[Chem. Pharm. Bull. 26(12)3832—3835(1978)]

UDC 546.466.2.08 : 543.52.062

Stimulated Incorporation of ^{14}C Amino Acids and ^{14}C Fatty Acids in Various Tissues of Ginsenoside-treated Rats

Yasuo Shibata, Yukiko Tatsuno, and Tokuhiko Higashi

School of Pharmaceutical Sciences, Showa University[1]

(Received July 15, 1978)

Rats were treated with ginsenoside-Rb_1 or -Rc, purified saponins from Ginseng, given intraperitoneally at a dose of 5 mg per 100 g body weight, and the incorporation of ^{14}C-amino acids or of ^{14}C-fatty acids was studied with adipose, intestine, liver, kidney, spleen and serum. The stimulation in protein synthesis by the ginsenoside was observed in the intestine and the liver, and that in lipid metabolism was detected in adipose tissue as well as intestine and liver. These three organs are assumed to be target organs of the ginsenoside, and the latter two to be probable sites for apolipoprotein synthesis.

Keywords——ginsenoside; lipid metabolism; serum lipoprotein; protein synthesis; stimulated tissue

Ginsenosides, the purified saponins of *Panax ginseng* C.A. Meyer, have been studied on their effects on cholesterol and serum protein metabolism to explore the pharmacological activities of *Panax ginseng*. It has been found that these ginsenosides, especially two major ones in Ginseng (Rb_1 and Rc), stimulate the synthesis of cholesterol in liver[2,3] and of protein moieties of serum lipoproteins[4] upon administration to rats. It is of interest that the stimulated metabolism of fats including cholesterol is associated with the enhanced synthesis of proteins transporting them. However, whether the one is a trigger to the other, or if so, which is a real trigger—has not decided yet. On the other hand, an origin of serum apolipoproteins has not been thoroughly clarified.

In the present study the incorporation of ^{14}C-amino acids and of ^{14}C-fatty acids into various tissues have been investigated with normal and ginsenoside-treated rats, for searching a target tissue of the drug which would be mostly responsible for the biosynthesis of apolipoproteins.

Materials and Methods

Ginsenoside Rb_1 and Rc were purified by thin-layer chromatography from a hot ethanol extract of the powder of *Panax ginseng* C.A. Meyer,[5] and dissolved in physiological saline at the concentration of 10 mg/ml for injection.

^{14}C(U)-labeled leucine, ^{14}C(U)-amino acid mixture, sodium 1-^{14}C-acetate and 1-^{14}C-palmitic acid were purchased from The Radiochemical Centre Amersham England.

Administration of Ginsenoside——Albino male rats (*ca.* 150 g) were given 5 mg of ginsenoside Rb_1 or Rc per 100 g of body weight intraperitoneally.

***In Vivo* Labeling**——After 0.5—3 hr of the ginsenoside administration, 10 μCi of ^{14}C-labeled compound per 100 g of body weight was injected intraperitoneally for labeling. Expired air was collected in an ethanolamine solution. The rats were killed by bleeding from Aorta abdominalis under ether anesthesia, and tissues were excised. Epididymis adipose tissue, upper portion of small intestine (around 10 cm from pylorus), kidney, liver and spleen tissues were sliced to 100 mg block and combusted directly by an automatic combus-

1) Location: *1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142, Japan.*
2) K. Sakakibara, Y. Shibata, T. Higashi, S. Sanada, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **23**, 1009 (1975).
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tion apparatus (Aloka ASC 111). Otherwise, the tissues were homogenized, treated with 5% trichloroacetic acid (TCA), washed 3 times and then combusted.

***In Vitro* Labeling**——Each 200 mg of minced tissue from rats receiving no isotope, were incubated with 2.5 ml of #199 media containing 1 μCi of ^{14}C-amino acid mixture for 1 hr and a TCA insoluble radioactivity was measured after 3 times washing.

Radioactivity of ^{14}C was measured by a liquid scintillation spectrometer (Aloka LSC 502).

