Effect of Ginseng Saponins on Cholesterol Metabolism. I. The Level and the Synthesis of Serum and Liver Cholesterol in Rats treated with Ginsenosides

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The in vivo effect on cholesterol metabolism of ginsenoside-Rb, -Re, -Rd, -Rg, purified from Ginseng (the root of Panax ginseng C.A. Meyers) was investigated. Four hours after the intraperitoneal injection of 5 mg of each pure ginsenoside into rats, the concentrations of serum and liver cholesterol and the incorporation of \(^{14}\)C-aceatate into serum and liver cholesterol were determined. The results indicated the enhancement of cholesterol biosynthesis by administered saponins, particularly by ginsenoside-Rb, a predominant component of Ginseng saponins.

Biochemical effect of an extract of Ginseng has been widely studied, and it has been revealed that the extract stimulates various metabolic reactions, as reviewed in recent papers.\(^2\)\textsuperscript{4} Since most of the active preparations from the extract contained saponins as main constituents, it should be investigated whether the stimulating effect is definitely due to the saponins or to other minor components. This has become possible by the success in isolating and purifying various saponins contained in Ginseng.\(^5\)\textsuperscript{7} It is also interesting to study the difference in biochemical effect of saponins having different chemical structures.

The present paper reports a research concerning with the effect of five purified saponins of Ginseng on the level and the biosynthesis of rat serum and liver cholesterol. A partially purified preparation of the extract of Ginseng was found by Yamamoto\(^8\) to reduce the concentration of cholesterol and triglycerides in blood of hyperlipemic rats, and to accelerate the rate of disappearance of \(^{14}\)C-labeled cholesterol from circulation. Administering the same preparation, Oura, et al.\(^4\)\textsuperscript{8} observed a stimulation in the incorporation of \(^{14}\)C-aceatate into the total lipid fraction of liver and an accumulation of fat in adipose tissues. The preparations used in these studies were rich in saponins. In addition, Yamamoto\(^10\) reported a similar effect of Saiko-saponin, a saponin of Bupleurum falcatum L.

**Experimental**

**Saponins**—Ginsenoside-Rb, -Re, -Rd, -Re, and -Rg,\(^11\) were isolated and purified from Ginseng.\(^5\)\textsuperscript{7}

All the preparation used in the present study was found to be pure by chemical and physicochemical analyses.

**General Procedures**—Unless otherwise indicated, 5 mg of each purified saponin was injected intraperitoneally into rats weighing 100–120 g. Rb, Re, and Rg were dissolved in saline, and Rd and Re in 20% ethanol. The solvents were found not to disturb the present experiments. Total and free cholesterol

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1) Location: 1-5-8, Hatanodai, Shinagawa-ku, Tokyo.
3) S. Hiai and H. Oura, Protein, Nucleic acid and Enzyme, 18, 333 (1973).
4) H. Oura and S. Hiai, Metabolism, 10, 564 (1973).
11) These are abbreviated as Rb, Re, Rd, Re, and Rg.
in serum and in liver were assayed 4 hr after the injection of saponins. In labeling experiments, 10 µCi of sodium acetate-1-14C (per 100 g of body weight) was injected intraperitoneally into normal and saponin-treated rats at a definite time prior to sacrifice. The saponin-treated rats were killed 4 hr after the injection of saponins. Incorporation of 14C-acetate into serum cholesterol was measured for the period of 30 min, and that into liver cholesterol was determined for the period of 90 min.

Assay of Cholesterol in Serum—Total and free cholesterol in serum was measured by a modification13 of Zak's method.13

Assay of Liver Cholesterol—Liver cholesterol was determined by a modification of the method reported by Ichida.14 One gram of liver was first homogenized with about 5 ml of ethanol and was diluted further with about 30 ml of ethanol. The mixture was treated at 50—60° in a water bath for 30 min, and was filtered through defatted filter paper (No. 7, Toyo Roshi). The residue was extracted again with about 15 ml of ethanol-ether (3:1) at 50—60° for 30 min. The combined filtrates were adjusted the volume to 50 ml. The concentrations of total and free cholesterol in this extract were assayed by the same method as employed for the serum cholesterol.

