Scutellariae Radix aggravates diclofenac sodium-induced enteropathy in mice

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We have reported that the oral administration of water extract of Scutellariae Radix (SR) enhances small intestinal ulceration and intestinal bleeding in the experimental enteropathy induced by subcutaneous injections of indomethacin (INDO) in mice. The present study was carried out to examine whether SR extract or SR-containing Kampo formula exerts similar effects in diclofenac sodium (Dic)-induced enteropathy in mice. We found that small intestinal ulceration and fecal hemoglobin (Hb) excretion in Dic-treated mice were enhanced by SR extract. However, such effects were not induced by pure baicalin (≥ 97%, w/w). Orangedokuto (OGT) did not enhance small intestinal ulceration but significantly enhanced fecal Hb excretion in Dic-treated mice. Our observations suggest a possibility that SR-containing Kampo formulas could aggravate non-steroidal anti-inflammatory drug-induced enteropathy.

Key words NSAIDs, ulceration, intestinal bleeding, baicalin, orangedokuto.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively used as anti-pyretics and anti-inflammatory analgesics. However, long-term intake of NSAIDs induces mucosal injury in the stomach and duodenum\(^1\) as well as small intestine.\(^2\) It is reported that severe blood loss due to bleeding from small intestine is frequently observed in rheumatic patients taking NSAIDs.\(^3,4\) It has been also demonstrated that the administration of NSAIDs in rats and mice can induce small intestinal damage associated with intestinal bleeding and blood loss.\(^5,6\)

There are reports indicating that the administration of Scutellariae Radix (roots of Scutellaria baicalensis Georgi) (SR) or SR-containing Kampo formulas ameliorates pathological responses during the experimental intestinal diseases.\(^8,10\) Flavonoids derived from SR such as baicalin and wogonin\(^12,14\) can be implicated to the amelioration of experimental intestinal diseases by SR or SR-containing Kampo formulas.\(^8,11\) However, we have demonstrated that SR extract enhances small intestinal ulceration and intestinal bleeding during the experimental enteropathy induced by subcutaneous injections of indomethacin (INDO)\(^15,16\). These results suggest that SR or SR-containing Kampo formulas could aggravate enteropathy induced by NSAIDs, although such effects of SR have been shown only in the limited experimental condition. Moreover, we have not explored the role of major chemical constituents of SR in the effects of SR on NSAID-induced enteropathy.

The present study was carried out to confirm whether SR and orangedokuto (OGT), a SR-containing Kampo formula, cause the enhancing effects on enteropathy induced by diclofenac sodium (Dic) in mice. In addition, the effects of baicalin, the most abundant flavonoid found in SR, on Dic-induced enteropathy were examined. Dic is used more frequently than INDO in hospital for the therapy of inflammatory disease\(^17\) but is also known to induce intestinal bleeding and subsequent blood loss in these subjects\(^18\) as well as in the experimental animals.\(^19\)

Materials and Methods

Drugs. OGT and SR extract were provided as freeze-dried powder from Tsumura & Co. (Tokyo, Japan). OGT is composed of 4 kinds of herbal medicines, SR, Coptidis Rhizoma, Gardeniae Fructus and Phellodendri Cortex, in the ratio of 3.0:2.0:1.5:2.0 (w/w/w/w). Three-dimensional HPLC patterns of the methanol extracts of SR and OGT were shown in Figs. 1a and 1b, respectively. OGT contained major compounds derived from SR such as baicalin and wogonin 7-O-glucuronide as well as oroxylin A 7-O-glucuronide. Berberine found in OGT is a compound derived from Coptidis Rhizoma and Phellodendri Cortex. Geniposide is a typical compounds derived from Gardeniae Fructus. Baicalin used in this study was isolated from SR by solvent partition and recrystallization as preporated previously.\(^10\) Purity of baicalin was estimated to be more than 97 % (w/w).\(^19\)

Animals and drug treatment. Male ddY mice at 5 weeks of age (SLC Japan, Shizuoka, Japan) were housed in plastic cages placed in an air-conditioned room (temperature at 23 ± 2 °C and humidity at 55 ± 5 %) under a 12hr:12hr light-dark cycle; the light was turned on at 0800 h. The
Fig. 1. Three dimensional HPLC patterns of SR extract (a) and OGT (b). Arrows indicate the peaks of major chemical constituents with their names and structures.
animals were maintained under the above conditions at least for 1 wk prior to the experiments below. Dic dissolved in distilled water was orally administered at 1300 h on day 0 at 20 mg/kg. OGT or SR was dissolved in distilled water and was orally administered two times at 1000 h and 1600 h on days -1 and 0. The doses used were 250 and 500 mg/kg for SR and 300 and 1000 mg/kg for OGT. Baicalin was suspended in 0.5% carboxymethylcellulose (CMC) in distilled water and orally administered at 50 and 150 mg/kg in a similar schedule to those for SR extract and OGT. All the procedures for the animal experiments in this study were performed according to the Guide for Animal Experiment, University of Toyama and approved by the Committee of Animal Care and Experiments of the University of Toyama.

**Assessment of small intestinal ulceration and intestinal bleeding.** The numbers and total area of ulcers in small intestine were determined as described previously.10 To highlight the location of ulceration in small intestine, Evans blue solution (0.2 % in saline) was intravenously injected 30 min before the mice were killed by cervical dislocation. Measurement was performed by an observer who could not identify the treatments for tissue specimens. Hemoglobin (Hb) content in feces and Hb concentration in blood were determined 24 hr after Dic administration as described previously.11

**Statistical analysis.** All data were expressed as the mean ± standard error. Statistical analysis was carried out by analysis of variance (ANOVA) followed by a Dunnet’s post hoc test. When p values were below 0.05, the difference between the two groups were considered statistically significant.

