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Studies on the Absorption, Distribution, Excretion and Metabolism of Ginseng Saponins. II.[1] The Absorption, Distribution and Excretion of Ginsenoside Rg_1 in the Rat

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The pharmacokinetic charactor of ginsenoside Rg_1, one of the main saponins of ginseng (*Panax ginseng* C.A. MEYER), was investigated in rats by using thin-layer chromatography (TLC) and a dual-wavelength TLC scanner.

Ginsenoside Rg_1 was absorbed rapidly from the upper parts of the digestive tract (accounting for 1.9—20.0% of the dose of Rg_1 administered orally). The serum level of ginsenoside Rg_1 reached its peak at 30 min, and the maximum levels of ginsenoside Rg_1 in tissues were attained within 1.5 h. However, ginsenoside Rg_1 was not found in the brain. Ginsenoside Rg_1 was excreted into rat urine and bile in a 2:5 ratio. It was also proved that ginsenoside Rg_1 was not significantly metabolized in the liver. However, the decomposition and/or metabolism of ginsenoside Rg_1 in rat stomach and large intestine were confirmed.

Keywords——ginsenoside Rg_1; TLC; pharmacokinetic study in rat; biliary excretion; decomposition and/or metabolism

The pharmacological activities of crude drugs used in oriental medicine have been gradually confirmed through clinical and pharmacological studies during the last decade. However, the pharmacokinetics, such as absorption, distribution, excretion and metabolism, of the main active components in crude drugs have been little studied. Although, for example, Han *et al.*[2] and Chen *et al.*[3] reported on the pharmacokinetics of ginseng saponins isolated from *Panax* species, the pharmacokinetics are still not known in any detail. Their reports involve several problems such as the analytical method and animal species used in experiments. Therefore, further experiments are necessary for evaluation of the pharmacokinetics of ginseng saponins.

In a previous paper,[1] we reported a thin-layer chromatography-dual-wavelength densitometry procedure for the quantitative determination of ginsenoside Rg_1 (Rg_1), one of the main ginsenosides, in biological samples of rats. This report presents the results of a study on the absorption, distribution and excretion of Rg_1 in rats after oral and intravenous administration.

Experimental

Most of the materials and methods were the same as described in our preceding paper.[1] As brain samples, bile samples and urine samples obtained from rat bladder were not previously studied, the procedures for the preparation of these samples are outlined below.

Brain——The whole brain was dissected out and homogenized in 6 ml of distilled water with a glass homogenizer. After defatting of the homogenate with 20 ml of benzene, 20 ml of methanol (MeOH) was added to the aqueous phase. The precipitate was removed by centrifugation at 3500 rpm for 10 min and the supernatant was subsequently treated in the manner reported in the previous paper[1] except for the use of the developing solvent $CHCl_3$/1-BuOH/MeOH/H_2O (20:40:15:20, lower phase) in thin-layer chromatography (TLC).

Bile——Male rats were anesthetized with sodium pentobarbital (25 mg/kg, *i.p.*). After laparotomy, the bile duct was cannulated with polyethylene tubing. Each rat was held in a Ballman's cage and the bile was collected periodically. The bile was treated in the same manner as urine.

Urine Sample obtained from Rat Bladder——Male rats were anesthetized with sodium pentobarbital (25 mg/kg, *i.p.*). After laparotomy, the bladder was cannulated with polyethylene tubing. Each rat was held in a Ballman's cage and the urine was collected periodically.

