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Absorption of Ginseng Saponin in Rats*

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인삼 사포닌의 동물(쥐) 체내흡수에 관한 연구

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Abstract

Ginseng saponin absorbed in rat blood and liver were analyzed by High Performance Liquid Chromatography.

The amount of saponin was estimated from peak area of the corresponding fraction and the specific radioactivity was then calculated.

The radioactivity of the methanol-water extract of blood serum and livers of ginseng saponin administered rats decreased rapidly during the first four hours after the saponin administration.

However, the radioactivity disappearance rate was relatively slow when the radioactivity was below a certain level.

It seemed that the glycosides of panax ginseng were absorbed partly in the undissociated form and the saponin level of the liver might be maintained at $10^{-6}\%-10^{-5}\%$ for a considerable period of time in ginseng administered rats.

Introduction

During the past two decades, physiological and biochemical approaches to elucidate the mechanism of ginseng saponin effect on the body have been made intensively, however, it is still controversial whether appreciable amounts of ginseng saponin could be absorbed orally to explain the results obtained from *in vitro* experiments.

Han and Chang[2] showed that oral and intraperitoneal administration of ^{3}H-panaxsaponin A (PSA) resulted in the rapid appearance and prolonged retention of ^{3}H-PSA in organs such as liver, brain, bone marrow and spleen of mice and the amount of

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cellular intake of ^{3}H-PSA were to have a certain level of saturation ranging from 0.4mg to 0.7mg per 20gm body weight of mouse. Administration of ^{3}H-PSA within the dosage of the saturation point did not give urinary excretion of ^{3}H-PSA, but the excessive administration of ^{3}H-PSA resulted in the urine of mice.

It was demonstrated that orally administered ginseng saponins were absorbed and distributed in various tissues such as liver, kidney, bone and muscles.[4] However, it is uncertain whether the saponins are absorbed as such unhydrolyzed form, or dissociated and/or metabolized forms. Furthermore, the previous paper suggested that a significant portion of the saponins absorbed might exist in unextractable forms from the tissue. It is true that the saponin level and fate in the tissue after the oral administration of saponin must be answered to explain *in vitro* as well as *in vivo* experimental results demonstrating the efficacies of ginseng saponins.

In this study, it was attempted to demonstrate the oral absorption of ginseng saponin in rats using radioactive saponin preparation. Furthermore the turnover rates of the saponins in blood and liver were also considered.

Materials and Methods

Total 6.5g of ginseng saponin mixture was obtained from 300g of powdered Korean white ginseng (Keumsan, 4 years, 50 pieces) according to the modified procedure[3] described elsewhere. The chromatogram of the saponin showed that it contained several saponins with Rf values of 0.71, 0.65, 0.49, 0.52, 0.47, 0.43, 0.41, 0.36, 0.34, 0.27, 022, 0.20, 0.17 on silica gel pre-made thin plate (Pre-Coated TLC plates, Silica gel 60 F-254) by using chloroform-methanol-H_2O (65:40:9, v/v/v) as a developing solvent. It appeared that the saponins with Rf values of 0.22, 0.27 were the most abundant, the saponins of Rf values of 0.47, 0.36, 0,34, 0.20, 0.17 were less abundant and the saponins of Rf values of 0.71, 0.65, 0.49, 0.52 were the least.

Radioactive ginseng saponins were prepared as follows. 2g of sliced raw ginseng root (4 years, Keumsan) were incubated in the reaction mixture (3,3ml) containing 0.1M sucrose, 5×10^{-3}M ATP, 3×10^{-4}M NADPH, 5×10^{-4}M Coenzyme A, 8×10^{-4}M NAD, 2.5×10^{-2}M nicotinamide, 1×10^{-2}M glutathione, 1.2×10^{-3}M Na-acetate containing 1,2-^{14}C acetate (150 uCi) at 37°C for 64 hrs. Following incubation, the reaction mixture was diluted with water and homogenated, followed by centrifugation to remove the fraction which extract was washed three times with a little water. The combined soluble extract was lyophilyzed. 5ml of hot methanol were then added to the above extract and mixed sufficiently and the mixture was allowed to stand overnight in a refrigerator to precipitate the insoluble fraction. Following centrifugation to remove the insoluble fraction, the precipitate was extracted three times with a small volume of hot methanol as described above. The combined methanol extract was mixed with 3 volumes of chloroform, vortexed and then centrifuged to remove the insolu-

ble fraction (mainly sugar). The insoluble fraction was washed twice with methanol-chloroform mixture (1:3, v/v). The combined extract was then concentrated under a reduced pressure and finally dissolved in 2.0 ml of methanol. The above methanolic solution was chromatographed by thin plate of silica gel (Merck nach Stahl) using chloroform: methanol: water mixture (65:40:9, v/v/v) as a developing solvent. The fractions corresponding to the standard saponins were scraped out and extracted with methanol. The radioactivity and Rf values of each fraction was investigated. The chromatogram of the product showed that the pattern of the radioactive saponin preparation prepared as described above was exactly the same as that of the saponin preparation from ginseng roots (4years, Keumsan, 50 pices per 300g).

