Mechanisms by Which Hange-shashin-to Reduces Prostaglandin E2 Levels

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To determine the mechanisms by which Hange-shashin-to (TJ-14) reduces prostaglandin E2 (PGE2) levels, the effects on blood corticosterone levels were examined in vivo and the effects on cyclooxygenase (COX) activity in vitro assessed. TJ-14, orally administered to rats at dose levels between 125 and 1000 mg/kg, caused a dose-dependent increase in blood corticosterone levels. We also showed that Glycyrrhiza Radix and Ginseng Radix, constituents of TJ-14, are involved in the increase in blood corticosterone. The activity of COX-1 was not inhibited by TJ-14 even at a dose of 1000 µg/ml, while COX-2 was inhibited at dose levels between 10 and 1000 µg/ml. The constituents Scutellariae Radix, Glycyrrhiza Radix and Coptidis Rhizoma were believed to be involved in COX-2 inhibition.

These results suggest that the effect of TJ-14 in decreasing PGE2 is partially mediated by corticosterone and inhibition of COX-2.

Key words Hange-shashin-to; corticosterone; cyclooxygenase-1 (COX-1); cyclooxygenase-2 (COX-2)

Hange-shashin-to (TJ-14) is a kampo medicine made of a mixture of seven herbs (Pinelliae Tuber, Scutellariae Radix, Glycyrrhiza Radix, Zizia Fruits, Ginseng Radix, Zingiberis Siccatum Rhizoma, and Coptidis Rhizoma). It is often used to treat acute and chronic gastrointestinal catarrh, fermentative diarrhea and acute gastroenteritis. We previously reported that TJ-14 markedly suppresses castor oil-induced diarrhea, and that it reduces colonic prostaglandin E2 (PGE2), leading to enhanced water absorption in large intestine. The medicine suppresses choler toxin-stimulated intestinal fluid secretion by inhibiting the production of PGE2 and is effective against delayed diarrheal symptoms caused by irinotecan hydrochloride (an anti-cancer agent). These previous findings suggest that the anti-diarrheal effect of TJ-14 is partially due to its ability to suppress PGE2 production.

Arachidonic acid, which is a precursor of PGs, is produced from phospholipids in the presence of activated phospholipase A2, and PGs are biosynthesized from arachidonic acid in the presence of cyclooxygenase (COX). The production of PGs is suppressed by inhibitory actions of nonsteroidal anti-inflammatory agents against COX and by inhibition of phospholipase A2 activity by glucocorticoids. The present study was undertaken to examine the mechanism by which TJ-14 reduces PGE2 levels. For this purpose, the effects of TJ-14 on blood levels of corticosterone in rats and its inhibitory action against COX in vitro were assessed.

MATERIALS AND METHODS

Animals Eight-week-old male Wistar rats (SLC Japan) were used for the experiment in vivo. The animals were bred in quarters in which the temperature and relative humidity were kept at 23±2°C and 55±10%, respectively, and which were lit between 7:00 and 19:00. The animals were allowed free access to food and drinking water.

Drugs TJ-14 is a dried powder of an extract manufactured by Tsumura Co., Ltd., and manufactured from a mixture of Pinelliae Tuber (mixture ratio: 5.0), Scutellariae Radix (2.5), Glycyrrhiza Radix (2.5), Zizia Fruits (2.5), Ginseng Radix (2.5), Coptidis Rhizoma (1.0) and Zingiberis Siccatum Rhizoma (2.5). The yield of the extract was 24%. Dried extract powders of the constituents of TJ-14 were prepared by the same method as for TJ-14 extract powders. Indomethacin and arachidonic acid were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). NS-398 (Bionol Research Laboratories, Inc., U.S.A.) was used as a selective COX-2 inhibitor. COX-1 derived from sheep seminal vesicles and COX-2 derived from sheep placenta were purchased from Cayman Chemical Company (Ann Arbor, MI, U.S.A.). Plasma corticosterone levels were measured using a RIA kit (Amersham, U.K.) and PGE2 was quantified using an EIA kit (Cayman Chemical Company). In the in vivo experiment, TJ-14 and the extract of its constituents were dissolved in distilled water before use. In the in vitro experiment, each test drug was dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO was kept at 1% to avoid its effects on COX activity.

