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Metabolism of Dammarane Triterpene Glycosides of Korean Ginseng(I)

Absorption, Organ Distribution and Excretion of ^{3}H-Panax Saponin A

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Abstract

Panax Saponin A(PSA), one of dammarane glycosides of Korean ginseng, was labeled with ^{3}H or ^{14}C by our previously reported procedures of organic synthesis. Tracer studies with ^{3}H-PSA in vivo yielded the following results: 1). *Oral and intraperitoneal administration of ^{3}H-PSA resulted in the rapid appearance and prolonged retention of ^{3}H-PSA in all organs such as liver, brain, bone marrow and spleen of mice.* 2). *The amount of cellular intake of ^{3}H-PSA was shown to have a certain level of saturation ranging from 0.4mg to 0.7mg per 20gm body weight of mice.*

Administration of ^{3}H-PSA within the dosage of the saturation point did not give urinary excretion of ^{3}H-PSA. On the contrary, excessive administration of ^{3}H-PSA resulted in rapid excretion of the substance in the urine of mice.

In the light of a number of experimental results concerning the pharmacology of Korean ginseng, the dammarane triterpene glycoside has been considered as one of biologically active components contained in ginseng. The chemical studies on this substance have been almost completed at present time.[1~3]

Among many biological and pharmacological activities of this component, its activities on central nerve system,[4~6] stimulatory effects on protein synthesis[7~9], enhancement of red blood cell production, and anti-inflammatory effect[11~12] happen to coincide with the ethnobotanical efficacies of ginseng. Nonetheless many ginseng sciensists have had doubts in the metabolic fate of the ginseng saponins. For example,

1) Does the ginseng saponin penetrate into gastro-intestinal tract ?

2) Does the ginseng saponin reach a specific organ in which the ginseng saponin gives some biological effects, especially in brain?

3) Is the ginseng saponin metabolized *in vivo* and excreted smoothly?

Thus we developed a method to label panax saponin A, one of the ginseng saponins

with radioactive ^{14}C and ^{3}H[17-18]. And we attempted to answer these questions by using the radioisotope-labeled ginseng saponin.

Materials and Methods

1. Materials

1) Ginseng

Korean ginseng produced in Geum San was purchased from the domestic market and total 18 kg were used for isolating panax saponin A(PSA).

2) Radioisotopes

Tritium oxide(T_2O), 5 curie/ml and ^{14}C-isopropyliodide, 1 mc/ample were purchased from the Radio-Chemical Center, Ltd., in Great Britain.

3) Animals

Ddd albino mice of about 20g and house rabbits of 1.8 kg in both sexes were used for the experiments.

4) Reagents

Hyamine hydroxide was used for tissue solubilizer, and PPO(6 g/1) and POPOP (75 mg/1) were dissolved in spectro-grade toluene and used for scintillation fluids.

2. Methods

1) Perparation of Ginseng Extract

Dried white ginseng, 18 kg, were crushed and 50 1 of methanol were added into it and refluxed for 5 hrs. Then methanol was evaporated fully. Such extraction procedures were repeated for three times. About 2.5 kg of syruppy residue were obtained.

2) Seperation of Panax Saponin A(Ginsenoside Rg_1)

Total 2.5 kg of ginseng extract were suspended in water and extracted with 4 l of ether. This etheral extraction was repeated for three times. After the ether layer was discarded, the water layer was extracted again with n-butanol(2 l each time) for three times. Then these butanol layers were combined and concentrated under reduced pressure. About 500g of crude saponin were obtained. Total 500g of crude saponin were dissolved in 1 litre of methanol and 3 1 of chloroform were mixed with it well. When small amount of water was added into this solution, two layers were formed.

The chloroform layer was separated and condensed under reduced pressure. About 120 g of residue remained. This residue contained mainly PSA. For further purifica-

tion, 30g of crude PSA were applied to column chromatography (silica gel 500g, 5×75 cm in size) and this column was eluted with chloroform-methanol(3:1) solvent. About 18.5g of pure PSA, mp 195°, were obtained.

3) Synthesis of Panax Saponin A Decaacetate(II)

Nine grams of PSA were first dissolved in 20 ml of pyridine and then a mixture of 20 ml acetic anhydride and 10 ml pyridine was slowly added into it. And this solution was refluxed for five hrs. After the solution was cooled to room temperature, one litre of water was added and mixed well and it was left at room temperature over-night. As the precipitate was formed, it was collected by filtration. The precipitate was crystalized from methanol. When it was recrystallilzed in methanol twice, the pure PSA decaacetate, 12.5g, was obtained(mp 252°).