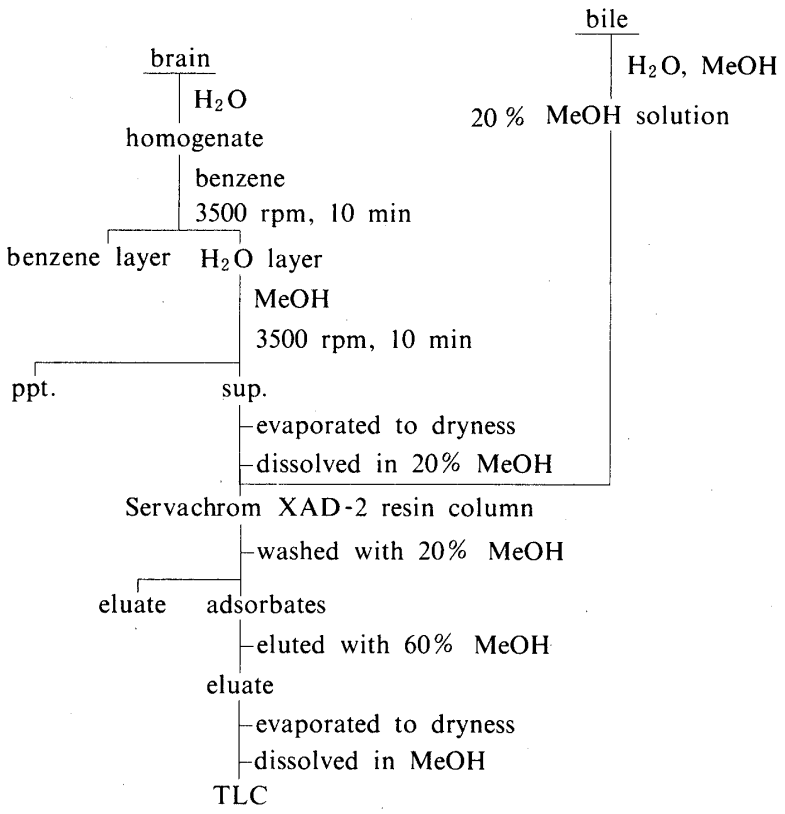

Chart 1. Assay Procedure for Ginsenoside Rg_1 in Brain and Bile

Administration Rg_1——Two percent aqueous solution of Rg_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, 0.2% solution of Rg_1 dissolved 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 Rg_1 was plotted semilogarithmically. The half-life was calculated from the linear region by means of linear regression analysis.

Results

Recovery of Rg_1 added to Brain and Bile

As shown in Fig. 1, added Rg_1 was separated perfectly from components contained in brain and bile samples obtained from non-administered rats.

Therefore, the recovery of Rg_1 added to brain and bile samples was examined in the same manner as for other biological samples.[1] The recoveries and standard deviations of 30 μg Rg_1 added were $94.5\pm6.5\%$ and $96.0\pm4.1\%$ in brain and bile samples, respectively. These methods for brain and bile samples were thus regarded as satisfactory and were employed throughout.

I. Oral Administration

Time Variation of Rg_1 in Rat Serum——Figure 2 shows the time variation of Rg_1 in rat serum after oral administration at a dose of 100 mg/kg. Rg_1 was first recognized in serum 15 min after administration and reached a maximum level, 0.9 μg/ml, at 30 min. After 6 h, Rg_1 was practically undetectable.

Time Variation of Rg_1 in Rat Tissues——Table I shows the tissues levels of Rg_1 after administration to rats. The Rg_1 concentrations in the liver and kidney were a little higher than those in the other tissues. The maximum levels of the liver and kidney were both

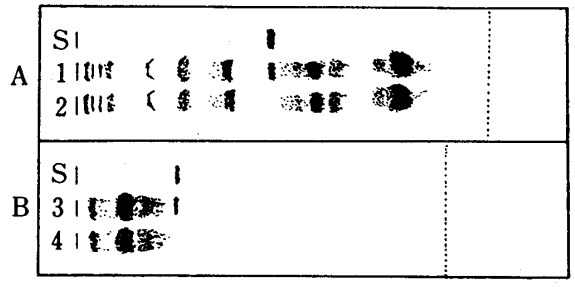

Fig. 1. Thin-Layer Chromatograms of Ginsenoside Rg_1 in Brain and Bile Samples of Rat

Developing solvents: A; $CHCl_3$/1-BuOH/MeOH/H_2O (20:40:15:20, lower phase), B; $CHCl_3$/MeOH/H_2O (65:35:10, lower phase). Plate: Merck precoated Kieselgel 60. Detection reagent: 8% vanillin-MeOH solution/72% H_2SO_4 (1:5), heating at 140°C for 3 min.
S, standard ginsenoside Rg_1; 1, brain+S; 2, brain; 3, bile+S; 4, bile.

