampo extracts on the PDE activity in the rat heart. TJ-1, TJ-20 and TJ-105, which exerted cardiotonic action in our previous study3, were assessed, whereas Chorei-to (TJ-40) was selected as a negative control that lacks cardiac effects5 (Table 1). A non-specific PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX), was used as a reference compound4.

MATERIALS AND METHODS

All experiments were 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 the University of Yamanashi.

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Preparation of the drugs and enzymes

The following four kinds of Kampo extracts: Kakkon-to (Formula puerariae, TJ-1), Boi-ogi-to (Formula astragali et sinomenii, TJ-20), Chorei-to (Formula polypori, TJ-40) and Tsu-do-san (Pulvis purgtionis sanguinolentiae, TJ-105) were provided by Tsumura Co., Ltd. (Tokyo, Japan) as freeze-dried powder made of boiled water-extracts of natural products (Table 1). One gram of each powder was dissolved or suspended in 40 ml of distilled water, mixed for 2 hr at room temperature, and centrifuged for 5 min at 10,000 × g. The top clear part of the fluid was passed through a filter with a pore size of 0.22 μm to obtain the desired solution of 25 mg/ml. Pentobarbital sodium was obtained from Tokyo-Kasei (Tokyo, Japan). All other enzymes and substrates were obtained from Sigma Chemical Company (St. Louis, MO, U. S. A.).

<table>
<thead>
<tr>
<th>Latin</th>
<th>Japanese</th>
<th>Chinese</th>
<th>Code*</th>
<th>Typical clinical application**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula puerariae</td>
<td>Kakkon-to</td>
<td>Ge-Gen-Tang</td>
<td>TJ-1</td>
<td>Cold</td>
</tr>
<tr>
<td>Formula astragali et sinomenii</td>
<td>Boi-ogi-to</td>
<td>Fang-Yi-Huang-Qi-Tang</td>
<td>TJ-20</td>
<td>Nephritis, Edema</td>
</tr>
<tr>
<td>Formula polypori</td>
<td>Chorei-to</td>
<td>Zhu-Ling-Tang</td>
<td>TJ-40</td>
<td>Hematuria, Diarrhea</td>
</tr>
<tr>
<td>Pulvis purgtionis sanguinolentiae</td>
<td>Tsu-do-san</td>
<td>Tong-Dao-San</td>
<td>TJ-105</td>
<td>Consipation, Lumbago</td>
</tr>
</tbody>
</table>

* Code numbers of Kampo extracts in Tsumura Co., Ltd. (Tokyo).
** Cited from drug information attached to the commercially available Tsumura Kampo Medicines.

Production of the membrane preparation

The heart specimens necessary for this study were obtained from male Sprague-Dawley rats weighing 200–300 g (n=4). The animal was anesthetized with intraperitoneal injection of 60 mg/kg of pentobarbital sodium and exsanguinated. Heart was harvested and placed in ice-cold SET buffer (0.25 mol/l sucrose; 0.1 mmol/l EDTA; 5.0 mmol/l Tris-acetate, pH 7.4). The apex region was trimmed and homogenized in 5 volumes of SET buffer. The homogenate was filtered (Nitex filter, Tetko, CA, U. S. A.) and centrifuged at 10,000 × g for 5 min at 4 °C. The pellet was resuspended in the SET buffer and the mixture was centrifuged under the same setting. After this procedure was repeated twice, the pellet was finally suspended in SET buffer. Protein analysis was performed using a commercially available protein assay reagent (Pierce, Rockford, IL, U. S. A.). The membrane suspension was diluted with SET buffer to a concentration of 3–5 mg protein/ml, and it was stored at −80 °C until its PDE activity was measured.

Measurement of PDE activity

Fifty microliters of reaction mix (100 mmol/l Tris-acetate, pH 7.4; 10 mmol/l MgCl2; 0.4 mg/ml bovine serum albumin; 20 μmol/l cyclic AMP) was added to each microcentrifugation tube in duplicate with or without either of IBMX (2, 20 and 200 μmol/l) or TJ-1, TJ-20, TJ-40 and TJ-105 (0.2 and 2 mg/ml). These concentrations of drugs were determined based on recent findings5,5), which could reflect clinical practice5). 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. Twenty-five microliters of the supernatant was diluted with 100 μl of distilled water (5 times
dilution). A volume of 5 μl of the diluted supernatant was transferred to the assay tube in triplicate and the cyclic AMP concentration was assayed using the enzymatic fluorometric method6).

Statistics

The values are presented as mean ± SE. The statistical comparisons of mean values were evaluated by one-way repeated measures ANOVA followed by Contrasts. A p-value of less than 0.05 was considered significant.

RESULTS

The effects of IBMX and Kampo extracts on the PDE activity are summarized in Fig. 1 (n=4). Basal PDE activity was 112 ± 9 pmol/min/mg protein. IBMX decreased the PDE activity in a concentration-related manner. TJ-1, TJ-20 and TJ-105 also suppressed the PDE activity, and significant changes were detected at high concentration. Meanwhile, TJ-40 did not affect the PDE activity.

DISCUSSION

In this study, we assessed effects of Kampo extracts on PDE activity in rat hearts to explore alternative mechanisms for the previously demonstrated cardiotonic effects of TJ-1, TJ-20 and TJ-1053). As clearly demonstrated in the results, TJ-1, TJ-20 and TJ-105 significantly attenuated the PDE activity. The inhibitory action was most prominent for TJ-105, which had been shown not to increase the adenylate cyclase activity3). Meanwhile, TJ-40 affected neither PDE activity in this study nor adenylate cyclase activity in the previous study, which supports the lack of its cardiotonic effect3).

Kampo extracts used in this study consisted of several crude drugs of natural origin. Among them, some components are known to possess inhibitory effects on the PDE. For example,
Puerariae radix (Kakkon) in TJ-1, Glycyrrhizae radix (Kanzo) in TJ-1, TJ-20 and TJ-105, and Aurantii nobilis pericarpium (Chinpi) in TJ-105 were reported to inhibit PDE activity\textsuperscript{7-10}, which could provide a rationale for the present results. It should be also noted that the extent of inhibitory action of PDE by 1 mg/mL of TJ-1 as well as TJ-105 is comparable to that by 10 \textmu mol/L IBMX, which is an experimentally standard concentration for PDE inhibition\textsuperscript{9}.

In summary, clinically available TJ-1, TJ-20 and TJ-105 inhibit PDE activity in addition to the previously demonstrated direct \(\beta\)-adrenoceptor stimulation and/or the norepinephrine release from the postganglionic nerve terminals in the heart. Thus, these cardiotonic Kampo extracts can be used as alternative therapies to PDE inhibitors, adenylate cyclase stimulators and \(\beta\)-adrenoceptor agonists in the modern medicine.

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References