Measurement of 14C Incorporated into Serum Cholesterol—14C-Labeled acetate was injected 30 min prior to sacrifice, i.e., 3.5 hr after the injection of saponins in the case of saponin-treated rats. Serum cholesterol was isolated as follows. The procedure was originally described by Entenman15 and later modified by De Matteis16 for isolating liver cholesterol. To 1 ml of serum were added 5 ml of 21% KOH and the mixture was kept in boiling water for 1 hr. Ethanol was added at a final concentration of 50%, and the mixture was heated to boil. After cooling extraction was carried out twice with use of each 10 ml of petroleum ether (for 5—10 min). The petroleum ether layer was evaporated to dryness under a reduced pressure. The residues were dissolved in 21 ml of ethanol and filtered through a defatted cotton-wool plug into 18 ml of acetone. Five milliliters of the extract were mixed with 1 ml of 1% digitonin solution in 50% ethanol, and the mixture was kept standing overnight in an incubator (37°). The precipitated digitonide of cholesterol was separated by centrifugation, washed once with a small volume of ethanol-acetone (1:1), and dried. For counting the radioactivity, the materials were dissolved in 9 ml of scintillation fluid and assayed with a scintillation spectrometer (Beckman LS-100 C). For quantitative determination of cholesterol, the materials were dissolved in 6 ml of glacial acetic acid and assayed by a modification of Zak's method. The data were expressed as cpm per mg of cholesterol.

Measurement of 14C Incorporated into Liver Cholesterol—14C-Acetate was injected 90 min before sacrifice, i.e., 2.5 hr after the injection of saponins in the case of saponin-treated rats. Liver cholesterol was isolated similarly as in the case of serum cholesterol. One gram of liver was digested and saponified with KOH and extracted with 50% ethanol at 80—100° for 1—4 hr until clear solution was obtained, followed by extraction with 20 ml of petroleum ether (twice). Cholesterol was separated as digitonide, as described above, and the amount and radioactivity were measured using appropriate aliquots.

Chemicals—Sodium acetate-1-14C (specific radioactivity: 48—49 mCi per m mole) was purchased from Daiichi Pure Chemicals Co., Tokyo. Digitonin was obtained from E. Merck, Darmstadt.

Result

Amount of Cholesterol in Serum

The amount of cholesterol in serum of the rats treated with various saponins was determined, and the results are presented in Fig. 1 (total cholesterol) and Fig. 2 (free cholesterol). Rb1 caused a slight elevation both in the total and the free cholesterol concentrations. Rc showed a similar effect as Rb1 for the total cholesterol, but decreased the amount of free cholesterol. Rg1 reduced only the free cholesterol level, whereas Rd and Re reduced both free and total cholesterol.

The ratios of free/total cholesterol were calculated, and shown in Fig. 3. Except for the case of Rb1, saponin-treated rats exhibited lower ratios of free/total cholesterol than those of normal rats.

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Fig. 1. Amount of Total Cholesterol in Serum
Cholesterol was assayed 4 hr after the injection of 5 mg of each ginsenoside. Data are expressed as mg of cholesterol in 109 ml of serum.
N: normal rat serum
Figures are the mean ± standard error.
○: p<0.01; ●: p<0.05.
The data without these marks are not statistically significant.

Amount of Cholesterol in Liver
Fig. 4 and 5 show the amounts of total and free cholesterol in the liver of rats injected with various saponins. Administration of Rc resulted in a considerable reduction of both types of cholesterol. Rb1 and Rg1 affected slightly the level of total cholesterol. Re caused a remarkable decrease in free cholesterol (50%) associated with an increase in esterified cholesterol, and on the contrary, Rd elevated the concentration of free cholesterol with concomitant decrease of the esterified one.
In Fig. 6 the ratios of free/total cholesterol in liver are shown. The ratio was found to be extremely low in Re-treated rats, and high to some extent in Rd-treated rats.