Results

Distribution of ^{14}C-leucine radioactivity incorporated into various tissues in 1 hr was studied, using rats treated with ginsenoside-Rb_1 for 4 hr. The results are presented in Table I (A: into whole tissue, B: into TCA-insoluble fraction). Higher radioactivities were found in intestine, spleen and liver in both normal and Rb_1-treated rats. Administration of the ginsenoside elevated the uptake of ^{14}C-leucine in all tissues analyzed, and its effect, represented as *b/a* ratio in the Table, was the greatest in the intestine followed by the liver and kidney. The ratio of the latter tissues was so close to the serum one.

TABLE I. *In Vivo* Uptake and Incorporation into Protein of Labeled Leucine in Various Tissues of Ginsenoside-Rb_1 Administered Rat

Tissue (100 mg)	Normal (cpm) (*a*)	Treated (cpm) (*b*)	*b/a*
A) Uptake of ^{14}C-leucine by tissue			
Adipose	6600	9450	1.43
Intestine	16940	39630	2.34
Kidney	9410	17800	1.89
Liver	14340	29210	2.02
Spleen	14860	28240	1.90
Serum (0.1 ml)	7120	14820	2.08
B) Incorporation of ^{14}C-leucine into TCA insoluble fraction			
Adipase	5900	8070	1.36
Intestine	12370	28760	2.33
Kidney	5690	11990	2.11
Liver	12180	24870	2.04
Spleen	12060	20120	1.67
Serum	5720	11600	2.09

Rats were given intraperitoneally 5 mg of ginsenoside-Rb_1 and 10 μCi of ^{14}C-leucine per 100 g of body weight at a 3 hr interval, and killed another 1 hr later. A) Tissues were excised, and each 100 mg of tissue was combusted directly and assayed for the radioactivity. B) Each 100 mg of tissue was homogenized and the TCA (5%) insoluble precipitates, after washed 3 times, was combusted and assayed for the radioactivity. All figures are the averages of each 2—3 samples.

Since no significant difference was seen between the *b/a* ratio in Table I A and B with respect to each tissue, it will be concluded that a stimulation in the uptake of amino acids leads to an increase in protein synthesis in ginsenoside-treated rats.

In order to study a distal effect of the ginsenoside treatment, the tissues from treated rats were incubated *in vitro* with ^{14}C-amino acid mixture, and the radioactivities incorporated in the TCA-insoluble form were assayed. The results are presented in Table II in comparison with those of control rats. Spleen and liver incorporated more amino acids *in vitro*, but the ratio of stimulation (*b/a*) by ginsenoside was the highest in the intestine, followed by the liver. Serum or blood sample of the treated rats was added to media in which tissues from normal rats were incubated with ^{14}C-amino acids, however no stimulation was observed on the incorporation, indicating no production of a transmittable stimulant by the ginsenoside.

Table II. *In Vitro* Incorporation of ^{14}C-Amino Acids into Protein in Tissue of Ginsenoside Administered Rat

Tissue (200 mg)	Normal (cpm) (*a*)	Treated (cpm) (*b*)	*b*/*a*
Adipose	1710	1500	0.88
Intestine	4050	5900	1.45
Kidney	3900	3350	0.85
Liver	12960	15680	1.21
Spleen	33310	31990	0.96

Rats were given ginsenoside-Rb_1 (5 mg/100 g of body weight) and killed after 3 hrs. Tissues were excised, and each 200 mg of minced tissue was incubated 1 hr with #199 media containing ^{14}C-amino acid mixture (1 μCi) triplicatedly. TCA insoluble radioactivity was measured after 4 times washing and the combustion.

Table III shows the distribution into several tissues of ^{14}C radioactivity given *in vivo* in the form of acetate or palmitate. In the case of ^{14}C-acetate, the liver was the highest in uptake among the tissues investigated, presumably indicating its high activity for utilizing acetate. The intestine contained a considerable amount of ^{14}C, being doubled by the treatment with the ginsenoside. In the case of palmitic acid, more fraction of the label was retained in tissues, and the liver showed the highest radioactivity, followed by the adipose tissue.