Effects of TJ-14 and Its Constituents on Plasma Corticosterone Levels Seven or eight animals were assigned to each group. To minimize the stress which could be induced by the manipulations required for oral administration of the test drug, a 7-d acclimatization period was incorporated into the experiment, following which TJ-14 at dose levels between 125 and 1000 mg/kg was orally administered smoothly. The rats were treated with distilled water in the same way as the control. Three or 5 h after administration, the animals were sacrificed by decapitation and blood treated with heparin was collected from each animal. TJ-14 at doses of 500 and 1000 mg/kg was orally administered, and the blood was collected in the same manner 24 h after administration. To evaluate the relationship between activities of TJ-14 and its constituents, each of the seven constituents was orally administered, and blood was collected by decapitation 3 h after administration. Blood collection by decapitation between 10:00 a.m. and 1:00 p.m. minimized the influence of daily variation of corticosterone. Corticosterone levels in plasma samples were determined using a RIA kit. The intact group was also incorporated into this experiment to assess

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Fig. 1. Effects of TJ-14 on Plasma Corticosterone Levels in Rats

TJ-14 at doses of 125 to 1000 mg/kg was orally administered to rats and the animals were sacrificed 3 h thereafter. They were treated with distilled water in the same way as the control. An intact group was also incorporated into this experiment to assess the influence of the manipulation associated with oral medication. Each column represents the mean±S.E. of 7 to 8 animals. * and ** significantly different from the control at p<0.05, p<0.01 and p<0.001, respectively.

Fig. 2. Changes in Plasma Corticosterone Levels Following TJ-14 Treatment in Rats

(A) 5 h after administration, (B) 24 h after administration. Each column represents the mean±S.E. of 7 to 8 animals. ** significantly different from the control at p<0.01.

Table 1. Effects of TJ-14 and Its Constituents on Plasma Corticosterone Levels in Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>N</th>
<th>Corticosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>7</td>
<td>42.4±10.4</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td></td>
<td>45.6±6.1</td>
</tr>
<tr>
<td>TJ-14</td>
<td>250</td>
<td>8</td>
<td>109.3±29.9*</td>
</tr>
<tr>
<td>Pinelliae Tuber</td>
<td>500</td>
<td>8</td>
<td>143.5±41.8**</td>
</tr>
<tr>
<td>Glycyrrhiza Radix</td>
<td>68</td>
<td>8</td>
<td>64.8±11.5</td>
</tr>
<tr>
<td>Glycyrrhiza Radix</td>
<td>500</td>
<td>8</td>
<td>111.5±16.8*</td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>68</td>
<td>8</td>
<td>79.6±11.7</td>
</tr>
<tr>
<td>Scutellariae Radix</td>
<td>500</td>
<td>8</td>
<td>135.2±26.7**</td>
</tr>
<tr>
<td>Zizyphi Fructus</td>
<td>500</td>
<td>8</td>
<td>52.1±11.6</td>
</tr>
<tr>
<td>Zingiberis Siccatum</td>
<td>500</td>
<td>8</td>
<td>56.1±16.0</td>
</tr>
<tr>
<td>Rhizoma</td>
<td>71.7±12.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coptidis Rhizoma</td>
<td>500</td>
<td>8</td>
<td>67.2±18.1</td>
</tr>
</tbody>
</table>

TJ-14 or one of its constituents was orally administered to the rats. Three hours after administration, the animals were sacrificed by decapitation and blood treated with heparin was collected from each animal. * and ** significantly different from the control at p<0.05 and p<0.01, respectively.

**Effects of TJ-14 and Its Constituents on Plasma Corticosterone Levels**

The plasma corticosterone levels did not significantly differ between the control group and the intact group. This indicates that the manipulations associated with oral medication did not greatly affect plasma corticosterone levels in this experiment. Oral administration of TJ-14 (125—1000 mg/kg) resulted in elevation of the plasma corticosterone levels after 3 h in a dose-dependent manner (Fig. 1). The levels of increase in corticosterone induced by TJ-14 decreased within 5 h after its oral administration except when used at a dose of 1000 mg/kg; however, no marked effects of TJ-14 at 1000 mg/kg were observed 24 h after administration (Fig. 2). Among the seven constituents of TJ-14, Glycyrrhiza Radix and Ginseng Radix significantly elevated the plasma corticosterone levels after their oral administration at a dose of 500 mg/kg, although at 68 mg/kg there was no marked increase in corticosterone, despite this being equivalent to their contents in TJ-14 at a dose of 500 mg/kg. The other constituents of TJ-14 did not cause any marked changes even at a dose of 500 mg/kg (Table 1).

