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Studies on the Absorption, Distribution, Excretion and Metabolism of Ginseng Saponins. III.[1] The Absorption, Distribution and Excretion of Ginsenoside Rb_1 in the Rat

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The pharmacokinetic character of ginsenoside Rb_1 (Rb_1), one of the main 20(*S*)-protopanaxadiol group saponins of ginseng (*Panax ginseng* C.A. Meyer), was investigated in rats. First, quantitative analysis of Rb_1 in biological samples was investigated and the most suitable assay procedure for each biological sample was established.

Little Rb_1 was absorbed from the digestive tract after oral administration (100 mg/kg) to rats. The serum level of Rb_1 in rats after intravenous injection (5 mg/kg) declined biexponentially, and the half-life of the β-phase was 14.5 h. The long persistence of Rb_1 in serum and tissues in rats after intravenous administration was assumed to correlate with the high activity of plasma protein binding. Rb_1 was gradually excreted into urine, but not bile. Unabsorbed Rb_1 in the digestive tract was rapidly decomposed and/or metabolized mainly in the large intestine.

These results are quite different from our results on ginsenoside Rg_1 in rats.

Keywords——ginsenoside Rb_1; quantitative analysis; TLC; pharmacokinetic study in rats; absorption; distribution; excretion; decomposition and/or metabolism

Ginseng (the root of *Panax ginseng* C.A. Meyer) contains more than ten saponins. It is well known that most of them can be classified into two groups according to their sapogenins, namely the 20(*S*)-protopanaxadiol (diol) group and 20(*S*)-protopanaxatriol (triol) group, except for ginsenoside Ro.

In a previous paper,[1] we reported on the absorption, distribution and excretion of ginsenoside Rg_1 (Rg_1), one of the main triol group saponins in ginseng. However, there is only one report[2] on the pharmacokinetics of diol group saponins, in spite of the many reports on pharmacological[3] and biochemical[4] studies. In the present paper, we chose ginsenoside Rb_1 (Rb_1) as a representative of diol group saponins, and we report the results of a study on the absorption, distribution and excretion of Rb_1 in rats after oral or intravenous administration.

Experimental

Materials——Experimental animals used were male Sprague–Dawley (JCL: SD, SPF) rats weighing 180—200 g.

Rb_1, isolated from ginseng and purified, was a gift from Dr. T. Tani and Mr. M. Higashino, Research Institute of Oriental Medicine, Kinki University, Japan. SEP-PAK®C_{18} cartridges (SEP-PAK) were purchased from Japan Waters Co., Ltd., Tokyo, Japan. Other chemicals used were similar to those used in our previous study.[1]

Thin-Layer Chromatography (TLC) and TLC-Densitometry (densitometry)——TLC and densitometry were done in much the same way as in the previous paper[1] except for the detecting wavelength (535 nm) and the developing solvents used for TLC. The developing solvents used for TLC were: solvent A [1-butanol (BuOH)/acetic acid/H_2O (4: 1: 5, upper phase)] for serum samples; solvent B [$CHCl_3$/BuOH/methanol (MeOH)/H_2O (20: 40: 15: 20, lower phase)] for kidney and spleen samples; solvent C [$CHCl_3$/MeOH/H_2O (65: 35: 10, lower phase)] for samples other than the above.

Determination of Rb_1 in Biological Samples——The biological samples, such as serum, urine, bile, feces, tissues (liver, kidney, heart, lung, spleen and brain) and digestive tract (stomach, small intestine and large intestine, including their contents), were obtained by the methods described in the previous paper.[1]

These biological samples were treated according to the procedures in Chart 1 and subjected to TLC. The procedures are briefly outlined below. Tissues samples (whole tissues except for liver; 2 g of liver;

3 g of feces) were homogenized with 5—9 ml of distilled water in a glass homogenizer. After homogenation, MeOH (25 ml) was added to extract Rb_1. In the case of the brain, the homogenate was defatted with 20 ml of benzene, then MeOH (25 ml) was added. The MeOH extracts of these samples were obtained by evaporation of MeOH under reduced pressure, and then the extracts were prepared as 20% acetonitrile aqueous solutions (5 ml). The solution were treated with SEP-PAK. The serum sample (3 ml) was treated according to the standard procedure for tissue samples. The urine (5 ml) and bile (1 ml) were also treated with acetonitrile and prepared as 20% acetonitrile aqueous solutions, which were treated with SEP-PAK.