4) Syntheis of PSA Decaacetate Glycol(III)

Nine grams of PSA decaacetate were dissolved in 25 ml of benzene. Two grams of osmium tetraoxide dissolved in 20 ml of benzene solution were added into the above and mixed well. Then 2 ml of pyridine were added. Reaction was allowed to proceed at room temperature for six days. Then H_2S gas was introduced slowly into the reaction solution until osmium sulfate was precipitated completely. After the precipitate was removed by filtration, the filtrate was applied to column chromatography(silica gel column 1×10cm). Then it was eluted with benzene-ethylacetate(1:1). The substance having R*f* value of 0.1 was separated and eluting solvent was evaporated under reduced pressure. The remaining residue was crystallized in methanol. A needle-form crystal showed mp 156-8°, and ν_{OH}, 3400 cm^{-1}.

5) Synthesis of PSA Decaacetate Trisnor Aldehyde

Firstly, three g of HIO_4 were dissolved in the solvent consisting of 10 ml of dioxane and one ml of water. Then seven g of PSA decaacetate glycol dissolved in 40 ml of dioxane were mixed with the above HIO_4 solution. When the reaction was allowed to proceed at room temperature, the glycol portion of (III) was oxidized completely. Deaerated water was added into the reaction mixture. Aldehyde (IV) was gradually crystallized as needle. For further purification, the above crystal was recrystallized in chloroform-ethanol solution, and then in ethanol for three times. All procedures were conductad in N_2 atmosphere.

Five grams of the final product were obtained. It showed mp 229° and its IR spectra showed the disappearance of -OH absorption band, but $\nu_{C=O}$ appeared at 1710 and $\nu_{CH(CHO)}$ at 2820 and 2720 cm^{-1}.

6) ^{14}C-Triphenylphosphorane Iodide(V)

One milli-curie sample of ^{14}C-isopropyl-iodide was diluted with one ml of radio-inactive-isoppopyl-iodide and then mixed with 5ml of xylene. This solution was carefully transfered into the glass vial in which 2g of triphenylphosphine was placed. After the vial was sealed, it was heated in boiling water bath for 6 hrs. Then the vial was cooled to room temperature. As the xylene was evaporated under reduced pressure, 1.14g of ^{14}C labeled isopropyltriphenyl-phosphorane iodide(V) was obtained.

7) Preparation of ^{14}C-Isopropyl Wittig Reagent

To 20ml of freshly distilled, anhydrous dimethylsulfoxide, 2 g of NaH (washed with 30ml of ether for three times) was dissolved to obtain sodium methylsulfinyl-carbanion(VI).

^{14}C-isopropyltriphenylphosphorane iodide(V) 1.14 g was placed in a small glass bottle and then air(oxygen) in the bottle was driven out by repetition of suctioning and N_2 replacement.

To this bottle containing compound (V), 3 ml of sodium methylsulfinyl carbanion (VI) were added to prepare Wittig reagent. Overall yield of reaction depends on the dehydration of dimethylsulfoxide, deaeration of reaction bottle and the purity of NaH.

8) Synthesis of ^{14}C-PSA(IX)

Anhydrous PSA trisnoraldehyde(IV) 1,9 g was placed in a glass bottle with rubber stopper and then N_2 gas was introduced into the bottle to drive out oxygen. To this bottle, 2 ml of DMSO were added. Then 2 ml of Wittig reagent were introduced through the rubber stopper by using syringe. After 12 hrs, 50 ml of 5% NaOH(in 50% ethanol) were added into the reaction solution and it was heated for two hrs till saponification was completed. After the ethanol in reaction solution was removed by evaporation, the remaining solution was extracted with 10 ml of butanol each for three times. As the butanol was evaporated under reduced pressure, the final product ^{14}C-PSA(IX) was obtained. For further purification, column chromatography was employed(silica gel 50 g, eluent, $CHCl_3$:MeOH:H_2O, 70 : 30 : 4). The yield was 0.25 g and specific activity showed 0.1 μci/mg. The final product was dissolved in 25 ml of ethanol and stored in refrigerator to avoid radiolysis.

9) Synthesis of PSA Nonaacetate

To the solution of nine g PSA dissolved in 35 ml of pyridine the mixture of acetic anhydride (11 ml) and pyridine(15 ml) was slowly added. The reaction was allowed in cold for three days to obtain the mixture of nonaacetate, decaacetate(II)

and octaacetate. The reaction was terminated by adding cold water and allowed for over night. The precipitate was collected by filtration, dissolved in chloroform and then the resulting solution was dehydrated with anhydrous sodium sulfate. The chloroform layer was separated and evaporated under reduced pressure to obtain 13gm residue. The residue was chromatographed over silica-gel column to obtain 3.5 gm nona-acetate (silica gel 250 g; eluent, benzene: ethylacetate 6 : 4).

