Corticosterone Secretion-Inducing Activity of Saikosaponin Metabolites Formed in the Alimentary Tract

Mitsuhiko Nose, Sakae Amagaya and Yukio Oghara*

Department of Pharmacoogy, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizhuo-ku, Nagoya 467, Japan.
Received March 18, 1989

The corticosterone secretion-inducing activities of saikosaponin a, saikosaponin c and saikosaponin d, isolated from the root of Bupleurum falcatum L., and 27 metabolites formed in the murine alimentary tract were studied in mice. Serum corticosterone was determined by high-performance liquid chromatography (HPLC). Intraperitoneal administration of saikosaponin a and its intestinal metabolite, prosaikogenin F, showed corticosterone secretion-inducing activity at a dose of 0.1 mmol/kg, and maximally increased it at a dose of 0.4 mmol/kg. On the other hand, the genuine sapogenin, saikogenin F, was inactive. Saikosaponin b2, b3 and saikosaponin g, gastric metabolites of saikosaponin a, and their intestinal metabolites, prosaikogenin A, prosaikogenin H, saikogenin A and saikogenin H, were also inactive. Serum corticosterone was increased by the administration of saikosaponin d and its intestinal metabolite, prosaikogenin G, at a dose of 0.04 mmol/kg, and it reached the maximal level at the dose of 0.1 mmol/kg. Saikogenin G also showed a slight activity. A gastric metabolite of saikosaponin d, saikosaponin b3, and its intestinal metabolites, prosaikogenin D and saikogenin D, were inactive. In the experiments on saikosaponin c and its metabolites, saikosaponin c was inactive but its intestinal metabolites, especially prosaikogenin E-2, showed activity almost equal to that of saikosaponin a. Saikosaponin h and saikosaponin i, gastric metabolites of saikosaponin c, were also inactive, but their prosaikogenins showed slight activities. When these compounds were orally administered, their corticosterone secretion-inducing activities were similar to those obtained in the intraperitoneal experiment.

These results suggest that a proper polar balance between the sugar moiety and the aglycone is important for the corticosterone secretion-inducing activity of saikosaponins and their metabolites.

**Keywords** corticosterone secretion; saikosaponin; prosaikogenin; saikogenin; gastric juice; intestinal flora; alimentary tract; structure—activity relationship; Bupleurum falcatum; HPLC

The root of Bupleurum falcatum L. (Umbelliferae) is one of the most important components in Kamphozi (Japanese and Chinese traditional medicines). Saikosaponins are the main components in the root of Bupleurum falcatum L. and their pharmacological actions have been reported by many investigators. Hiai et al.1,2) reported that saikosaponins a and d increased the plasma adrenocortico tropin (ACTH) and corticosterone levels when given by intraperitoneal injection. Further, they reported3,4) that the action of low doses of saikosaponins a and d on the pituitary-adrenocortical system was suppressed by dexamethasone, and that high doses of saikosaponins a and d released the suppressive effect of dexamethasone. They concluded that the site of action of saikosaponins a and d is closely related to that of dexamethasone. On the other hand, most Kamphozi (Japanese and Chinese traditional medicines) are orally administered. After oral administration, the components of Kamphozi are exposed to gastric juice and intestinal flora in the alimentary tract and some of them should be transformed before absorption into the blood. Therefore, we studied the structural transformations of saikosaponins a, c and d in rat gastric juice and intestinal contents, and clarified the structures of 27 metabolites5,6) derived from saikosaponins a, c and d as shown in Chart 1. Namely, saikosaponin a is transformed to saikosaponin b2 and saikosaponin g, possessing hetero- and homoanular diene moieties, respectively, in gastric juice. Saikosaponins b2, h and i are also formed from saikosaponins d and c in gastric juice. Moreover, these saponins are transformed to corresponding prosaikogenins and saikogenins in the intestinal contents. When saikosaponin a was administered to rats, serum saikosaponin a peaked 30 min later, and serum prosaikogenin F and saikogenin F, intestinal metabolites of saikosaponin a, peaked at 480 min.7) Judging from our studies, other metabolites are also expected to be absorbed into the blood similarly.

In the present study, we investigated the corticosterone secretion-inducing activities of saikosaponins and their metabolites in order to identify the active forms of oral saikosaponins and the relationship between the structures and activities.