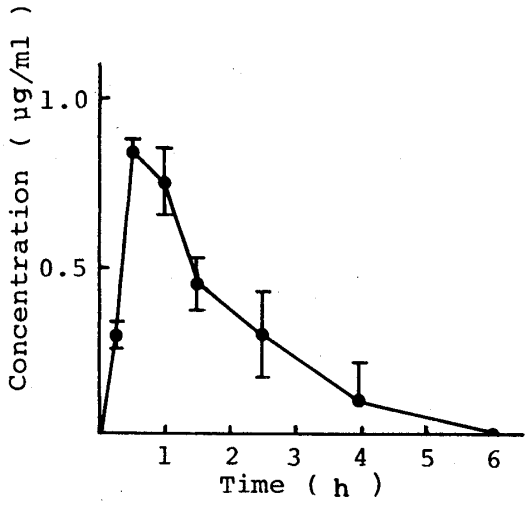

Fig. 2. Serum Concentration of Ginsenoside Rg_1 after Oral Administration of Ginsenoside Rg_1 (100 mg/kg) to Rats

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

TABLE I. Tissue Levels of Ginsenoside Rg_1 after Oral Administration of Ginsenoside Rg_1 (100 mg/kg) to Rats

Tissue	Concentration (μg/g) Time after administration (h)					
	0.5	1.0	1.5	2.5	4.0	6.0
Brain	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Heart	N.D.	1.1±1.1	1.5±0.8	N.D.	N.D.	N.D.
Lung	N.D.	1.2±0.9	1.5±1.1	N.D.	N.D.	N.D.
Liver	1.1±0.7	2.3±1.4	3.5±2.0	2.2±1.5	1.4±0.9	N.D.
Kidney	0.9±0.7	1.8±1.0	2.6±1.5	1.9±1.1	1.5±0.9	N.D.
Spleen	N.D.	0.8±0.5	1.1±0.7	N.D.	N.D.	N.D.

Each value represents the mean±S.E. of 4 animals.
N.D.; not detectable.

attained within 1.5 h and were 3.5±2.0 μg/g and 2.6±1.5 μg/g, respectively. On the other hand, the Rg_1 levels in the heart, the lung and the spleen were below 1.5 μg/g at all times after administration. However, no Rg_1 was found in the brain at any time after administration.

Rg_1 Amount in Digestive Tract of Rat——The variation of Rg_1 contents in the stomach, small intestine and large intestine after administration are shown in Fig. 3. The amounts of Rg_1 in the stomach and the small intestine at 15 min after administration were 42.3±1.6% of the dose and 35.6±4.3% of the dose, respectively. At 30 min after, most of the Rg_1 administered moved to the small intestine and 56.7±8.5% of the dose existed in the large intestine 4 h after administration. No Rg_1 was found in the small intestine at 6 h after administration and 52.2±2.7% of the dose remained in the large intestine at that time.

On the other hand, degradation products and/or metabolites of Rg_1 were also found in the stomach and large intestine by TLC. Figure 4 shows the TLC chromatogram.

Urinary, Fecal and Biliary Excretions——The time variation of urinary and fecal excretions of Rg_1 administered to rats is shown in Fig. 5.

The cumulative urinary and fecal excretions of Rg_1 within 24 h were 0.40±0.04% of the dose and 41.2±2.6% of the dose, respectively. Fifty-seven percent of total urinary excretion and 71% of total fecal excretion occurred at 6 to 12 h after administration.