Each sample of stomach, small intestine and large intestine was minced, and 25 ml of MeOH was added. The supernatant obtained after centrifugation at 3500 rpm for 10 min was poured into a measuring flask, which was then filled up to 50 ml with MeOH. Ten ml of the solution was evaporated to dryness under reduced pressure, 5 ml of 20% acetonitrile aqueous solution was added to the residue, and the solution was treated with SEP-PAK.

Before use, the SEP-PAK was prepared with 10 ml of MeOH and then 10 ml of 20% acetonitrile aqueous solution. The sample solution was injected into the SEP-PAK. Then, the SEP-PAK was washed with 20 ml of 20% acetonitrile aqueous solution, and eluted with 30 ml of 30% acetonitrile aqueous solution for the serum and tissue samples, with 50 ml of acetone for the bile sample, and with 15 ml of MeOH for the urine, feces and digestive tract samples. Each eluate was evaporated to dryness under reduced pressure, and redissolved in a definite volume of MeOH. The MeOH solution was used as a sample for TLC, and the amount of Rb_1 was measured by densitometry.[1)]

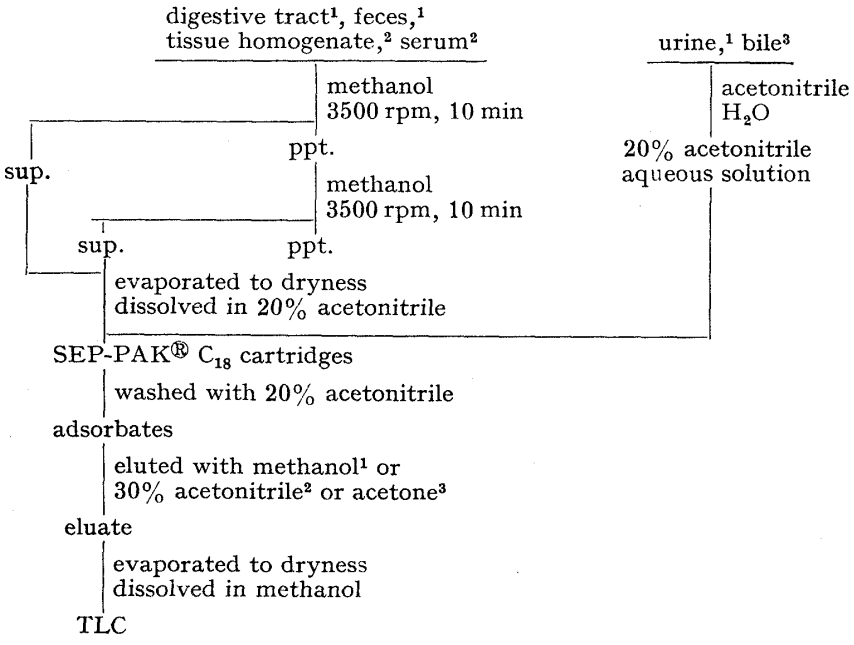

Chart 1. Assay procedure for Ginsenoside Rb_1 in Biological Samples

Recovery Test of Rb_1——The recovery of Rb_1 added to biological samples was determined according to the procedure for Rg_1 reported previously.[1)]

Administration of Rb_1——A 2% aqueous solution of Rb_1 was administered orally at a dose of 100 mg/kg to rats deprived of food but given free access to water for 18 h before the experiments. For intravenous experiments, a 0.2% solution of Rb_1 in 0.9% saline was given *via* the femoral vein at a dose of 5 mg/kg to non-fasted rats.