Albino rats (Sprague Dawley, male, 180-200g) were fed with normal diet (Jaiilsaryo Co. product, composition: crude protein above 19.6%, crude cellulose bellow 7.0%, crude ash below 9.0%, Ca below 0.6%, P below 0.4%, DCP above 16.5% TDN 7.3%, antibiotic below 40ppm) until required.

1mg of ginseng saponin containing ^{14}C-labelled saponin (5,600,000 DPM) was administered by stomach tubing to a rat and then killed on time course after the saponin administration. The blood was taken directly from the heart and the serum was prepared as usual way. The livers were homogenated in water and made up to a known volume. Radioactivities of serum and liver homogenates were counted according to Mahin and Lofberg[6]. Blood serum and the liver were then extracted by Bligh-Dyer method[1] and the radioactivities of individual fractions were counted as usual way[4] descrived elsewhere. The radioactive ginseng saponins prepared from ^{14}C-acetate using sliced raw ginseng roots as an enzyme source described above and saponins from blood serum and livers were chromatographed by High Performance Liquid Chromatography using Waters Model 244 (Column: u-Bondapak carbohydrate Analysis, Solvent system: Acetonitril-H_2O-Butanol (80:20:15, v/v/v), Detector: RI, Chart speed: 1cm/min., Flow rate : 1.5ml/Min.).

Results and discussion

Radioactivities recovered from blood sera of ^{14}C-labelled ginseng saponin administered rats on time course are given in Table 1. The radioactivity of the blood surum was rapidly decreased during the first four hours with a half time of approximately 2.5 hours assuming the radioactivity disappearance is proportional to time. However, it seemed that the radioactivity disappearance rate was very slow when the radioactivity was below a certain level. Again, in the liver, the radioactivity disappearance rate was very fast with a half time of approximately 3 hours during the first four hours but it became considerably slower as shown in Table 2.

Table 3 showed the distribution of individual glycosides of the saponin fraction extracted from panax ginseng C.A. Meyer containing ^{14}C-labelled saponins prepared

Table 1. Radioactivities recovered from the blood sera of ginseng saponin (1mg) containing ^{14}C-labelled saponin (56,000,000 DPM/mg) administered rats on time course. The figures in blackets are recovered % of administered total radioactivity

(DPM)

	Total serum	MeOH-Water insoluble fraction (mainly proteins)	$CHCl_3$ soluble fraction (sapogenins and others)	MeOH-Water soluble fraction (mainly saponins)
1hr.	9,301 ± 264 (0.17)	2,304 ± 187 (0.04)	1,060 ± 660 (0.02)	2,492 ± 279 (0.5)
4hrs.	8.640 ± 435 (0.15)	6,243 ± 902 (0.11)	1,453 ± 461 (0.03)	792 ± 264 (0.01)
8 hirs.	7,453 ± 348 (0.13)	5,964 ± 770 (0.11)	667 ± 67 (0.01)	287 ± 88 (0.005)

Table 2. Radioactivities recovered from the liver of ginseng saponin (1mg) containing ^{14}C-labelled saponin (5,000,000 DPM/mg) administered rats on time course. The figures in blackets are recovered % of total radioactivity

(DPM)

	Total homogenates	MeOH-Water insoluble fraction (mainly proteins)	Chloroform soluble fraction (sapogenin and others)	MeOH-Water soluble frafion (containg saponin)
1hr.	204,030 ± 19,341 (3.64)	69,120 ± 7,410 (1.23)	41,415 ± 9,611 (0.74)	94,906 ± 12,990 (1.69)
4 hrs.	157,340 ± 23,198 (2.81)	68,440 ± 9,924 (1.22)	46,683 ± 7,408 (0.83)	48,900 ± 14,807 (0.87)
8 hrs.	142,540 ± 34,266 (2.55)	59,560 ± 7,888 (1.06)	44,350 ± 7,330 (0.79)	42,980 ± 10,902 (0.77)

Table 3. Radioactivities of individual glycosides of saponin preparation containing ^{14}C-labelled saponin prepared from ^{14}C-acetate. Individual glycosides were fractionated by HPLC and the amounts were estimated from the peak area of the corresponding fraction

Fraction	Amounts (μg)	Radioactivity (DPM)	Specific radioactivity (DPM/mg)
Rg_2, Rg_1 and Rf	110	566,131	5,146
Re	102	137,155	1,344
Rd	94	2,321,770	23,935
Rc	165	42,867	260
Rb_2	97	409,940	4,226
Rb_1	110	67,633	614
Rh_1 Ra, Ro and others	—	—	—
Total	1,000	5,600,000	5,600

from ^{14}C-acetate.