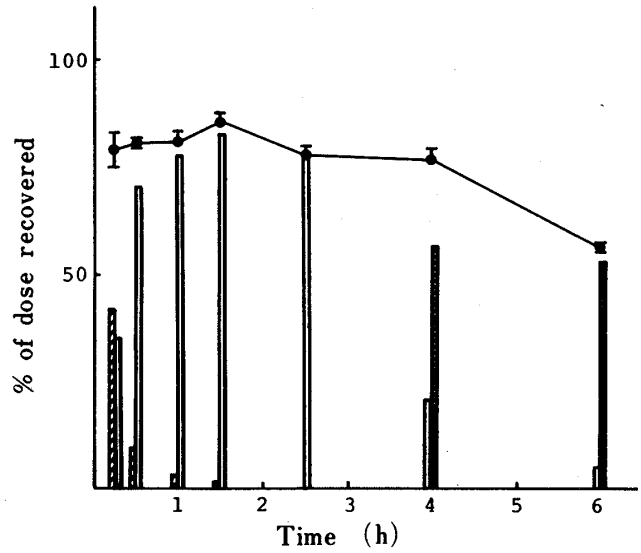

Fig. 3. Amounts of Ginsenoside Rg_1 in the Digestive Tract of Rats after Oral Administration of Ginsenoside Rg_1 (100 mg/kg)

▨, stomach; □, small intestine; ▩, large intestine —●—, total.

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

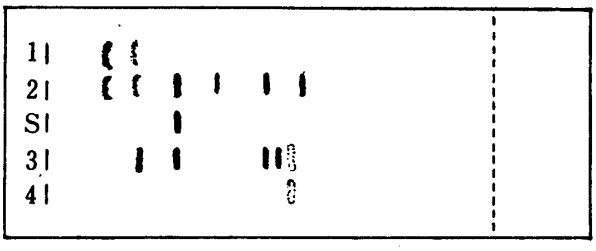

Fig. 4. Thin-Layer Chromatogram of Degradation Products and/or Metabolites of Ginsenoside Rg_1 in the Stomach or Large Intestine

Developing solvent: $CHCl_3/MeOH/H_2O$ (65:35:10, lower phase).

1, normal stomach; 2, stomach 1.5 h after oral adminstration of ginsenoside Rg_1; 3, large intestine 6.0 h after oral administration of ginsenoside Rg_1; 4, normal large intestine; S, standerd ginsenoside Rg_1

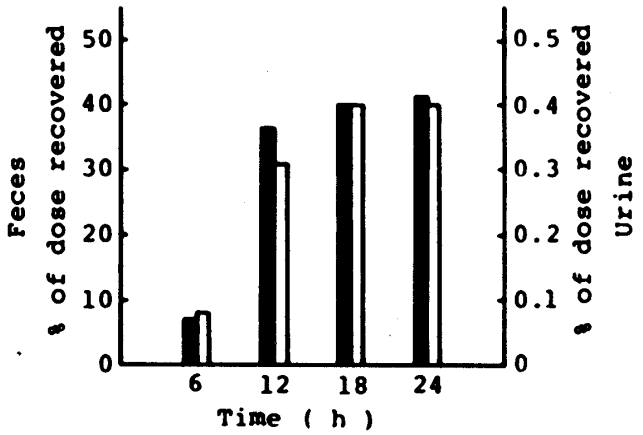

Fig. 5. Cumulative Excretions of Ginsenoside Rg_1 into Urine and Feces after Oral Administration of Ginsenoside Rg_1 (100 mg/kg) to Rats

■, feces; □, urine.

Each point represents the mean of 3 animals.