Pharmacokinetic Analysis——The concentration–time curve of Rb_1 was plotted semilogarithmically. The half-life was calculated from the linear region by means of linear regression analysis.

Results

Calibration Curve

Rb_1 on the TLC plate exhibited the maximum absorption at 535 nm, so this wavelength was adopted as the detecting wavelength. A linear relationship was obtained between the amount (0.5—4.0 μg) of Rb_1 applied and the integration value.

Isolation of Rb_1 in Biological Samples

Fig. 1 shows the TLC chromatograms of the biological samples obtained from non-administered rats and standard Rb_1. Rb_1 was separated perfectly from biological components on the TLC plates. The *Rf* values of Rb_1 were 0.10 with solvent A, 0.09 with solvent B and 0.08 with solvent C.

Elution of Rb_1 from SEP-PAK

SEP-PAK was used for the pretreatment of the biological samples in preference to TLC. As the components contained in different biological samples were different from each other,

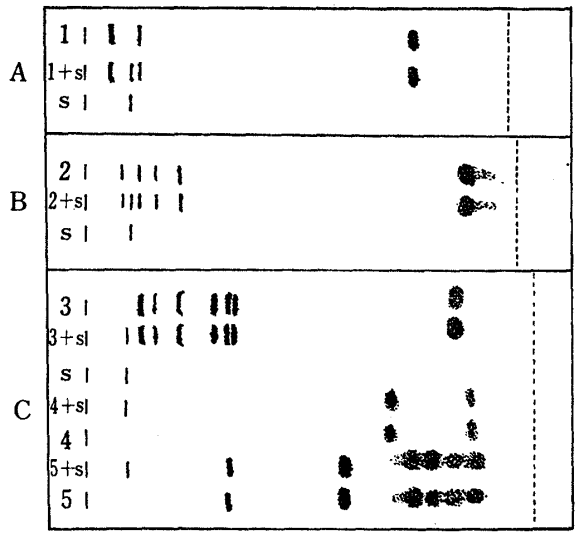

Fig. 1. Thin-Layer Chromatograms of Ginsenoside Rb_1 in Biological Samples of Rat

Developing solvents—A, BuOH/acetic acid/H_2O (4:1:5, upper phase); B, $CHCl_3$/BuOH/MeOH/H_2O (20:40:15:20, lower phase); C, $CHCl_3$/MeOH/H_2O (65: 35: 10, lower phase). Plate—Merck precoated silica gel 60.
Detecting reagent—8% vanillin–MeOH solution/72% H_2SO_4 (1:5), with heating at 140°C for 3 min. S, standard ginsenoside Rb_1; 1, serum; 2, kidney; 3, liver; 4, urine; 5, large intestine.

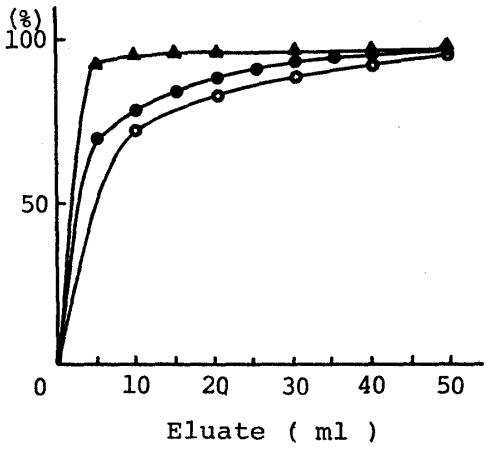

Fig. 2. Cumulative Recovery of Ginsenoside Rb_1 from SEP-PAK® C_{18} Cartridge with Various Eluting Solvents

Five thousand μg of ginsenoside Rb_1 was adsorbed on a SEP-PAK® C_{18} cartridge.
▲, MeOH; ●, 30% acetonitrile; ○, acetone.