10) Synthesis of PSA-Ketone(XII)

To prepare Sarett reagent, one g of CrO_3 was dissolved in 10ml of pyridine. To 5 ml of this reagent the solution of 3.5 g of PSA nonaacetate dissolved in 8ml of pyridine was added and mixed well. After 36 hours, the reaction was ceased by adding cold water. Then the reaction mixture was extracted three times with 70 ml of ether. And all three etheral extracts were combined and washed with water. 3% HCl and 5 % $NaHCO_3$ successively. The washed etheral extract was evaporated and the remaining residue was crystallized in methanol to give 3.2 g PSA nonaacetate ketone (XI) (mp 233-5^0). In order to saponify it, 3.2 g of compound(XI) were added into 100ml of ethanol containing 50 ml of 5% NaOH. Then the solution was refluxed for five hrs on boiling water bath. After the evaporation of ethanol the remaining solution was extracted with 50 ml portion of butanol for four times. As the butanol was evaporated under reduced pressure to give 2.1 g of PSA ketone(XII). The final product showed a single spot on TLC with Rf 0.16($CHCl_3$:MeOH:H_2O, 75 : 25 : 2.5)

11) Synthesis of H^3-PSA(XIV)

PSA ketone(XII) 1.9 g dissolved in 7.2 ml of absolute alcohol was placed in a 10-ml glass vial. To this soluton, 100 mg sodium metal in 2.5 ml of ethanol and 0.5 ml T_2O(2.5 Ci) were added and then the vial was sealed and heated on ethanol bath for three hrs. The reaction solution was cooled and ethanol was evaporated fully. The remaining solution was adjusted to pH 7 with 5% HCl and 15 ml of water were added. The whole solution was extracted with 10 ml portion of butanol for four times. Then the butanol portion was evaporated, and the remaining residue was dissolved in ethanol/benzene(1 : 1) and evaporated for several times to remove water completely. About 1.6 g of H^3-PSA ketone(XIII) were obtained.

In order to reduce the ketone of compound (XIII), 1.5 g of compound (XIII) dissolved in anhydrous isopropanol(20ml) were reacted with three g of powdered sodium metal under N_2 atmosphere. The powdered sodium metals used in this reaction were specially prepared as follows: three g of sodium metal were dissolved in 30 ml of xylene heated until metals were completely melted and then vigorously shaken

to obtain fine particles. After the removal of unreacted sodium from reaction vessel, the reacton was ceased by adding cold water. The water layer was extracted with 70 ml butanol each for four times. After the removal of butanol the remaining residue was applied to column chromatography to obtain ^3H-labeled PSA.(silica gel, 100g, eluent, $CHCl_3$: MeOH: H_2O, 75 : 25 : 2.5). The yield of ^3H-PSA was 1.3 g. Total amount of H^3-PSA synthesized was dissolved in ethanol and stored in refrigerator until use. This ^3H-PSA in ethanol solution contained 2.61% of PSA and radioactivities appeared to be 111 μci/ml(10% counting efficiency). The PSA content in ethanol solution was determined by vanillin-H_2SO_4 color reaction. Details of this assay method were reported by the author elsewhere.

12) Measurement of Radioactivity

Radioactivities were measured by Nuclear Chicago Liquid Scintillation Spectrometer. All the tissue samples were treated specially to minimize quenching effects as follows:

A) Treatment of tissue samples: The organs and tissues like brain and liver were dried in an oven at 105^0 until they showed consistent average weight. Then these dried tissues were pulverized by using mortar and pestle. And the dried tissue powders were incubated in Hyamine hydroxide solution at 50~60° until they were completely dissolved. To this clear solutions, 10 ml of toluene based scintillation fluid were added and mixed well. Then radioactivities were counted.

B) Measurement of radioactivities in urine and blood samples: About 0.1~1.0ml of urine or blood samples were spotted on filter paper. The filter papers were dried fully in air-stream, and counted the radio-activity of the strip in toluene scintillation solution.

C) Measurement of radioactivities on TLC plate: Tissue extracts of liver, brain and other organs were spotted on pre-coated silica gel TLC plates. TLC plates were developed in the solvent system, $CHCl_3$:MeOH:H_2O(75 : 25 : 2.5) up to 15 cm from the base line. After drying the plates, they were cut by one cm length. Then these pieces were placed in the counting vials and the radioactivites were strip-counted.

