The Effects of Hange-shashin-to on the Content of Prostaglandin E₂ and Water Absorption in the Large Intestine of Rats

Yoshio Kase,* Terumasa Hayakawa, Atsushi Ishige, Masaki Aburada, and Yasuhiro Komatsu

Central Research Laboratories, Tsumura & Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan.
Received February 19, 1997; accepted May 28, 1997

The effects of Hange-shashin-to (TJ-14) were examined regarding the amount of prostaglandin E₂ (PGE₂) and the water absorbing capacity in the large intestine of rats. Repeated oral administration of TJ-14 at doses of 125 to 1000 mg/kg revealed a significant decrease in PGE₂ content in the colonic mucosa and also the promotion of colonic water absorption in a dose dependent manner. However, there was no remarkable influence on the concentrations of aldosterone and electrolytes in the serum, even at 1000 mg/kg. From these results, it was considered that some of the anti-diarrheal effects of TJ-14 might be based on a repression of the increase in the amount of PGE₂ as well as promotion of the water absorbing capacity of the large intestine. Moreover, it was also suggested that it is possible, by the application of TJ-14, to prevent the loss of water content caused by diarrhea.

Key words  Hange-shashin-to; diarrhea; colon; prostaglandin E₂; water absorption

Hange-shashin-to (TJ-14) is composed of seven crude drugs: Pinelliae Tuber, Scutellariae Radix, Glycyrrhizae Radix, Zizyphi Fructus, Ginseng Radix, Coptidis Rhizoma and Zingiberis Siccatum Rhizoma. TJ-14 is frequently used for acute or chronic gastrointestinal catarrh, fermentative diarrhea and acute gastroenteritis.1,2 Recently, TJ-14 has been reported to be effective against castor oil-induced diarrhea and to have no influence on intestinal motility.3 Furthermore, it has become evident that TJ-14 is effective against diarrhea present as a side effect of carcinostatic drugs.4 It is generally known that prostaglandin E₂ (PGE₂) can cause diarrhea and a decline in the water absorbing capacity of the large intestine, which is an important factor in diarrhea.5,6,7

This paper presents the relationship between the anti-diarrheal effects of TJ-14, especially its effect on the PGE₂ content and the water absorbing capacity of the large intestine.

MATERIALS AND METHODS

Animals  Male Wistar rats, seven weeks old (SLC, Japan), were housed in an environment of 23 ± 2°C, 55 ± 10% humidity, with a 12 h light-dark cycle (7:00—19:00) and free access to drinking water and rodent chow (F-2, Funabashi Farm, Japan). All rats were allowed 1 week for acclimatization before any treatment.

Drugs  TJ-14 was supplied in the form of dry powdered extract created in our company from a mixture of the following proportions: Pinelliae Tuber (5.0), Scutellariae Radix (2.5), Glycyrrhizae Radix (2.5), Zizyphi Fructus (2.5), Ginseng Radix (2.5), Zingiberis Siccatum Rhizoma (2.5) and Coptidis Rhizoma (1.0), and the mixture was dissolved in distilled water.

Indomethacin and aldosterone were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). These compounds were suspended in 0.5% carboxymethyl cellulose sodium solution. PGE₂ was quantified using a RIA kit (NEN Research Products, Boston, U.S.A.). Aldosterone was measured by a SPAC-S aldosterone RIA kit (Daichi Radioisotope, Tokyo, Japan). All other chemicals were of the highest grade commercially available.

The Effect of TJ-14 on the Amount of PGE₂ in the Large Intestine  TJ-14 at doses of 125 to 1000 mg/kg was given orally once daily for 4 consecutive days, while indomethacin at 5 mg/kg was subcutaneously injected once a day for the same period. The animals were sacrificed 24 h after the final administration. Immediately after the sacrifice the descending colons were taken out, then the PGE₂ was extracted according to the method of Kobayashi et al.8 for its quantification using the RIA kit. Nine samples of each group were used for this test.

The Effect of TJ-14 on the Water Absorption in the Large Intestine  Eleven to sixteen rats were used from each group in this test. TJ-14 at doses of 125 to 1000 mg/kg was orally administered once a day for 4 consecutive days, while indomethacin at 5 mg/kg was subcutaneously injected once a day for the same period. Twenty-four hours after the final treatment, measurement of the water absorbing capacity in the large intestine was carried out by the method of Fedorak et al.9 with slight modification. By celiotomy of the anesthetized animals (pentobarbital-Na [50 mg/kg, i.p.]), the large intestine was exposed and tied at the cecum. The large intestine was cleaned by infusing 2 ml of 0.9% NaCl at 37°C into the lumen, which was subsequently excreted through the anus. A loop of large intestine was then generated by tying the large intestine at one end, about 0.5 cm from the cecum, and at the other end, about 3 cm from the anus. The loop was filled with 2 ml of 0.9% NaCl at 37°C, then removed for weighing 1 h after the NaCl injection. Water absorption was expressed as the difference between the amount of the solution injected and that retained in the lumen, which was estimated by subtracting the wet weight of the tissue from the weight of the loop.