Table I. Recovery of Ginsenoside Rb_1 added to Rat Tissues, Fluids and Feces

Tissues or fluids[a]	Ginsenoside Rb_1 added (μg)	Recovery[b] (%)
Liver	30	96.3±4.9
Kidney	30	95.4±5.5
Heart	30	96.5±5.6
Lung	30	94.0±4.8
Spleen	30	96.7±4.9
Brain	30	56.4±2.1
Stomach	1000	94.2±3.1
Small intestine	1000	86.7±1.3
Large intestine	1000	81.6±0.8
Serum	9	95.8±2.0
Urine	30	87.2±2.3
Bile	30	90.3±2.8
Feces	1000	73.6±4.1

a) The amounts or volumes employed were as follows: liver (2 g), other tissues (whole organs), serum (3 ml), urine (5 ml), feces (3 g).
b) Each value represents the mean±S.D. of 3 experiments.

a suitable eluting solution for each biological sample had to be selected.

As shown in Fig. 2, the recoveries of added Rb_1 (5000 μg) adsorbed on SEP-PAK were more than 90% after elution with 40 ml of acetone, 25 ml of 30% acetonitrile aqueous solution and 5 ml of MeOH. Therefore, the volumes of eluting solutions were fixed at 50 ml, 30 ml and 15 ml of acetone, 30% acetonitrile aqueous solution and MeOH, respectively.

Recoveries of Rb_1 Added to Biological Samples

Recoveries of Rb_1 (9, 30 or 1000 μg) added to homogenates or solutions of biological samples listed in Table I were investigated. As shown in Table I, the mean recoveries of Rb_1 in all samples except for the samples of brain, small intestine, large intestine, urine and feces were more than 90%, and the standard deviation was generally below 6%. Therefore, data obtained were not corrected. In the cases of the brain, small intestine, large intestine, urine and feces, the mean recoveries of Rb_1 were less than 90%, and so the data for these samples were corrected for recoveries in subsequent experiments.

I. Oral Administration

Concentrations of Rb_1 in the Serum and Tissues

The concentrations of Rb_1 in the serum and tissues (the liver, kidney, heart, lung, spleen and brain) were determined at 15, 30, 60, 150, 240 and 360 min after administration of Rb_1. However, the concentration of Rb_1 in every sample was less than 0.2 μg/(ml or g) at any time after administration.

Rb_1 in the Digestive Tract

The amounts of Rb_1 found in the digestive tract are shown in Fig. 3. The amounts of Rb_1 in the stomach and the small intestine at 15 min after administration were 35.5±8.5 and 43.0±13.6% of the dose, and at 30 min after, 17.2±5.6 and 65.2±13.9% of the dose, respectively. At 60 min after, most of the Rb_1 administered had moved to the small intestine, and remained there until 150 min after administration. At 4 h after, 34.8% of the Rb_1 administered was found in the small intestine and 28.4±10.7% in the large intestine, and at 6 h after administration, only 3.8±1.3% in the small intestine and 0.7±0.2% in the large intestine. On the other hand, decomposition products and/or metabolites of Rb_1 were also found in the stomach and large intestine by TLC. The TLC chromatogram is shown in Fig. 4.

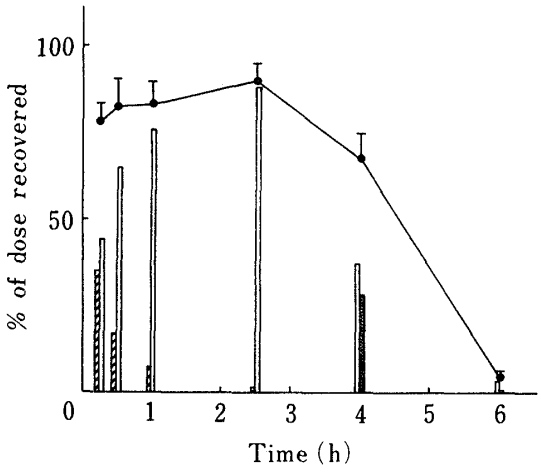

Fig. 3. Amounts of Ginsenoside Rb_1 in the Digestive Tract after Oral Administration of Ginsenoside Rb_1 (100 mg/kg) to Rats

▨, stomach; □, small intestine; ■, large intestine; ——, total.
Each point represents the mean±S.E. of 3 animals.