Incorporation of 14C-Acetate into Serum Cholesterol
Time course of the incorporation of 14C-acetate into serum cholesterol was investigated with the normal and the Rb1-treated rats. The results are illustrated in Fig. 7. An apparent peak in the specific radioactivity was observed 30 min after the injection of radioactive acetate in Rb1-treated rats. Based on this evidence the labeling of serum cholesterol by injected 14C-acetate was compared at 30 min after the injection between the normal and the saponintreated rats.

Fig. 8 demonstrates the effect of various saponins on the labeling of serum cholesterol by injected 14C-acetate. Serum cholesterol in Rb1-treated rats was labeled almost 10 times as highly as that in normal rats, and in Rc-treated rats 5 times as much 14C-radioactivities were incorporated compared with the control animals. The effect of other three saponins were found comparatively less than those of Rb1 and Rc.
Fig. 3. Ratio of Free/Total Cholesterol in Serum

mean 26.6 28.9 17.9 22.9 19.5 17.6
± S.E. 1.16 1.15 0.97 0.87 1.06 1.49

Fig. 4. Amount of Total Cholesterol in Liver

Cholesterol was assayed 4 hr after the injection of 5 mg of each ginsenoside. Data are expressed as mg of cholesterol per g of liver.

N: normal rat liver
Figures are the mean ± standard error.
○: p < 0.01, ●: p < 0.05.
The data without these marks are not statistically significant.

mean 5.12 4.58 4.15 4.42 4.86 5.54
± S.E. 0.21 0.10 0.08 0.17 0.10 0.16

Fig. 5. Amount of Free Cholesterol in Liver

See the legend to Fig. 4.

mean 1.86 1.86 1.47 1.79 2.29 0.88
± S.E. 0.11 0.05 0.07 0.09 0.10 0.09

Fig. 6. Ratio of Free/Total Cholesterol in Liver

mean 36.2 40.8 35.6 40.4 47.1 15.8
± S.E. 1.01 1.47 1.24 0.85 1.66 1.22
**Incorporation of $^{14}$C-Acetate into Liver Cholesterol**

Kinetics of the incorporation of radioactive acetate into liver cholesterol are shown in Fig. 9. The curve for the specific radioactivity of liver cholesterol in Rb$_1$-treated rats revealed a peak at 90 min after the injection of $^{14}$C-acetate, as is the case in normal rats. However, the values in the former were significantly higher than the corresponding ones in the latter. From this finding, it was decided that the effect of saponins on the labeling of liver cholesterol by injected radioactive acetate was investigated 90 min after the isotope injection.

In Fig. 10 are summarized the specific radioactivities of liver cholesterol in saponin-treated rats. As seen in the labeling of serum cholesterol, Rb$_1$ enhanced the labeling of liver cholesterol most extensively. The other four ginsenosides also stimulated the incorporation to some extent.

**Dose Response for Ginsenoside-Rb$_1$ in Incorporation of $^{14}$C-Acetate into Liver Cholesterol**

Varying amounts of Rb$_1$ were administered to rats and their effects on the incorporation of $^{14}$C-acetate into liver cholesterol were investigated. The results are presented in Fig. 11, where the data are expressed as percentage of the incorporation in the rats receiving 5 mg of Rb$_1$.