**Materials and Methods**

**Animals** Male ddY mice (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), 6 weeks old, were used. They were fed on laboratory chow (CE-2, Clea Japan Inc., Tokyo, Japan) and tap water ad libitum, and maintained at 24 °C for more than 5 d.

**Reagents** Saikosaponin a, saikosaponin c and saikosaponin d were isolated from the root of Bupleurum falcatum L. according to the reported method.8,9) Their metabolites were obtained by means of alcoholic alkaline and acid treatments.8,10)

**Experimental Procedure** To avoid stress-induced release of corticosterone, mice were handled once a day for 5 d and transferred into individual cages the day before the experiment. Saikosaponins and their metabolites were suspended in 5% Tween-80 saline, and a 0.2 ml aliquot was intraperitoneally or orally administered to mice. In the case of oral administration, a stomach tube was used. Sixty minutes after the administration of saikosaponin or its metabolite, mice were decapitated and blood was collected. These procedures were performed at 10:00—12:00.

**Determination of Serum Corticosterone** Blood samples were collected and allowed to stand at room temperature for 30 min. After centrifugation at 7400 x g for 10 min, 200 µl of serum was transferred to a 10-ml separating funnel, and internal standard (dexamethasone; Sigma, St. Louis, MO, U.S.A.), 50 ng/5 µl, was added. Then, a few drops of 0.5% sodium hydroxide and 4 ml of methylene chloride were added. After shaking of the mixture for 15 min, the organic layer was washed with 2 ml of water and evaporated in vacuo at 30 °C. The residue was dissolved in 50 µl of methanol and 30 µl was injected into a high performance liquid chromatography (HPLC) column. For HPLC, a Shimadzu model 5A chromatograph with a Shimadzu model SPD-2A UV detector was employed. A stainless-steel column (25 cm x 4.6 mm i.d.) packed with Hypersil ODS (5 µm; Erma Inc., Tokyo, Japan) was used. The mobile
Results

Corticosterone Secretion-Inducing Activity by Intraperitoneal Injection

1) Saikosaponin a and Its Metabolites: Saikosaponin a (0.1—0.4 mmol/kg) and prosaikogenin F (0.1—0.4 mmol/kg), an intestinal metabolite of saikosaponin a, induced a marked increase of the serum corticosterone level 60 min after the treatment (Fig. 1). On the other hand, saikogenin F, an intestinal metabolite of saikosaponin a or prosaikogenin F, did not increase the serum corticosterone level. Saikosaponins b1 and g, gastric metabolites of saikosaponin a, and their intestinal metabolites, prosaikogenin A, prosaikogenin H, saikogenin A and saikogenin H, were also inactive (Figs. 2, 3). However, high doses (1.2—2.0 mmol/kg) of these metabolites except saikogenin H increased the serum corticosterone level. In a control group, the serum corticosterone level was 5.6 ± 1.0 μg/100 ml (vehicle treatment).

2) Saikosaponin d and Its Metabolites: Both saikosaponin d and prosaikogenin G, an intestinal metabolite of saikosaponin d, significantly increased the serum corticosterone level at a low dose of 0.04 mmol/kg. These actions were markedly stronger than that of saikosaponin a (Fig. 4). Saikogenin G also showed the activity at doses of more than 0.2 mmol/kg. Saikosaponin b2 and its intestinal metabolites, prosaikogenin D and saikogenin D, did not increase the serum corticosterone level (Fig. 5), but high doses (1.2—2.0 mmol/kg) of prosaikogenin D and saikogenin D induced an increase of serum corticosterone.

3) Saikosaponin c and Its Metabolites: Saikosaponin c did not increase the corticosterone level in the range of...
doses used. On the other hand, prosaikogenins, which are intestinal metabolites of saikosaponin c, were active. In particular, prosaikogenin E-2, whose sugar moiety is composed of one glucose and one rhamnose, increased the serum corticosterone level at doses of 0.1–0.4 mmol/kg. Prosaiogenin E-1 also showed the activity at a dose of 0.4 mmol/kg (Fig. 6). Saikosaponin h, a gastric metabolite of saikosaponin c, was inactive, but its intestinal metabolites, prosaikogenin C-2 and prosaikogenin C-3, induced an increase of serum corticosterone at a dose of 0.4 mmol/kg (Fig. 7). Saikosaponin i, another gastric metabolite of saikosaponin c, was also inactive, but its intestinal metabolite, prosaikogenin B-3, increased the serum corticosterone level at a dose of 0.4 mmol/kg (Fig. 8).
Effects of Saikosaponin i, Prosaikogenin B-1, Prosaikogenin B-2, Prosaikogenin B-3 and Saikogenin B on Serum Corticosterone Level after Intraperitoneal Injection in Mice

- ●, saikosaponin i; ○, prosaikogenin B-1; △, prosaikogenin B-2, ▲, prosaikogenin B-3; ■, saikogenin B. Data are means ± S.E. of 6 mice.

b) p < 0.01 versus the control.