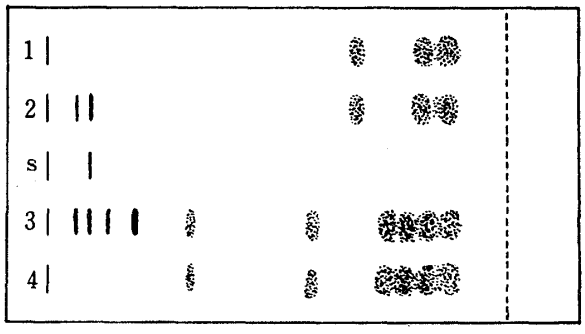

Fig. 4. Thin-Layer Chromatogram of Decomposition Products and/or Metabolites of Ginsenoside Rb_1 in the Stomach or Large Intestine

Developing solvent: $CHCl_3/MeOH/H_2O$ (65: 25: 10, lower phase). 1—2, stomach (1, normal; 2, at 30 min after oral administration of ginsenoside Rb_1); 3—4, large intestine (3, at 4 h after oral administration of ginsenoside Rb_1; 4, normal); S, standard ginsenoside Rb_1.

Urinary, Fecal and Biliary Excretions of Rb_1

Cumulative urinary and fecal excretions of Rb_1 are shown in Fig. 5 and Fig. 6.

The maximum excretions of Rb_1 into urine and feces were observed at 12 h to 18 h and at 6 h to 12 h after administration, respectively. The cumulative urinary excretion of Rb_1 within 48 h was 0.05±0.01% of the dose. The cumulative fecal Excretion of Rb_1 within 24 h was 10.8±1.5% of the dose. On the other hand, the concentration of Rb_1 in the bile sample was less than 0.5 μg/ml throughout.

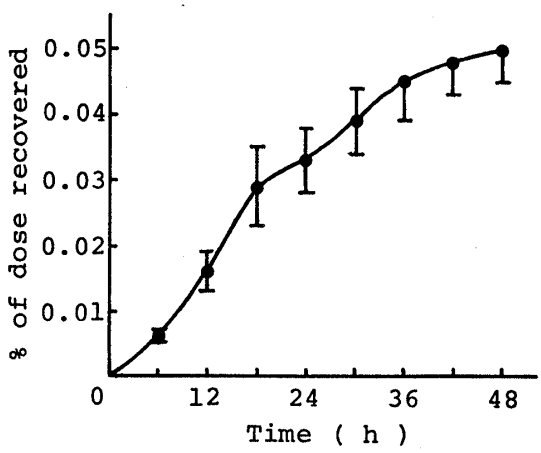

Fig. 5. Cumulative Excretion of Ginsenoside Rb_1 into Urine after Oral Administration of Ginsenoside Rb_1 (100 mg/kg) to Rats

Each point represents the mean ± S.E. of 3 animals.

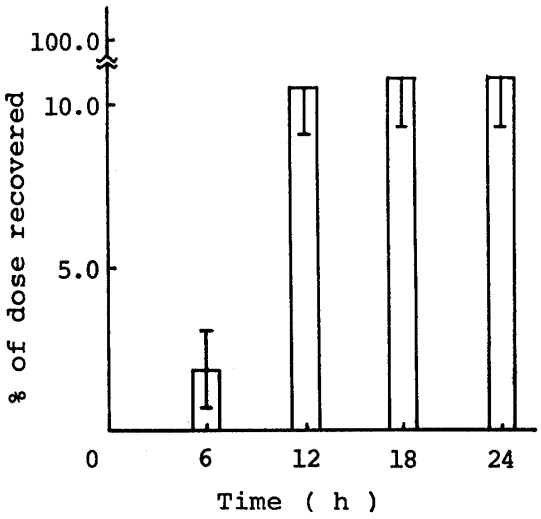

Fig. 6. Cumulative Excretion of Ginsenoside Rb_1 into Feces after Oral Administration of Ginsenoside Rb_1 (10 mg/kg) to Rats

Each point represents the mean ± S.E. of 3 animals.