**Effects of TJ-14 and Its Constituents on COX Activity**

TJ-14 did not inhibit COX-1 even at a dose level of 1000 µg/ml, while COX-2 was inhibited at dose levels between 10 and 1000 µg/ml (Figs. 3 and 4). Treatment with NS-398 (10—100 µg/ml) selectively inhibited COX-2 activity,
while indomethacin at doses of 10 and 100 μg/ml inhibited both COX-1 and COX-2. Scutellariae Radix, Glycyrrhizae Radix and Coptidis Rhizoma significantly inhibited COX-2 activity at a dose of 100 μg/ml, however, other constituents of the medicine showed no marked inhibition against COX-2 (Fig. 5).

**DISCUSSION**

Prostaglandins (PGs) are biologically active substances and play important roles in the regulation of cell functions. It has been reported that PGE2 in the digestive tract is closely correlated with diarrhea. An increase in PGE2 enhances intestinal peristalsis, inhibits Na+ absorption (through inhibition of Na+, K+-ATPase activity), enhances Cl- secretion and reduces water absorption, leading to the onset of diarrhea. Regarding the relationship between PGE2 and the anti-diarrheal action of TJ-14, our previous studies demonstrated that TJ-14 suppressed castor oil-induced diarrhea, and that it also reduced the colonic PGE2 level. We have also shown that TJ-14 suppresses the cholera toxin-induced increase in PGE2 as well as diarrhea and the increase in PGE2 induced by irinotecan hydrochloride (an anti-cancer agent). The present study was undertaken to determine the mechanism by which TJ-14 reduces these levels.

When stimulated by factors such as infection, various cells produce arachidonic acid from cell membrane phospholipids in the presence of phospholipase A2. PGs are then biosynthesized from arachidonic acid in the presence of COX. The production of PGs is suppressed by COX inhibitors such as indomethacin and by the inhibition of phospholipase A2 activity by glucocorticoids. It has been shown that blood corticosterone levels rise when the animal experiences stress, infection, etc., and that corticosterone manifests anti-inflammatory, anti-allergic and immunosuppressive actions. We therefore studied the effects of orally administered TJ-14 on plasma corticosterone levels and noted no marked differences between the control group and the intact group under conditions in which stress or other stimuli are minimized. The experiment revealed a dose-dependent rise in the blood corticosterone levels following TJ-14 treatment. There was a significant increase in plasma corticosterone levels 3 or 5 h after its oral administration, although no marked effects were observed 24 h after administration. TJ-14 was reported to suppress the diarrhea caused by castor oil, in which enhanced PGE2 production is involved, in a dose-dependent manner and also the enhancement of intestinal fluid secretion and the increase in PGE2 production induced by the cholera toxin. In these earlier studies, the effects of TJ-14 were evaluated 3 or 5 h after its oral administration. In the present study, blood levels of corticosterone increased approximately 150 ng/ml after oral administration of TJ-14. Flower reported that the release of eicosanoids was inhibited by phospholipase A2 inhibitory action of dexamethasone, a glucocorticoid, 24-fold stronger than that of corticosterone. In their in vitro study, Lin et al. demonstrated that phospholipase A2 activity was inhibited by the application of 3.9 ng/ml of dexamethasone. Based on these reports and observations, the increased blood corticosterone levels after TJ-14 administration might inhibit phospholipase A2 activity. It was reported that endogenous glucocorticoids suppressed the appearance of COX-2, and that COX-2 expression was completely inhibited by the application of 1 μM of dexamethasone. Although the drug efficacy of corticosterone is weaker than that
of dexamethasone, it may be possible that the increase in corticosterone induced by TJ-14 was responsible for the suppression of COX-2 induction. Sapolsky et al. reported that the blood levels of corticosterone reached 172 ng/ml 4 h after subcutaneous injection of corticosterone at a dose of 5 mg/kg. They also confirmed that subcutaneous treatment with corticosterone at 5 mg/kg inhibited castor oil-induced diarrhea (data not shown). Furthermore, taking into consideration a report that glucocorticoids enhance intestinal electrolyte absorption\(^{21,22}\) and our previous report that TJ-14 enhances water absorption via the digestive tract,\(^{23}\) the increase in corticosterone may be involved in the enhancement of water absorption by TJ-14 in this tract. Therefore, the effect of TJ-14 in reducing PGE2 may be partially mediated by corticosterone.