13) Detection of ^{3}H-PSA in TLC of Tissue Extract

^3H-PSA, 0.2 ml (PSA, 5.2 mg; 22.4μci) was diluted with 0.4 ml of normal saline and injected i.p. into mice. One hour later, the organs were removed as reported elsewhere.[15]

The organ samples were homogenized in 30 ml of MeOH, and whole homogenates were heated on a water bath for five min. Whole homogenates were filtered and the filtrates were condensed to approximately one ml volume. The radioactivities were

measured as the procedures in 12)-C.

14) Distribution of ^{3}H-PSA *in vivo*

^{3}H-PSA 0.8 ml (PSA, 4.7 mg, 20μci) was slightly heated to remove ethanol. The remaining residue was dissolved in physiological saline for oral administration. Each group consisted of three mice was received p.o. ^{3}H-PSA. After a specified time, mice were sacrificed by cervical dislocation, and organs and urine were harvested. The radioactivities of organ samples were measured as the experiment (12)-A.

15) Studies on Gastrointestinal Absorption of ^{3}H-PSA

Mice were fasted for 24 hrs before experiments. All mice in the experiment were divided into two groups. One group received p.o. ^{3}H-PSA 0.1 ml (PSA, 2.61 mg, 11.1 μci). At each specified time later, mice were sacrificed and gastrointestines from esophagus to anus were harvested. The gastrointestinal contents were harvested by washing with 30ml portion of 50% MeOH, filtered and 100 ul of MeOH filtrate were spotted on TLC sheets. The radioactivities of the sheets were checked by method as in (12)-B.

The other group received p.o. 0.01 ml of PSA (PSA 0.26 mg, 1.11 uci). After each specified time, mice were sacrificed and the contents in gastro-intestine were collected and washed with 30 ml of 50% MeOH. The methanol washings 500 ul were spotted on TLC sheets. The next preparations were followed as the above.

16) Repetitive Administrations of ^{3}H-PSA

This experiment was conducted to know whether the cellular uptake of ^{3}H-PSA might be increased by repeated administration or by elevated doses of ^{3}H-PSA.

Total 12 mice were divided into four groups, A, B, C, and D. On the first day the mice in group A received i.p. ^{3}H-PSA(PSA 2.61 mg, 11,1 uci), on the second day the mice in groups A and B, and on the third day the mice in groups A, B, and C received the same doses of H^3-PSA as on the first day. Then on the third day the mice in group D were injected i.p. with ^{3}H-PSA (PSA 7.83 mg, 33.3 μci), three-fold dose at once. On the fourth day, all mice were sacrificed and various tissues and organs were harvested. The subsequent preparations of samples were followed as in experiment (12)-A.

17) Prolonged Retention of ^{3}H-PSA

Each mouse was injected i.p. with 0.2 ml of ^{3}H-PSA (PSA 2.61 mg, 22.2 μci). One week or two weeks later, three mice were sacrificed and various organs were harvested. The organ samples were homogenated. The subsequent sample preparations were followed as in experiment (12)-A.

18) Urinary Excretion of ^{3}H-PSA

A male house rabbit (1.75 kg, body wt) was anesthesized and its urinary tract received cannulation surgery. Then the rabbit received 2 ml of ^{3}H-PSA (52.2 mg, 222 uci) through stomach by injection. Urine samples were collected every one hour up to ten hours period. The rabbit was sacrificed and its organs were harvested. The organ samples were prepared and treated as in experiments (12)-A and (12)-B.