**Results**

Small intestinal ulceration was not observed in the mice not treated with Dic but was found in Dic-treated mice (data not shown). It was found that the numbers of ulcers in the small intestine of Dic-treated mice were significantly greater in the low SR group than in the vehicle-treated group (p<0.05) (Fig. 2a). However, no further increase in the number of ulcers was induced in the high SR group. The total area of ulcers in small intestine was significantly higher in the high SR group than the vehicle-treated group (p<0.05) (Fig. 2b). Fecal Hb excretion in normal mice was below 1mg/day (data not shown) but that in Dic-treated mice was markedly elevated (>50 mg/day)(Fig. 2c). The treatment of
low and high doses of SR extract in the Dic-treated mice further enhanced fecal Hb excretion in a dose-dependent manner (p<0.05 in the low SR group and p<0.01 in the high SR group vs. vehicle group). The elevation of fecal Hb excretion in Dic-treated mice was associated with the reduction of blood Hb concentration (Fig. 2d)(p<0.01 vs. normal group), which was further reduced by low and high doses of SR extract (p< 0.05 vs. vehicle group).

Administration of baikalin at 50 and 150 mg/kg did not change the number ulcers (Fig. 3a). A trend toward the elevation of total area of ulcers by the doses of baikalin was shown, although this effect was not statistical significant (p>0.05) (Fig. 3b). In addition, intestinal bleeding and the reduction of blood Hb concentration were not changed by these doses of baikalin (Figs. 3c and 3d).

When OGT was administered at 300 and 1000 mg/kg, the number and total area of ulcers in small intestine of Dic-treated mice were not changed compared with the control mice treated Dic alone (Figs. 4a and 4b). However, both low and high doses of OGT significantly enhanced fecal Hb excretion compared with the vehicle group (Fig. 4c). A trend toward to the reduction of blood Hb concentration by these doses of OGT in Dic-treated mice was shown, although such effect was not statistically significant (0.05 < p <0.1) (Fig. 4d).

**Discussion**

Our previous and the present studies have confirmed that major pathological changes, small intestinal ulceration and intestinal bleeding, induced by different NSAIDs (INDO and Dic) administered through different routes (subcutaneous and oral) were similarly enhanced by SR extract. These results suggest that common mechanisms are involved in the aggravation of the two enteroopathy models by SR extract. It has been reported that NSAID-induced enteroopathy requires the inhibition of both cyclooxygenase (COX)-1 and COX-2 activities. It is also known that the enteroopathy induced by NSAIDs depends on their biliary excretion and enterohepatic circulation as well as on potencies as an inhibitor of prostaglandin synthesis. In addition, recent investigations demonstrate that NSAIDs can induce directly mucosal damage by enhancing bile acid-mediated toxicity against intestinal epithelial cells, which does not require their inhibition of prostaglandin generation. Thus, the complicated mechanisms have been proposed in the NSAID-induced enteroopathy and therefore the influences of SR extract on the above steps should be further investigated.

Since baikalin content in the SR extract used in this study was 26.1 % (w/w), the doses of SR extract adminis-

![Fig. 3. Effects of baikalin on small intestinal ulceration and intestinal bleeding in Dic-treated mice](image)

Mice (6 mice/group) orally administered with baikalin (Ba) (50 or 150 mg/kg) or CMC solution (vehicle) and then treated with Dic (20 mg/kg) or distilled water (normal). Small intestine was harvested 24 h after Dic administration. The number (a) and the total area (b) of ulcers were determined. Fecal excretion of hemoglobin (Hb) (c) and blood Hb (d) for 24 h after Dic treatment were shown. Statistically significant difference was represented by symbols (\#; p<0.01 vs. vehicle group).
tered (250 and 500 mg/kg) correspond to 65 and 130 mg/kg as pure baikalin. Therefore, an inability of the administration of baikalin at 50 and 150 mg/kg to enhance small intestinal ulceration and intestinal bleeding (Fig. 3) is not due to its insufficient administration. Thus, baikalin seems not to play a major role in these effects of SR extract. The effects of other chemical constituents of SR on Dic-induced enteropathy should be further investigated.

Administration of lower dose of OGT (300 mg/kg) enhanced intestinal bleeding to a similar extent by the higher dose of SR extract (500 mg/kg) without changing ulceration in small intestine (Figs. 2 and 4). Therefore, OGT might enhance intestinal bleeding in Dic-treated mice through the mechanism(s) distinct from those by SR extract. It is reported that platelet aggregation is inhibited by several kinds of flavonoids derived from herbal constituents of OGT,24,25 which may be implicated to the enhancement of intestinal bleeding by this formula without enhancing ulceration in small intestine. Further detailed analyses are necessary to explore the differential effects of SR extracts and OGT on enteropathy in NSAID-treated animals.

Miura et al. recently demonstrated that the experimental enteropathy induced by subcutaneous injections of INDO in mice was attenuated by OGT.26 This observation is consistent with our previous data showing that intestinal bleeding was attenuated by OGT in the enteropathy induced by subcutaneous INDO.13 Nevertheless, we observed that intestinal bleeding induced by oral INDO was enhanced by OGT (unpublished observation), which was consistent with the present observations (Fig. 4). Thus, difference in the routes of NSAID administration, rather than the types of NSAIDs (INDO vs Dic), can be attributed to the conflicting results for the effects of OGT on the enteropathy. Differential modulation of OGT on the experimental enteropathy induced by NSAIDs administered through different routes is of great interest. However, it should be mentioned that more frequently used are oral Dic than parenteral INDO in hospital.17 The effects of other SR-containing Kampo on NSAID-induced enteropathy need to be examined under appropriate experimental conditions.

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References