The Effects on the Concentrations of Aldosterone and Electrolyte in the Serum  Nine samples were tested from each group. TJ-14 at 125 to 1000 mg/kg was given orally once a day for 4 consecutive days. Twenty-four hours after the final treatment, the blood was taken out from the abdominal aorta of animals anesthetized with pentobarbital-Na (50 mg/kg, i.p.).

© 1997 Pharmaceutical Society of Japan
Aldosterone at 1 mg/kg was subcutaneously administered once, and 1 h later the blood was taken out. The blood was centrifuged and the concentration of aldosterone was measured by use of a SPAC-S aldosterone RIA kit and the concentrations of electrolytes were measured by means of an Automatic Electrolytes Analyzer (Model 710, Hitachi, Japan).

Statistical Analysis Results were expressed as the mean ± S.E. and analyzed by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference procedure.

RESULTS

The Effect of TJ-14 on the Amount of PGE₉ in the Large Intestine TJ-14 at doses of 125 to 1000 mg/kg dose-dependently decreased the amount of PGE₂ in the large intestine. Treatment with indomethacin at 5 mg/kg significantly lowered the level of PGE₂ (Fig. 1).

The Effect of TJ-14 on the Water Absorbing Capacity of the Large Intestine TJ-14 at doses of 125 to 1000 mg/kg accelerated the water absorbing capacity of the large intestine in a dose-dependent manner. The promotion of the water absorption was also observed in the group treated with indomethacin at 5 mg/kg (Figs. 2 and 3).

The Effects on the Concentrations of Aldosterone and Electrolytes in the Serum Treatment with TJ-14, even at 1000 mg/kg, had no significant influence on the concentrations of aldosterone and electrolytes in the sera. In the group treated with aldosterone at 1 mg/kg, the amount of aldosterone in the serum was significantly increased, but no such influence was seen on the electrolyte concentration (Tables 1 and 2).

DISCUSSION

There are various causes of diarrhea, but in all cases, in the stimulated intestine the intestinal secretion and peristalsis are so greatly promoted that the contents in

![Fig. 1. Effects of TJ-14 and Indomethacin on Colonic Mucosal PGE₂ Content in Rats](image1)

![Fig. 2. Effects of TJ-14 on Colonic Water Absorption in Anesthetized Rats](image2)

![Table 1. Effects of TJ-14 and Aldosterone on the Concentration of Aldosterone in the Serum](table1)

![Table 2. Effects of TJ-14 and Aldosterone on the Concentration of Electrolyte in the Serum](table2)
the intestine are discharged before they are sufficiently digested and absorbed, resulting in diarrhea. There are many reasons for diarrhea such as stress, bacterial toxins, cold stimulants, the intake of certain foods or drugs, colonic cancer or ulcerative colitis.\textsuperscript{10–12} In each case, both an increase in the amount of PGE\textsubscript{2} in the digestive tract and its accompanied lowering of the water absorbing capacity are considered to be important factors responsible for the diarrhea.\textsuperscript{3–7}

This report described the effect of TJ-14 on both the PGE\textsubscript{2} amount in the large intestine and the water absorbing capacity in relation to its anti-diarrheal effect. We have orally administered TJ-14 in combination with an anti-cancer agent, irinotecan hydrochloride (CPT-11), for 4 consecutive days and assessed its efficacy 24 h after the last dose. This study revealed that TJ-14 suppressed the CPT-11-induced increase in colorectal PGE\textsubscript{2} levels and improved colorectal water absorption 24 h after the last dose (submitted for publication). Therefore, we adopted the regimen of once daily oral treatment for 4 consecutive days, and assessed the efficacy of this drug 24 h after the last dose in this study.