**Discussion**

The present results clearly demonstrate that the saponins contained in Ginseng generally stimulate the biosynthesis of cholesterol when administered to rats. The data are summarized in Table I, being expressed as percentage of the control ones. Among five ginsenosides so
Fig. 9. Kinetics of Incorporation of $^{14}C$-Acetate into Liver Cholesterol in Normal and Rb$_1$-treated Rats
See the legend to Fig. 7.
○: Rb$_1$-treated rats
●: normal rats

Fig. 10. Incorporation of $^{14}C$-Acetate into Liver Cholesterol
Saponin-treated rats were killed 4 hr after receiving the saponins, and were injected with $^{14}C$-acetate 90 min before sacrifice. Data are expressed as cpm per mg of cholesterol.
N: normal rats receiving $^{14}C$-acetate 90 min before sacrifice.
Figures are the mean ± standard error.
○: $p < 0.01$; ●: $p < 0.05$

Fig. 11. Incorporation of $^{14}C$-Acetate into Liver Cholesterol in Rats Receiving Various Doses of Ginsenoside-Rb$_1$
Ginsenoside-Rb$_1$ and $^{14}C$-acetate were injected 4 hr and 90 min prior to sacrifice, respectively. The incorporation with rats receiving 5 mg of Rb$_1$ was taken as the standard (100%).

far examined, ginsenoside-Rb$_1$ was most strikingly active. This is a predominant saponin in quantity in Ginseng, thus it can be concluded that the activity of Ginseng for stimulating the cholesterol metabolism is mainly accounted for by ginsenoside-Rb$_1$.

As seen in Chart 1, the difference in the structure between ginsenoside-Rb$_1$ and -Rc is only in a sugar component at the end of each molecule, i.e., glucose in ginsenoside-Rb$_1$ and arabinose in ginsenoside-Rc. Replacement of arabinose by glucose doubles the incorporation of $^{14}C$-acetate into cholesterol. The sugar component at this site in the molecule of ginsenoside seems to be significant for exerting the enhancement of cholesterol synthesis, because ginsenoside Rd lacking a sugar molecule in this position showed a less activity.

It may be considered that ginsenosides containing panaxatriol (Rg$_1$ and Re) appeared to be less effective than those containing panaxadiol (Rb$_1$, Rc, and Rd). However, ginsenoside-Re containing rhamnose showed
TABLE I. Effect of Various Ginsenosides on Cholesterol Metabolism

<table>
<thead>
<tr>
<th>Ginsenoside</th>
<th>Rb₁</th>
<th>Rc</th>
<th>Rg₁</th>
<th>Rd</th>
<th>Re</th>
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<tr>
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<td>amount total</td>
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<td>114</td>
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<td></td>
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<td>67</td>
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<td>433</td>
<td>133</td>
<td>201</td>
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<tr>
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<td>86</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>free</td>
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<td>79</td>
<td>96</td>
<td>123</td>
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<tr>
<td></td>
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<td>155</td>
<td>132</td>
<td>111</td>
</tr>
</tbody>
</table>

The results in Fig. 1—10 were summarized in this table. The values are expressed as percent of the control.

* a) free/total cholesterol × 100

Chart 1. Structure of Ginsenosides Used in the Present Study
rather unique features in affecting the free cholesterol level in liver and the labeling of serum cholesterol by injected $^{14}$C-acetate.

Compared to the significant increase in biosynthesis of cholesterol, particularly by ginsenoside-Rh$_2$, the amounts of cholesterol in serum and in liver were affected to a lesser extent. This fact will evidently suggest that the saponins stimulate not only the synthesis but also the catabolism of cholesterol, including the conversion into other steroid compounds. Yamamoto$^8$ observed an increased rate in the excretion of injected $^{14}$C-cholesterol into bile and feces by administering the partially purified preparation of Ginseng extracts. In this connection, glycyrrhizin in licorice was reported to behave similarly.$^8$

Following the experiments reported in the present paper, the authors have been studying in vitro cholesterol synthesis using liver slices from the ginsenoside-treated rats. The results so far obtained indicate an enhanced synthesis by those slices compared with slices from normal rats. A report dealing with such study will follow this paper shortly.

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