II. Intravenous Administration

Time Course of Rb_1 in Rat Serum

As shown in Fig. 7, two phases of decline were observed, a rapid decline (α-phase) followed

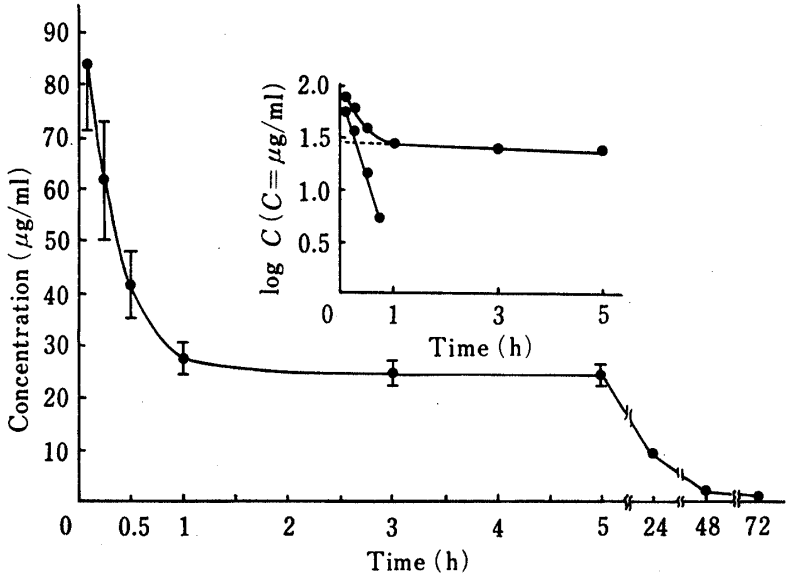

Fig. 7. Serum Concentration of Ginsenoside Rb_1 after Intravenous Administration of Ginsenoside Rb_1 (5 mg/kg) to Rats

Each point represents the mean ± S.E. of 3 animals.

by a slow decline (β-phase). The half-lives of Rb_1 were 11.6 min for the α-phase and 14.5 h for the β-phase.

Time Course of Rb_1 in Rat Tissues

Fig. 8 shows the time course of Rb_1 in the kidney, heart, lung and liver. The concentrations of Rb_1 at 5 min after administration were 9.0±1.6 μg/g, 5.3±0.9 μg/g and 2.9±0.6 μg/g in the kidney, heart and liver, respectively. Rb_1 distributed in these tissues declined approximately in parallel with that in serum. On the other hand, the concentration of Rb_1 in the lung was 3.3±0.5 μg/g, and reached a maximum (5.0 μg/g) at 30 min to 1 h after administration, then declined slowly. In the brain and spleen, the concentration of Rb_1 was less than 0.5 μg/g even at 5 min after administration, and Rb_1 was not detectable at any time after that.