It is well known that PGE\textsubscript{2} suppresses the absorption of Na\textsuperscript{+} by interfering with the Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity, and lowers the body’s water absorbing capacity, finally causing diarrhea.\textsuperscript{13–16} Previously, we reported that TJ-14 does not affect intestinal motility but is effective against diarrhea caused by castor oil.\textsuperscript{31} PGE\textsubscript{2} and platelet activating factor (PAF) are known to be involved in causing castor oil diarrhea,\textsuperscript{17–19} and since TJ-14 dose-dependently decreased the PGE\textsubscript{2} amount of the large intestine in rats, its anti-diarrheal action can be considered to be related to PGE\textsubscript{2} suppression. The effects of TJ-14 continued to be seen 24 h after its last dose. It is more likely that TJ-14 continues to have some influence and remains effective even after the blood levels of its active ingredients have become lower than their peak levels. It has been reported that PGE\textsubscript{2} production was suppressed by Scutellariae Radix, Zingiberis Rhizoma, 6-gingerol and 6-shogaol (components of Zingiberis Siccatum Rhizoma).\textsuperscript{20–22} Glycyrrhizae Radix and its active ingredient, glycyrrhizin, have been shown to suppress PGE\textsubscript{2} production through their steroid-like actions.\textsuperscript{23} Therefore, the suppression of PGE\textsubscript{2} production observed in the present study may be attributable to these herbs.

In general, anti-inflammatoryatories such as indomethacin are known to have side effects such as gastric lesions and these side effects are caused by the suppression of cyclooxygenase-1 (COX-1).\textsuperscript{24} Indomethacin has been reported to aggravate ethanol-induced gastric lesions,\textsuperscript{25} while TJ-14 actually suppressed ethanol-induced gastric damage.\textsuperscript{26} In this study, we also confirmed that the repeated oral administration of distilled water increased the PGE\textsubscript{2} content in colonic mucosa compared with non-treated rats (data not shown). Although the action of TJ-14 is less potent than existing anti-inflammatory agents, it selectively suppress COX-2 without affecting COX-1 (submitted for publication). It is possible that the effect of TJ-14 on cyclooxygenase might be different from that of indomethacin. Further investigation is necessary to elucidate the mechanisms by which TJ-14 suppresses the amount of PGE\textsubscript{2} in the large intestine in rats.

Minematsu et al. reported that when normal rats were orally treated with TJ-14 at doses of 125 to 2000 mg/kg for 5 weeks, their serum Na\textsuperscript{+} and Cl\textsuperscript{−} levels changed but returned to normal after the drug was discontinued.\textsuperscript{26} The exact mechanism of the changes observed in their study, designed to assess the toxicity of this drug, is unknown. TJ-14 was also found to enhance colonic water absorption in the present study. Aldosterone, known to affect electrolyte metabolism, may be involved in the effects of TJ-14. The relationship between the promotive action of TJ-14 on the water absorption in the large intestine and aldosterone was also examined. Aldosterone is an adrenocortical hormone which promotes the reabsorption of Na\textsuperscript{+} by its action on the distal tubule and encourages the excretion of K\textsuperscript{+}, which plays an important role in the regulation of water and electrolytes.\textsuperscript{27,28} In this study, blood was sampled from animals allocated to the aldosterone treatment group as positive controls for the measurement of aldosterone levels 1 h after a dose. The concentration of aldosterone in the serum was significantly elevated in this group. On the other hand, TJ-14 had no noticeable effect on the concentrations of either aldosterone or electrolytes. These results suggest that the promotive action of TJ-14 on the water absorbing capacity of the large intestine is not related to aldosterone. It is known that the water absorbing function lowered by the cholera toxin is sometimes responsible for vital problems, and the prevention of such water loss is regarded as important. Beubler et al. reported that the water absorbing function lowered by the cholera toxin was improved using indomethacin in animal experiments.\textsuperscript{12} Therefore, it can be considered that drugs such as TJ-14, which have no influence on intestinal motility as one of their characteristics and suppress PGE\textsubscript{2} along with the promotive action on the water absorbing capacity, are likely to be effective against the diarrhea caused by bacterial infections or the like. It is suggested by these results that the suppressive effects of TJ-14 on PGE\textsubscript{2} could be one of mechanisms involved in promoting water absorbing capacity of the large intestine.

Bastl et al. reported that glucocorticoids regulate adrenalin-dependent electroneutral Na\textsuperscript{+} absorption in rat colon.\textsuperscript{29} Since it is possible that TJ-14 may also promote the water absorbing capacity by influencing Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activity and the renal function, the effects of TJ-14 on colonic water absorption should be examined in more detail.

In conclusion, TJ-14 decreases the PGE\textsubscript{2} amount in the large intestine, which is closely related to the occurrence of diarrhea, and it also promotes intestinal water absorbing capacity; these effects are considered to contribute to its anti-diarrheal effect.

REFERENCES

4) Takasuna K., Kasai Y., Kitano Y., Mori K., Kobayashi R.,