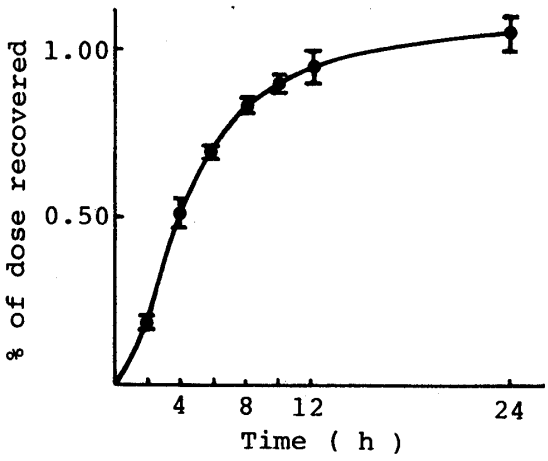

Fig. 6. Cumulative Excretion of Ginsenoside Rg_1 into Bile after Oral Administration of Ginsenoside Rg_1 (100 mg/kg) to Rats

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

As shown in Fig. 6, the cumulative biliary excretion within 24 h was 1.1±0.1% of the dose and 34.1% of total excretion in the bile occurred at 2 to 4 h after administration.

II. Intravenous Administration

Time Variation of Rg_1 in Rat Serum——As shown in Fig. 7, the level of Rg_1 in rat serum was 8.9±1.0 μg/ml 2 min after administration at a dose of 5 mg/kg, and declined with a half-life of 6.3 min. After 60 min, Rg_1 was practically undetectable.

Time Variation of Rg_1 in Rat Tissues——Figure 8 shows the time variation of Rg_1 in the liver and the kidney after intravenous administration to rats. In both organs, a two-phase decline of Rg_1 was observed, namely a rapid α-phase and a slow β-phase. The half-lives of Rg_1 in liver were 5.3 min for α-phase and 34.7 min for β-phase, while those in the kidney were 5.7 min for α-phase and 36.1 min for β-phase.

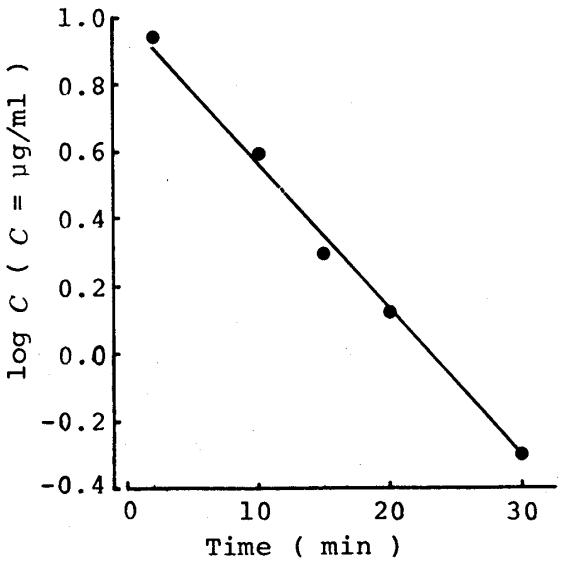

Fig. 7. Serum Concentration of Ginsenoside Rg_1 after Intravenous Administration of Ginsenoside Rg_1 (5 mg/kg) to Rats