Glycyrrhiza Radix has been reported to increase corticosterone levels in rats.\(^{23}\) Glycyrrhizin and glycyrrhetinic acid, which are contained in Glycyrrhizae Radix, are known to inhibit phospholipase A\(_2\).\(^{24,25}\) Saponin contained in Ginseng Radix is also reported to enhance corticosterone secretion by stimulating the release of adrenocorticotropic hormone (ACTH) from the pituitary adrenal system.\(^{26,27}\) It was confirmed in this study that Glycyrrhiza Radix and Ginseng Radix caused the increase in corticosterone, indicating that both constituents are involved in the enhancement of corticosterone by TJ-14. Since administration of either of these constituents alone at a dose equivalent to their concentrations in TJ-14, which showed marked activity, caused only a slight increase in corticosterone, it is suggested that they exhibit their effects only in the form of TJ-14. We previously demonstrated that TJ-14 accelerated gastric emptying.\(^{28}\) Pinelliae Tuber, a major component of TJ-14, was reported to increase gastric movement.\(^{29}\) Therefore, other components of TJ-14 were speculated not to markedly affect the blood levels of corticosterone by themselves, but might accelerate the transition of TJ-14 from the stomach to the intestine to increase the absorption of crude drug components in that organ. Although a more detailed study will be needed, these findings suggest that Glycyrrhiza Radix and Ginseng Radix are involved in the effect of TJ-14 in promoting corticosterone secretion.

We then analyzed the effects of TJ-14 on COX activity, an enzyme involved in the production of PGs. Cyclooxygeneases can be divided into COX-1 (a type of COX ubiquitously found in vivo) and COX-2, which is induced by stimuli such as tissue damage. In this study, TJ-14 inhibited COX-2, resembling the action of NS-398 (a selective COX-2 inhibitor), although the effects of TJ-14 were weaker than those of existing anti-inflammatory agents. Many anti-inflammatory agents inhibit not only COX-2 but also COX-1, and for this reason can induce adverse reactions such as gastrointestinal damage.\(^{30,31}\) We cannot rule out that enhanced secretion of endogenous corticosterone may cause such damage; however, in view of the results of this study and our previous finding that TJ-14 suppressed acute gastric mucosal lesions,\(^{32}\) it seems unlikely that the medicine would induce this type of damage.

Yanagisawa et al. reported that the blood level of baikalin, a major component of Scutellariae Radix, peaked 2 h after oral administration, and that this peak might reach approximately 0.007% of its initial dose.\(^{33}\) Orally administered TJ-14 demonstrated an effect at a dose of 500 mg/kg. If the absorption rate of TJ-14 is identical to that of major components such as baikalin, the former's effect in vitro is estimated to be expressed around a blood level of 35 \(\mu\)g/ml. In the present in vitro study, TJ-14 inhibited COX-2 activity at a dose between 10—1000 \(\mu\)g/ml, and it was speculated that its absorbed effective components were partly involved in this inhibition. However, a significant effect of TJ-14 in vitro was observed at doses above 100 \(\mu\)g/ml; therefore, the effective dose cannot be completely explained by the blood levels of TJ-14 alone. Further detailed evaluations are necessary to understand the correlation between the COX-2 inhibitory effect of TJ-14 and the blood levels of its effective components.

The relationship between COX-2 inhibition of TJ-14 and its components was also examined. Scutellariae Radix and Glycyrrhizae Radix significantly inhibited COX-2 activity. Scutellariae Radix is known to inhibit COX, and isoliquiritigenin contained in Glycyrrhizae Radix has been shown to have similar activity.\(^{33,34}\) However, these reports apparently did not distinguish the effects of these components on COX-1 activity from the effects on COX-2 activity. Our results were believed to show the inhibition of this activity by Scutellariae Radix and Glycyrrhizae Radix. Coptidis Rhizoma also inhibited COX-2 activity though more weakly than Scutellariae Radix and Glycyrrhizae Radix. Berberine, a component of Coptidis Rhizoma, has been shown to be effective against diarrhea caused by castor oil, in which the enhancement of PGE2 production is involved, and to suppress the enhancement of intestinal fluid secretion by the choler toxin.\(^{35,36}\) These results indicate that the COX-2 inhibiting action of TJ-14 may be attributable to all three components: Scutellariae Radix, Glycyrrhizae Radix and Coptidis Rhizoma.

In conclusion, the results of this study suggest that the effect of TJ-14 in reducing PGE2 is partially mediated by its actions of promoting corticosterone secretion and inhibiting COX-2 activity.

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REFERENCES