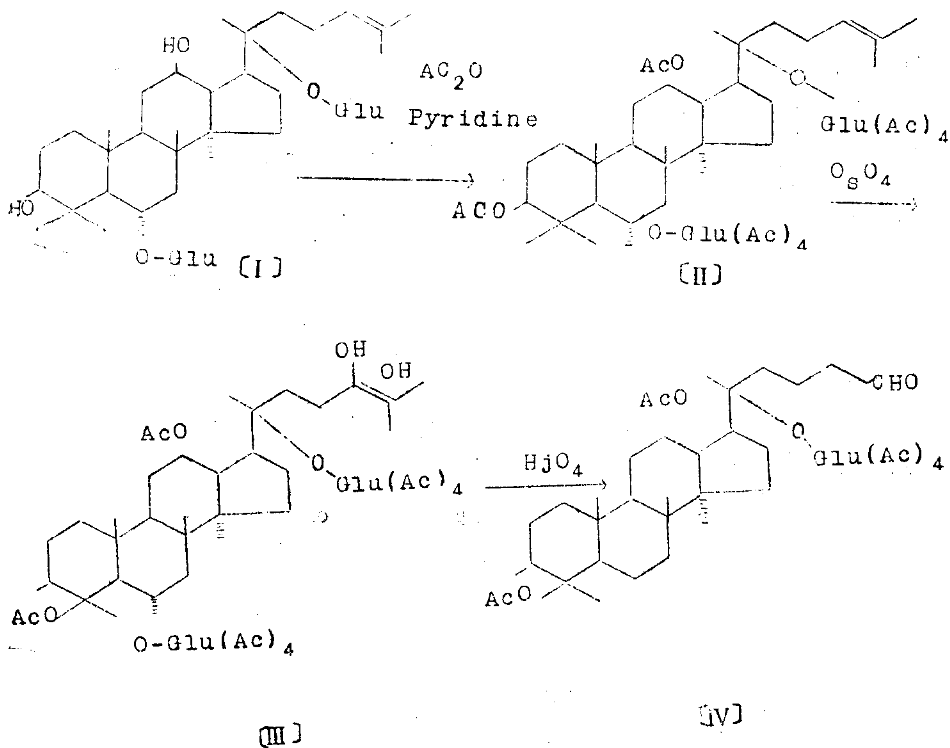
Results and Discussion

1. Synthesis of ^{14}C-PSA

As Scheme I shows, synthesis was divided into eight steps and finally carbon stoms of isopropropylidene portion in PSA side chain were replaced with radioactive isotope ^{14}C.

From step(I) to (IV), PSA trisnoraldehyde was synthesized and ^{14}C-isopropyl Wittig reagent was prepared through step(V) to (VII).

^{14}C-isotope could be introduced into the side chain of PSA by simple mixing the trisnoroldehyde [IV] and the ^{14}C-Wittig reagent [VII]. Overall chemical yield was



$(C_6H_5)_3{\equiv}P + (C^{14}H_3)_2C(H)J \longrightarrow (C_6H_5)_3{\equiv}P(J){-}CH(C^{14}H_3)_2$ [V]

$(CH_3)_2SO + NaH \longrightarrow NaCH_2(CH_3)S{=}O$ [VI]

[V] + [VI] $\longrightarrow (C_6H_5)_3{=}P{=}C(C^{14}H_3)_2$ [VII]

[IV] + [VII] $\longrightarrow$ [VIII] $\xrightarrow{NaOH}$ [X]

AcO, AcO, O-Glu(Ac)$_4$, O-Glu(Ac)$_4$, $C^{14}H_3$, $C^{14}H_3$ [VIII]

HO, HO, O-Glu, O-Glu, C 14, C 14 [X]

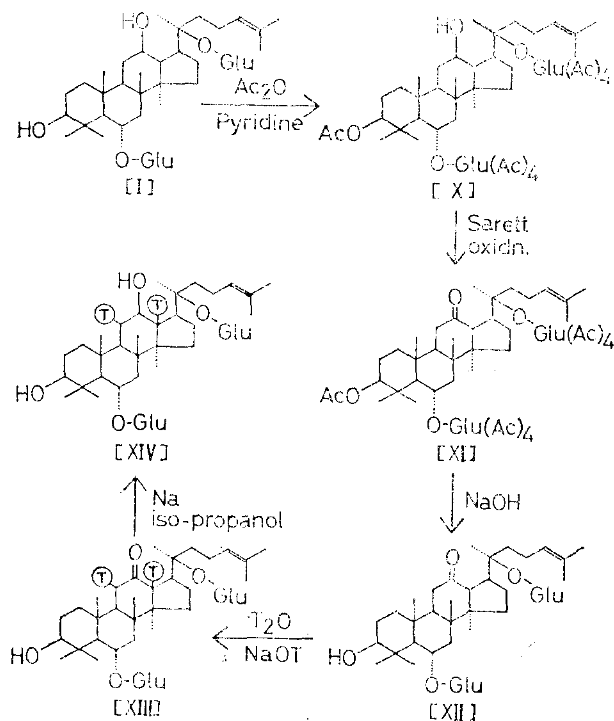
Scheme I. ^{14}C-Labeling Synthesis of PS-A

20% with respect to PSA as the starting materials, and radio-chemical yield was about 5%. Such relatively low yield of labeling seems due to: (1) Before receiving ^{14}C-isopropyliodide from the manufacturer, appreciable amount of radiolysis occurred; (2) Wittig reagent was decomposed by moisture and oxygen, (3) Excess amount

of Wittig reagent caused such a low yield.