Table III. Uptake of ^{14}C-Acetic Acid and ^{14}C-Palmitic Acid by Tissues of Ginsenoside-Rc Treated Rat

Tissue (100 mg)	Normal (cpm) (*a*)	Treated (cpm) (*b*)	*b*/*a*
A) Na-^{14}C-Acetate (10 μCi injected)			
Adipose	970	3030	3.12
Intestine	7490	16500	2.20
Liver	11600	26600	2.29
Serum (0.1 ml)	2880	6380	2.22
Expired	1.54×10^7	1.56×10^7	1.01
B) ^{14}C-Palmitic Acid (1 μCi injected)			
Adipose	4360	13520	3.10
Intestine	1410	4250	3.01
Liver	5090	5610	1.10
Serum (0.1 ml)	260	540	2.13
Expired	7.26×10^5	8.75×10^5	1.20

Rats were administered ginsenoside-Rc (5 mg/100 g of body weight), and 30 min later 1-^{14}C–Na acetate (10 μCi) or 1-^{14}C-palmitic acid (1 μCi) was injected intraperitoneally. Expired air was collected for 2.5 hrs by ethanolamine trap, then rats were killed and the distribution of radioactivity in tissues was measured.

The effect of the ginsenoside (*b*/*a* ratio in Table III) was most evident in adipose tissue in both cases. The liver gave a high *b*/*a* ratio in acetate uptake, but a low ratio in plamitate utilization. The ratio of the intestine was significantly high, particularly in palmitic acid uptake being almost equal to that of the adipose tissue.

Discussion

Adipose, intestine, kidney, liver, and spleen of the rat were surveyed for the stimulatory effect of ginsenosides on the metabolism of protein and lipid. Although these tissues appeared to be stimulated in amino acid uptake and protein synthesis *in vivo* by the ginsenoside-treatment, the labeling experiment *in vitro* freed from the contamination of blood radioactivity revealed no significant enhancement except for the intestine and the liver. There-

fore, these two organs are assumed to be the most probable target organ of the ginsenoside. From the data on the incorporation of ^{14}C-fatty acids, on the other hand, the adipose tissue is undoubtedly an another target organ of the ginsenoside.

In adipose tissue, a high stimulation was observed in the uptake both acetate and palmitate, indicating the role of this tissue in the lipid metabolism. However, an increase in protein synthesis was poorly associated with that. The intestine and the liver were stimulated by the ginsenosides in both lipid metabolism and protein synthesis. Therefore, these two organs are the most probable sites of lipoprotein synthesis.

Some of the apolipoproteins, for instance human A-I subunit, have been reported to be synthesized in the intestine and function as a constituent of various lipoproteins (VLDL and HDL).[6]

The previous report with ginsenoside-treated rats[4] showed that the incorporation of radioactive amino acids into VLDL and chylomicrons was more intensely enhanced than that into LDL. From this and the present evidence, it is safe to suppose a stimulated production of chylomicrons in the intestine of the ginsenoside-treated rats. The recent progress in the research of serum apolipoproteins have elucidated an interconvertibility of apoprotein subunits among all species of lipoproteins.[7,8] Therefore, the stimulation in the synthesis of VLDL and chylomicron apoproteins would be followed by an elevation in LDL or HDL, which are proposed to play a major role in cholesterol transport in circulation. Some of the plasma apolipoproteins have been reported to increase by a dietary cholesterol in rats[9] and in guinea pigs.[10] The interrelationship between the synthesis of cholesterol and that of apoprotein to transport it will be further studied using animals treated with the ginsenoside.

Acknowledgement The authors thanks are due to Dr. Junzo Shoji and Dr. Shuichi Sanada of this School for providing the purified ginsenosides, and to Mr. Toshinori Toya for his technical assistance.

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