Effects of Saikosaponin a, Saikosaponin c, Saikosaponin d and Their Intestinal Metabolites on Serum Corticosterone Level after Oral Administration in Mice

(A) Saikosaponin a (●-●) and its intestinal metabolites, prosaikogenin F (△-△), and saikogenin F (●-●); (B) prosaikogenin d (●-●) and its intestinal metabolites, prosaikogenin G (△-△), and saikogenin G (●-●); (C) saikosaponin c (●-●) and its intestinal metabolites, prosaikogenin E-1 (○-○), prosaikogenin E-2 (△-△), prosaikogenin E-3 (△-△) and saikogenin E (■-■). Data are means ± S.E. of 6 mice. a) p < 0.05 versus the control.

ministration The corticosterone secretion-inducing activity of saikosaponin a, saikosaponin c, saikosaponin d and their 8 intestinal metabolites which were effective in i.p. treatment were examined after oral administration (Fig. 9).

In a control group, the serum corticosterone level was 4.4 ± 2.4 μg/100 ml (vehicle treatment). Saikosaponin a and saikosaponin d showed the corticosterone secretion-inducing activities, but their effective doses in the oral experiment were about 20 times as high as those in the intraperitoneal experiment. The effects of prosaikogenin F and prosaikogenin G (intestinal metabolites of saikosaponin a and saikosaponin d, respectively) were weaker than those of saikosaponin a and saikosaponin d. Prosaikogenin E-2 and prosaikogenin E-3 (intestinal products of saikosaponin c) were also effective, as in the intraperitoneal experiment.

Discussion

The actions of saikosaponins are significant, although they are not enough to explain the action of Kampophozai, a mixture of several herbal drugs. We have already reported that saikosaponin a, saikosaponin c and saikosaponin d were transformed into at least 27 metabolites in the alimentary tract, and thus a mixture of at least 30 compounds is available to be absorbed into the blood stream when they are orally administered. The pharmacological actions of these metabolites have not been investigated. Therefore, we synthesized these metabolites from saikosaponin a, saikosaponin c and saikosaponin d enzymatically and organic chemical methods, and studied their pharmacological actions. In this paper, corticosterone secretion-inducing activities of them are reported.

Saikosaponin a, saikosaponin d and their intestinal metabolites, prosaikogenin F and prosaikogenin G, showed strong activities, although their aglycones, saikogenin F and saikogenin G, and the metabolites in which ether ring of aglycones was cleaved, such as saikosaponin b1, prosaikogenin a, saikogenin a, saikosaponin g, prosaikogenin h, saikogenin h, saikosaponin b2, prosaikogenin d and saikogenin d, were inactive. Among the active compounds, saikosaponin d (which possess an z-hydroxyl function at the C-16 position) is more active than saikosaponin a (which possess a β-hydroxyl function at C-16 position). In the experiments using saikosaponin c and its metabolites, the existence of a two sugar moiety (prosaikogenins E-1 and E-2) is preferable to a three sugar moiety (saikosaponin c) for corticosterone secretion-inducing activity. Further, prosaikogenin E-2 possessing one glucose and one rhamnose showed stronger activity than prosaikogenin E-1 possessing two glucose residues in the sugar moiety. These data indicate that a proper polar balance between the sugar moiety (polar part) and the aglycone moiety (non-polar part) is necessary for the activity. To prove this hypothesis of the correlation of corticosterone secretion-inducing activity and the polar balance, further biochemical and biophysical studies will be required.

The lower activities of prosaikogenins F and G in the oral experiment suggest that prosaikogenins are easily converted into inactive saikogenins in the alimentary tract, although the structural transformation to saikogenins requires some time. Furthermore, the ether ring of prosaikogenins may be easily cleaved relative to that of saikosaponins. Judging from these data, the results obtained in the intraperitoneal treatments with saikosaponin metabolites should accurately reflect the in vivo actions of saikosaponins a, c and d.

References