2. Synthesis of ^{3}H-PSA

As shown in Scheme II, when PSA ketone(XII) was reacted with T_2O and NaOT in heat, H atoms of C_{11} and C_{13} were replaced by tritium by keto-enol tautomerism. Although it was said that this reacton occurs in good yield according to various literatures published, our yield appeared to be only 3%. Perhaps the easy exchange between tritium and hydrogen during solvent extraction steps could result in such a low yield.



Scheme-II

3. Purity of ^{3}H-PSA

The column A in Table I represents radiogram of ^{3}H-PSA itself on TLC plate. PSA spot is located in 6~8 cm from the base line of TLC plate. The radioactivities of this spot(PSA) were counted as representing 60% of total radioactivities. Such decrease of radioactivities could result from radiolysis which could occur during purification of ^{3}H-PSA by using column chromatography and during evaporation step for removing solvents.

Table I. TLC-Radiogram Tables of ^{3}H-PSA sample, liver extract and urine. (radiograms of TLC).

Distance from Start Line	A	B	C
	^{3}H-PSA	Liver	Urine
2	2152	102	4559
4	910	207	5898
6	4793*	645*	32305*
8	6334*	473	30113*
10	530	504	3315
12	1437	67	2743
14	2714	28	482
		27	

* This indicates the spot of ^{3}H-PSA.

4. Radiogram of Mouse Liver Extracts and Urine

The column B in Table I shows the radiogram of mouse liver extract. Mice were injected i.p. with ^{3}H-PSA. One hour later, livers were removed from mice, washed with normal saline and homogenated in alcohol. Then, small amount of cold PSA was added into the liver homogenate. An exact amount of liver homogenate was chromatographed by TLC. The location of PSA on TLC plate was identified by placing the TLC plate in iodine chamber. The PSA spot appeared as brown spot. When this spot was cut and strip-counted, it showed very strong radioactivities. Such result indicated that PSA was indeed transported into tissue and taken by liver.

It was also found that another spot having greater *Rf* value than that of authentic PSA showed somewhat strong radioactivity.

However, it was uncertain whether this spot represented a metabolite of PSA or just a radiolysis product of PSA.

The column C in Table I shows a radiogram of urine. The urine sample was collected one hour after ^{3}H-PSA was administered. This urine radiogram appeared to be quite similar to ^{3}H-PSA radiogram itself. Thus most of ^{3}H-PSA might be excreted as intact form.

5. Time Course Distribution of ^{3}H-PSA in Various Organs

Each mouse was injected i. p. with ^{3}H-PSA(PSA 4.7 mg, 20 μci) and various organs were removed from mice at specified time later.

Table Ⅱ. Time Course Change in Organ Distribution of H^3- PSA.
H^3-PSA 4.7mg, 20μc was administered by intraperitoneal injection to 20g mouse. Radio activity; cpm/mg dry wt organs

Organs \ Hours	1	3	$5\frac{1}{2}$	12	24
Spleen	99.1	98.4	80.7	106.8	84.8
Stomach	53.7	101.5	54.9	28.1	36.8
Parcreas	50.2	90.7	65.9	106.0	94.7
Liver	109.5	124.5	53.1	135.0	90.3
Kidney	99.15	152.8	78.0	118.3	88.1
Lung	46.9	117.5	66.7	98.0	89.5
Spinal	50.7	26.3	12.9	72.8	67.7
Brain	45.9	55.8	47.5	40.2	43.7

As shown in Table II, H^3-PSA administered by intraperitoneal injection was absorbed very rapidly and distributed into various organs.

One of interesting results was that the radioactivities which appeared in various organs did not change relatively throughout time course.

The mean radioactivity of this table (77 cpm) should be therefore considered as the maximum value of radioactivity at the saturation of ^{3}H-PSA in the tissue of various organs. Based on this mean radioactivity and on the arbitrarily estimated counting efficiency 10%, the amount of ^{3}H-PSA retained in 20g mouse appears to be 3 μci(0.7mg of ^{3}H-PSA). This indicates that 16% of total ^{3}H-PSA administered were absorbed immediately after iniection and the concentration was retained without significant changes during next 24 hrs. Thus it appears to establish a dynamic equilibrium between absorption and excretion. Nonetheless, 4.7 mg of ^{3}H-PSA administered to 20g mouse might be equivalent to 14.1 g of PSA in 60kg human, if we computed it roughly. Certainly such amount of PSA corresponds to excess dosage to human.