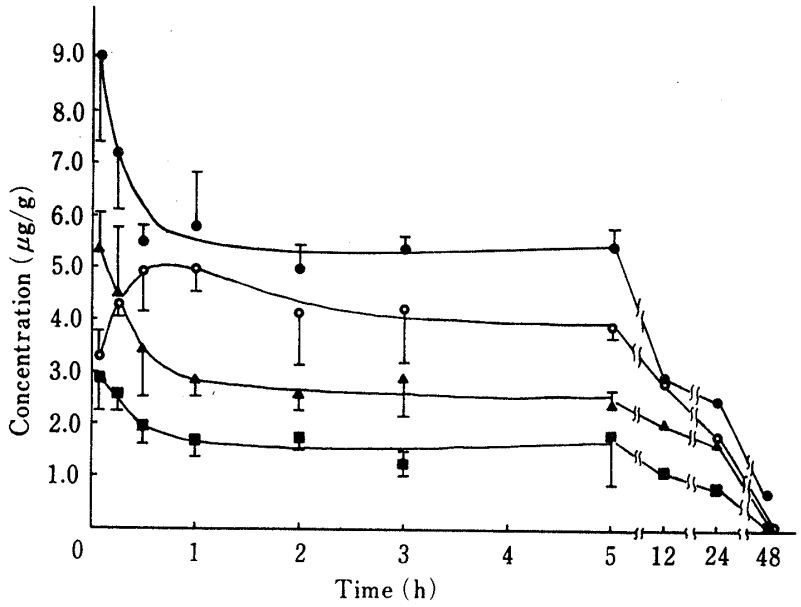

Fig. 8. Tissue Levels of Ginsenoside Rb_1 after Intravenous Administration of Ginsenoside Rb_1 (5 mg/kg) to Rats

—●—, kidney; —▲—, heart; —○—, lung; —■—, liver.
Each point represents the mean ± S.E. of 3 animals.

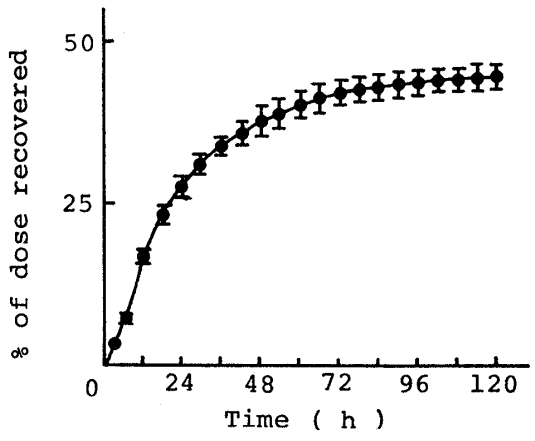

Fig. 9. Cumulative Excretion of Ginsenoside Rb_1 into Urine after Intravenous Administration of Ginsenoside Rb_1 to Rats

Each point represents the mean ± S.E. of 3 animals.

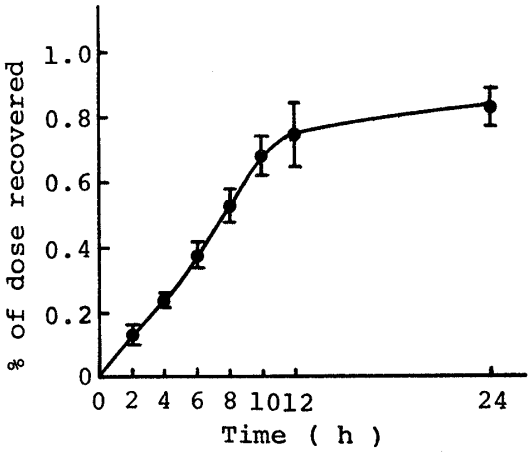

Fig. 10. Cumulative Excretion of Ginsenoside Rb_1 into Bile after Intravenous Administration of Ginsenoside Rb_1 to Rats

Each point represents the mean ± S.E. of 3 animals.

Urinary and Biliary Excretions of Rb_1

The cumulative urinary and biliary excretions of Rb_1 after administration are shown in Fig. 9 and Fig. 10. Most of the urinary excretion of Rb_1 occurred within 48 h after administration, but excretion of Rb_1 continued after that. Cumulative urinary excretion of Rb_1 was 44.4±2.6% in 120 h. On the other hand, most of the biliary excretion of Rb_1 occurred within 12 h, and the cumulative biliary excretion of Rb_1 was 0.8±0.6% in 24 h.