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

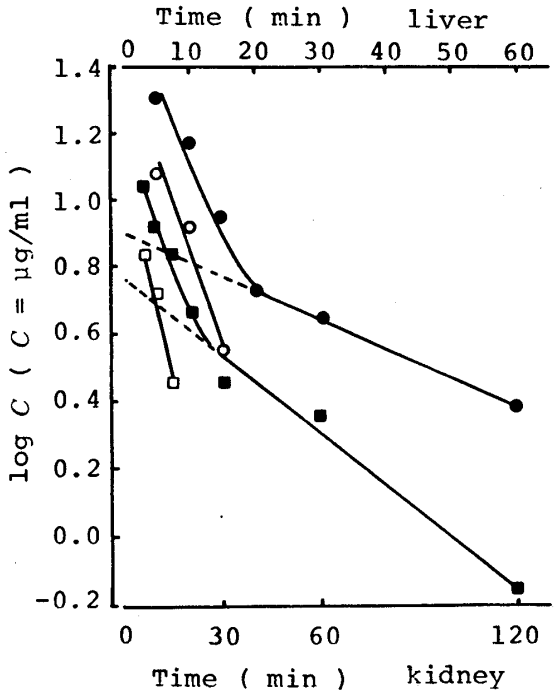

Fig. 8. Tissues Levels of Ginsenoside Rg_1 after Intravenous Administration of Ginsenoside Rg_1 (5 mg/kg) to Rats

○, ●, liver; ■, □, kidney.

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

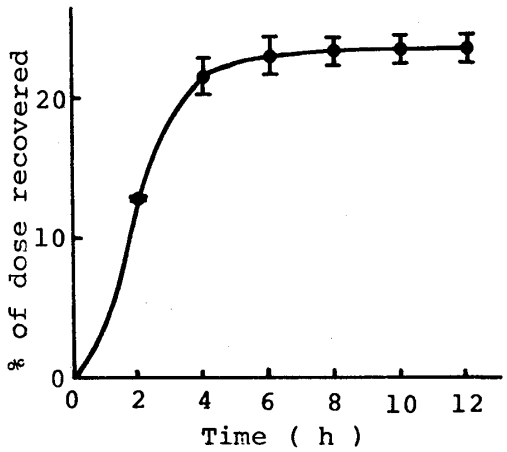

Fig. 9. Cumulative Excretion of Ginsenoside Rg_1 into Urine after Intravenous Administration of Ginsenoside Rg_1 (5 mg/kg) to Rats

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

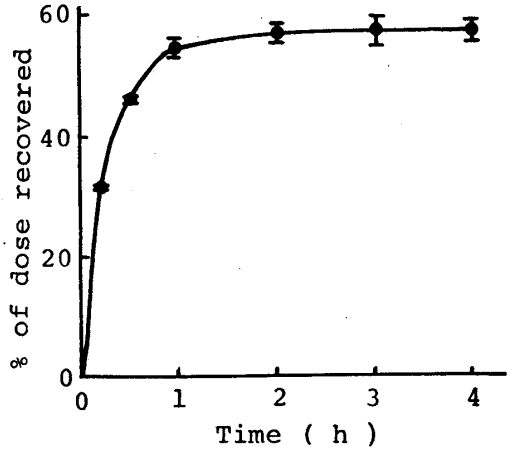

Fig. 10. Cumulative Excretion of Ginsenoside Rg_1 into Bile after Intravenous Administration of Ginsenoside Rg_1 (5 mg/kg) to Rats

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

Urinary and Biliary Excretions——The time variations of cumulative urinary and biliary excretions of Rg_1 administered to rats are shown in Fig. 9 and 10, respectively.

The urinary excretion of Rg_1 was examined by using urine obtained from rat bladder. The greatest urinary excretion of Rg_1 was observed during the 4 h after administration. The cumulative urinary excretion of Rg_1 within 12 h was 23.5±10.9% of the dose.

On the other hand, the biliary excretion of Rg_1 was faster than the urinary excretion, that is, more than 50% of the biliary excretion of Rg_1 occurred within 15 min after administration. The cumulative biliary excretion of Rg_1 within 4 h was $57.2 \pm 1.7\%$ of the dose.