6. Oral Administrations of ^{3}H-PSA

Each mouse received orally ^{3}H-PSA(PSA 2.6mg, 11.1 μci).

As shown in Table III, the average radioactivities were 23.5 cpm/mg dry weight. This value appeared to be far less than the results described in the preceeding pages. Low counting efficiency(5%) due to decreased POPOP content in scintillation solution in this experiment would result in such low radioactivity counting. The ^{3}H-PSA content retained in mouse (20g body weight) was computed to be about 0.43 mg. As shown in the previous experiment, the radioatcivities in various organ did not change through time course as well. Such results indicate that dynamic equilibrium

Table Ⅲ. Time Course Change in Organ Distribution of ^{3}H-PSA in Mouse. Oral Administration of ^{3}H-PSA, 2.6mg, 11.1μC per. mouse. Radioactivity: cpm/mg. dry wt. Organs

Organs \ hours	1	2	4	6
Stomach	20.4	26.3	16.5	16.2
Liver	19.6	25.7	27.2	22.6
Kidney	26.3	36.2	24.0	26.9
Brain	21.9	22.4	21.4	20.7

between absorption and excretion must be established also in case of oral administration.

7. Ascertaining the Dynamic Equilibrium

In order to ascertain dynamic equillibrium, the following experiments were conducted. One group of mice received orally ^{3}H-PSA(PSA 2.6mg, 11.1 uci), and at specified time the mouse was sacrificed. G. I. tract from esophagus to anus were removed, their contents were irrigated with normal saline and unabsorbed ^{3}H-PSA was measured. And the other group of mice received 1/10 dose of the above group's dose.

Table Ⅳ. ^{3}H-PSA in Gastro-Intestinal Tract Unabsorbed. ^{3}H-PSA(2.6mg 11.1μc) was administered orally to mouse and total radioactivity in the gastrointestinal tract was recorded by cpm.

Amount of ^{3}H-PSA administered	Blank	0 Hour	2	4	6	8	20
2.6mg 11.1μc	73	8872×300	6703×300	2622×300	6641×300	6415×300	598×300
0.26mg 1.11μc	73	2906×50	2104×50	1401×50	2047×50	1719×50	764×50

As shown in Table IV, no matter how much doses were administered, most of ^{3}H-PSA was absorbed completely from G. I. tract.

8. Prolonged Retention of ^{3}H-PSA

Above results suggest the earliest formation of dynamic equillibrium of absorption and excretion with cellular uptake.

In order to estimate an approximate biological half life of ^{3}H-PSA taken by cells, prolonged retention experiment was conducted.

Table Ⅴ. Prolonged Retention of ^{3}H-PSA in Mouse. H^3-PSA(5.2mg, 22.2μc) was administered by a intraperitoneal injection. The radioactivities of organs were determined one or two weeks later(radioactivity: cpm/mg dry wt organs)

weeks \ organs	Stomach	Liver	Kidney	Brain	Lung	Pancreas	Spleen
1 week	58.9	55.1	56.9	50.2	53.2	51.3	57.3
2 week	25.3	30.7	33.0	20.2	39.2	57.1	22.5

Each mouse was injected i.p. with ^{3}H-PSA(PSA 5.2mg, 22.2 μci) at once. One week later, one group was sacrificed and various organs were harvested. Then, radioactivities of the samples were measured after all samples were treated properly as mentioned before. And two weeks later, the other group was also followed as the same in the above. As shown in Table V residual radioactivities were observed even two weeks later very strong. Such results indicate that ^{3}H--PSA once absorbed in the cells of tissue remains there for long time.

9. Repeated Administration of ^{3}H-PSA

All mice were divided into four groups, A, B, C, and D. The A group mice received ^{3}H-PSA once a day. The B group mice received ^{3}H-PSA once a day for two days. The C group mice were administered with ^{3}H-PSA once a day for three days, and the D group mice received three-fold dose once on the third day(see Experiment 17).

As the results were shown in Table VI, regardless of dose-schedules, the residual radioactivities remained in the organs appeared to be almost the same. Therefore these results indicate that there might exist a certain saturation concentration limit for ^{3}H-PSA uptake within a cell. Consequently, the dose 2.6mg/mouse(20g) might exceed such saturation point, because the saturation concentration per mouse appeared to be roughly 0.4mg.

Table VI. Repeated Administration of ^{3}H-PSA and Saturation of Cellular Intake. ^{3}H-PSA (2.6mg, 11.1μc) was administered repeatedly by intraperitoneal injection to mouse; Once to A group mouse, twice to B group, three times to C group and once of three-fold dosage to D group mouse. Radioactivity cpm/mg. dry wt.