Discussion

As the pharmacological[3] and biochemical[4] activities of ginseng saponins have been clarified, differences have been found between diol group saponins and triol group saponins in binding activity to plasma protein,[2] toxicity,[3a] stimulating effect on the central nervous system[3b] and hemolytic activity.[5] It is thus of interest to know what differences exists between the diol and triol group saponins in absorption, distribution, excretion and metabolism.

In the previous paper,[1] we reported on the absorption, distribution and excretion of Rg_1, one of the main triol group saponins, in rats. In the present work, we attempted to clarify the absorption, distribution and excretion in rats of Rb_1, one of the main diol group saponins (it is soluble in water, like Rg_1), and to compare the results with those for Rg_1.

In the quantitative analysis of Rg_1, we previously used Servachrom XAD-2 resin column (XAD-column) chromatography for the pretreatment of the biological samples from rats. However, we could not separate Rb_1 from other biological components in TLC after the use of XAD-column chromatography for pretreatment. Thus, we employed SEP-PAK for the pretreatment of biological samples containing Rb_1, and tried to elute Rb_1 adsorbed on SEP-PAK with various eluting solutions. Acetone, 30% acetonitrile aqueous solution and MeOH eluted Rb_1 increasingly effectively, in that order. The eluates were applied to TLC with a suitable developing solvent as described in "Experimental," and it was found that Rb_1 was well separated from other biological components. By employing this method, reasonable recoveries of Rb_1 added to biological samples of rats were obtained, except for a few samples. The concentration of Rb_1 in the serum and tissues after oral administration of Rb_1 (100 mg/kg) was less than 0.2 μg/(ml or g). This result suggests that the absorption of Rb_1 from the digestive tract is extremely slight. The percentage (P) of Rb_1 absorbed after oral administration was calculated using the following equation: $P\ (\%)=U_o/U_v\times 100$, where U_o is the cumulative urinary excretion (% of the dose) after oral administration and U_v is that after intravenous administration. As U_o is 0.05% and U_v is 44.4% in this experiment, P is 0.11%. This value is quite low compared with that of Rg_1,[1] and clearly, little Rb_1 is absorbed from the digestive tract of rats.

On the other hand, unabsorbed Rb_1 showed similar behavior to Rg_1 in the stomach and small intestine of rats. However, Rb_1 was quite rapidly decomposed and/or metabolized in the large intestine of rats as compared with Rg_1. Rb_1 was also observed to be decomposed and/or metabolized in the stomach of rats. The details will be reported elsewhere.

The serum concentration of Rb_1 after intravenous administration declined biexponentially and the half-life of the β-phase was extremely long. This is in contrast to the exponential decline of Rg_1 with a half-life of 6.3 min.[1] As Chen *et al.*[2] reported, diol group saponins possess higher plasma protein binding activity than triol group saponins, so the longer half-life of Rb_1 might be related to its higher plasma protein binding activity. Further studies are desirable.

The concentrations of Rb_1 in tissues such as the kidney, heart and liver after intravenous administration of Rb_1 (5 mg/kg) to rats were relatively high, and appeared to be correlated with the concentration of Rb_1 in the serum. On the other hand, the elimination of Rb_1 from the lung was different from those from the kidney, heart and liver. The Rb_1 levels in the brain and spleen were extremely low.

Rb_1 in the serum was excreted more into urine than into bile. In general, it is known that drugs which have large molecular weight are excreted into bile more easily than into urine.[6] In addition, it has been suggested[7] that compounds which are strongly bound with serum albumin are more easily excreted into bile than into urine. However, Rb_1, which is a large-molecular-weight compound (1109.3) and is assumed to have high plasma protein binding activity, was mainly excreted into urine.

From the present results, we conclude that little Rb_1 is absorbed from the digestive tract of rats. Once it enters the serum, Rb_1 persists for a long time in serum and tissues, and is gradually excreted into urine. Unabsorbed Rb_1 in the digestive tract of rats is rapidly decomposed and/or metabolized, mainly in the large intestine. These results are quite different from our results[1] on Rg_1 in rats.

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