Discussion

Ginseng saponins, isolated from the root of *Panax ginseng*, have been regarded as principal components manifesting the pharmacological activities of the drug. There are many reports of pharmacological and chemical studies on ginseng saponins. However, in spite of the investigations of Han *et al.*[2] and Chen *et al.*,[3] the absorption distribution, excretion and metabolism of ginseng saponins have still not been completely elucidated. Han *et al.*[2] studied the absorption, distribution and excretion of Rg_1 in rabbits by means of TLC-colorimetry and the radioisotopic method. However, there were problems with their analytical method and the use of (U-^{3}H) Rg_1, as pointed out by Chen *et al.*[3] On the other hand, the GLC method developed by Chen *et al.*[3] had too low a sensitivity for determination of ginsenoside in biological samples. Thus, they reported that no Rg_1 was found in the plasma and urine of rabbit after oral administration. In contrast, there are reports that both Rg_1 injected intraperitoneally and a saponin fraction containing Rg_1 administered orally stimulated the syntheses of nucleic acid, protein and lipid in the rat bone marrow.[4] These findings are inconsistent with those of Chen *et al.*[3] even when the difference of animal species is taken into account.

Therefore, we attempted to investigate the absorption, distribution and excretion of Rg_1 by employing our microdetermination method for Rg_1 in biological samples of rats. Rats were chosen sinse they have been widely used in pharmacological studies of Rg_1.

The amount of an absorbed drug after oral administration can be calculated by determination of the drug in the digestive tract. Since about 80% of the dose was present in the digestive tract until 2.5 h after administration of Rg_1, and degraded and/or metabolized forms of Rg_1 were found in the stomach, the amount of Rg_1 absorbed seems to be less than 20% of the dose.

In general, the amount of absorbed drug after oral administration can also be estimated from the urinary excretions of the drug after oral and intravenous administrations. However, Rg_1 was excreted more in the bile than in the urine. Therefore, the percentage (P) of absorbed Rg_1 after oral administration was calculated using the following equation: $P\ (\%) = UBo/UBv \times 100$, where UBo is the sum of urinary and biliary excretions (% of the dose) after oral administration and UBv is the same sum after intravenous administration. As UBo is 1.5% and UBv is 80.6% in this experiment, P is calculated as 1.9% of the dose, and hence the amount of absorbed Rg_1 seems to be more than 1.9% of the dose even if the minimum value is taken. Consequently, the amount of Rg_1 absorbed after oral administration seems to be in the range of 1.9—20.0% of the dose. In addition, the absorption of Rg_1 from rat digestive tract was assumed to occur rapidly in the upper part of the gastrointestinal tract. Rg_1 was found in the plasma as early as 15 min after administration and only 80% of the dose of Rg_1 was present in rat digestive tract at that time.

The serum level of Rg_1 reached its peak 30 min after oral administration and then declined rapidly. The levels of tissues (except for the brain) reached a maximum around 1.5 h after oral administration and then declined smoothly. There were no tissues to which Rg_1 was distributed specifically. Therefore, Rg_1 administered orally was concluded to be absorbed rapidly from rat digestive tract and to be distributed widely in rat tissues except for the brain. In spite of Kaku *et al.*'s reports[5] which indicated action of Rg_1 on the central nervous system of rats, Rg_1 was not detected in rat brain at any time after oral administration of Rg_1. This indicates that the amount of Rg_1 in rat brain was less than 0.2 μg/g tissues weight, which was the lower limit of detection in our method.[1] Rg_1 may affect the central nervous system at the hormonal level.

The urinary and biliary excretions of Rg_1 occurred in a 2:5 ratio in both oral and intravenous administrations of Rg_1 to rats. This result is consistent with the known characteristics of biliary excretion, since the minimum threshold of molecular weight in rat biliary excretion is said to be 325 ± 50[6] and the molecular weight of Rg_1 is 801.

As about 80% of the dose of Rg_1 was excreted into urine and bile after intravenous administration to rats, it seems that Rg_1 is hardly metabolized in rat liver. On the other hand, it is obvious that degradation and/or metabolism of Rg_1 occurs in the stomach and large intestine of rats. The details of this phenomenon will be reported elsewhere.

From the results of the present experiments, it seems reasonable to draw the conclusion that Rg_1 can be absorbed rapidly from the upper part of the digestive tract after oral administration and be distributed widely in the whole body except for the brain, and is then lost quickly through urinary and biliary excretions.

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