Repeated administration \ Organs	Spleen	Kidney	Liver	Brain
A (1)	40.7	41.1	37.1	33.6
B (2)	47.2	35.3	40.9	35.9
C (3)	47.1	41.9	32.5	30.9
D (1 × 3)	41.4	38.2	45.3	21.0

10. Urinary Excretion of ^{3}H-PSA in Rabbit

Table VII shows the results of two separate experiments on the time course of urinary excretron of PSA(Ginsenoside Rg_1).

The data in first row are the results of present experiment using ^{3}H-PSA and the data in second row indicate the results of already published one using unlabeled saponin. Present experiment was designed to administer the saponin in a relatively

small dose 52 mg/rabbit by oral route and the former experiment was conducted by administering the saponin in large dose 200 mg/rabbit via intravenous route.

Table Ⅶ. Urinary Excretion of PSA in Rabbit(oral administration of ^{3}H-PSA, and i.v. administn. of PSA)

Samples	Route of Administ.	Amounts of administered.	Assay \ Hours	1	2	3	4	5	6	7	8	9	10	11
H^3-PSA	Oral	52 mg 222μc	Radioactivity cpm	8	2472	2326	5977	4800	7935	2914	3334	3272	3148	2330
PSA*	Intravenous	200mg	Chemical assay	70	32	15	6	3.5	2					

* This refers to our previous report[10]

Urinary excretion of PSA appeared to be very rapid within one hour and to be almost completed within three hrs in case of intraveneous injecton of PSA. In contrast to such previous results, when ^{3}H-PSA was given orally, we could observe very neglisible urinary excretion of ^{3}H-PSA immediately after administration, but it reached maximum excretion six hrs after administration. Therefore this result indicated that the ^{3}H-PSA concentration in blood and body fluid increased very gradually by means of oral administration in comparison with that of intraveneous injection. In addition, if the total radioactivity collected in urine during 10 hrs was computed into PSA content, it appeared to be about 0.1 mg. Therefore, this result indicates that very negligible amount of PSA was indeed excreted into urine when the dosage (52mg/rabbit) is small. After urinary excretion experiment, the rabbit was sacrificed and various organs were harvested.

Table Ⅷ. Organ Distribution of H^3-PSA in Rabbit.
H^3-PSA(52mg, 222μc) was administered by oral route and the radioactivity was measured 13 hours later.

Organs	Kidney	Liver	Brain	Bone Marrow	Muscle
Radioactivity cpm/dry wt	25.9	19.7	23.7	12.2	34.2

The radioactivities the measured in the organs were shown in Table VIII. The average radioactivities per mg of dry weight appeared to be 23.7 cpm. If this value was converted to total PSA content retained in the rabbit, it became approximately 25~50 mg. Therefore, most of ^{3}H-PSA must be absorbed or being absorbed into body, when 52 mg dosage of ^{3}H-PSA was administered orally to rabbit.

Conclusion

1) Panax saponin A(ginsenoside Rg_1) one of glycosides in ginseng dammarane series was labeled with radioactive ^{14}C or with ^{3}H.
2) When ^{3}H-PSA was administered to mouse orally, it was absorbed easily through GI tracts and distributed in almost all organs and tissues and constituted a dynamic equillibrium between absorption, cellular uptake and renal excretion for long time.
3) The uptake of PSA by a cell appeared to reach a concentration of saturation point at 0.4 mg per 20 mg mouse.
4) Excess PSA absorbed beyond the saturation point was excreted rapidly into urine and PSA taken by the cell remained in the cell and tissues for a significantly long period of time.

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인삼 사포닌의 체내 대사에 관한 연구(I)

동위원소 표지 사포닌의 흡수, 체내 분포 및 배설에 관하여

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초 록

1. 인삼중에 함유된 dammarane계 glycoside 성분들중의 한 성분인 panax saponin A(ginsenoside Rg_1)(약자 : PSA)에 ^{14}C 또는 ^{3}H를 도입한 방사능 표지 사포닌을 합성하였다.
2. ^{3}H-PSA의 방사능을 생체내에서 추적한 결과 PSA는 위장관내에서 쉽게 흡수되어 생체내 전장기에 골고루 분포된다.
3. 각 장기에 분포된 PSA는 세포내에 섭취되며 세포내에 섭취되는 량은 일정한 포화점이 있다.
4. 포화점을 초과하여 섭취된 PSA는 즉시 뇨를 통하여 배설되며 섭취된 PSA는 세포내에 장기간 잔유한다.