Individual glycosides were fractionated by HPLC and the amounts were estimated from the peak area of corresponding fractions.

When the radioactivities of the individual glycosides of blood serum at 1 hour after the saponin administration were investigated. It was found that the degree of the amounts absorbed was differ from one another ranging from 0.1% up to 1.0% both in blood and in liver as shown in Table 4.

Table 4. Glucoside levels of blood serum of rat at 1 hr after the oral administration of 1 mg of ginseng saponin containing ^{14}C-labelled saponin (5,600,000 DPM) prepared from ^{14}C-acetate. Individual glycosides were fractionated by HPLC and the amounts were calculated from the radioactivities of corresponding fractions

Fractions	Fed Saponin (μg)	Recovered saponin (ng)	% recovered	Saponin level (×10^{-5}%)
Rg_2, Rg_1, and Rf	110	28 ± 1.55	0.025	0.22
Re, Rd	196	720 ± 34.06	0.367	5.54
Rc	165	1,640 ± 61.91	0.994	12.60
Rb_2	97	370 ± 18.03	0.381	2.84
Rb_1	110	102 ± 6.19	0.093	0.78

The radioactivity recovered from the liver on time course, however, showed that the individual saponins were degraded with a half time of less than 3 hours during the first four hours after the oral administration of the saponin as shown in Table 5.

Table 5. Glycoside levels of hepatocytes of rats which were administered with 1 mg of ginseng saponins containing ^{14}C-labelled saponin (5,600,000 DPM/mg) prepared from ^{14}C-acetate. Individual glycosides were fractionated by HPLC and the amounts were calculated from the radioactivity of the corresponding fraction

Fractions	Fed Saponins (μg)	1hr. Recovered saponin (ng)	1hr. Recovered %	1hr. Saponin level (10^{-5}%)	4hrs. Recovered saponin (ng)	4hrs. Recovered %	4hrs. Saponin level (×10^{-5}%)
Rg_2, Rg_1, Rf	110	160 ± 9.28	0.145	0.29	68.11 ±0.11	0.062	0.13
Re, Rd	196	1.270 ± 37.15	0.647	2.37	27.86 ± 0.84	0.014	0.06
Rc	165	2.700 ± 12.38	1.636	5.02	334,86 ± 4.36	0.202	0.65
Rb_2	97	148 ± 6.19	0.153	0.27	6.19 ± 0.91	0.006	0.01
Rb_1	110	360 ± 9.30	0.327	0.67	27.86 ± 0.61	0.025	0.06

This and previous data[4,5] suggest that the glycosides of panax ginseng are partly in the undissociated form and the saponin level of the liver might be maintained at 10^{-6} -10^{-5}% for a considerable period of time in ginseng administered rats.

요 약

인삼(*Panax ginseng C. A. Meyer*) 뿌리 전편을 효소원으로 사용하여 ^{14}C-acetate로부터 제조한 방사성 인삼 사포닌을 함유한 인삼 사포닌 분획 조제물을 High performance liquid chromatography 방법으로 분리하여 각 glycosides의 함량과 비 방사능을 측정하였다.

1 mg의 ^{14}C-표지사포닌을 함유한 인삼 사포닌을 쥐에게 구경투여하고 1시간 후 혈청및 간장으로부터의 추출물을 분석한 결과 혈청이나 간의 경우 모두 사포닌 투여후 회수된 메탄올-물층의 방사능이 빠른속도로 경감되었으나 방사능이 어느 수준 이하가 되면 방사능 소멸속도가 크게 저하되는 것으로 보아 일정수준의 인삼 사포닌은 비교적 오래 혈청이나 간에 잔재하는 것으로 사료된다. 각 glycoside에 따라 흡수정도는 다르나 0.1%-1%정도 흡수된 것으로 확인되었고 간에서의 인삼 사포닌의 농도는 인삼 사포닌 투여후 상당기간 10^{-6}%-10^{-5}%수준을 유지할 것으로 예상된다.

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