Note

Hypcholesterolemic Action of Dietary Grifolin on Rats Fed with a High-cholesterol Diet

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The hypcholesterolemic action of grifolin was investigated in terms of its structure-activity relationship with rats fed on a high-cholesterol diet. The results show that the structure of farnesylorcincolin was required for the hypcholesterolemic action, and that the effect of grifolin might be elicited, at least in part, through the augmented excretion of cholesterol into the feces.

It has been shown that certain species of mushrooms have the capacity for lowering the plasma cholesterol level when fed to experimental animals.1) As a hypcholesterolemic factor, eritadenine (2(R),3(R)-dihydroxy-4-(9'-adenyl)-butylic acid) has been isolated from Shiitake (Lentinus edodes) mushroom by several groups of investigators.8-9) In addition, we recently found that grifolin (2-trans,trans-farnesyl-5-methylresorcinol, 1) and neo-grifolin (4-trans,trans-farnesyl-5-methylresorcinol) present in Ningyotake (Polyporous confluens or Albatrellus confluens) mushroom had a plasma cholesterol-lowering effect on rats fed on a high-cholesterol diet.10) However, little is known about the mechanism for the hypcholesterolemic action of grifolin and its isomer, neoegrifolin. The structure-activity relationship of these compounds is also not known.

In this study, therefore, we investigated the comparative effects of grifolin and its constituent compounds orcinol (2) and farnesol (3) on the plasma and liver lipid levels and on the steroid excretion into the feces of rats fed on a high-cholesterol diet.

Male rats of the SL C: Wistar strain (Japan SL C, Hamamatsu) weighing about 120g were used as the experimental animals. They were fed on the experimental diets ad libitum for 14d in a temperature- and humidity-controlled room with a 12h cycle of light (0600-1800) and dark. The composition of the basal diet (25C) was as follows (%): casein, 25; sucrose, 20; lard, 10; corn oil, 2; mineral mixture, 11); vitamin mixture, 11); choline chloride, 0.2; cholesterol, 1; sodium cholate, 0.25; cellulose, 2; and corn starch to make up to 100%. The mineral and vitamin mixtures were obtained from a commercial source (Oriental Yeast Co., Tokyo, Japan). Grifolin was isolated from Ningyotake mushroom as described previously.10) and orcinol and farnesol were obtained from a commercial source (Tokyo Kasei Kogyo Co., Tokyo, Japan). Grifolin was added to the basal diet at a level of 0.5% at the expense of starch. Orcinol and farnesol were added to the basal diet singly or in combination at levels of 0.19% and 0.34%, respectively, which are equivalent to the 0.5% grifolin supplement on a molar basis. At the end of the feeding period, the animals were killed by decapitation between 1100 and 1200 following 11 to 12h of starvation. Feaces were collected for the last 3d. The plasma concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride and phospholipid were measured enzymatically with kits: Cholesterol C-Test Wako, HDL-Cholesterol-Test Wako, Triglyceride G-Test Wako and Phospholipid-Test Wako, respectively (Wako Pure Chemical Ind., Osaka, Japan). The difference between the total cholesterol and HDL cholesterol was assumed to be cholesterol associated with very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). Liver lipids were extracted by the method of Folch et al.,12) and the cholesterol, triglyceride and phospholipid in the extracts were measured by the methods of Zak,13) Fletcher,14) and Bartlett,15) respectively. Steroids in the feces were extracted by the method described by Van Beresteyn et al.,15) and the cholesterol and bile acids in the extracts were respectively measured colorimetricaly16) and enzymatically.17) Grifolin in the feces was extracted with 30 volumes (v/w) of ethyl acetate, and an aliquot (1.0ml) of the extract was dried in vacuo, before being dissolved in 4ml of methanol-water (9:1, v/v). An aliquot (15μl) was assayed for grifolin by HPLC with an ODS column (6.0×150 mm, Shimazu Seisakusho, Kyoto, Japan), using detection at 228 nm. The mobile phase was methanol-water (9:1, v/v) and the flow rate was 1.0ml/min. The retention time for grifolin was 10.8min. Data were subjected to an analysis of variance, and the differences between means were tested at p<0.05, using Duncan's multiple-range test when the F-value was significant at p<0.05.

The results are summarized in the Table. The addition of grifolin, orcinol or farnesol to the diet did not cause any deleterious effect on the growth and food consumption of the animals. The relative liver weight was slightly higher in those rats fed on the diets supplemented with farnesol. The contents of cholesterol and triglyceride in the liver were significantly decreased by dietary supplementation with grifolin, but not with orcinol, farnesol or both. The liver phospholipid content was significantly higher in those rats fed on the diets supplemented with grifolin or farnesol. Dietary supplementation with grifolin significantly decreased the plasma total cholesterol and conversely increased the HDL cholesterol level, whereas dietary supplementation with orcinol, farnesol or both had no significant effect on the plasma cholesterol level, although farnesol tended to slightly decrease the plasma cholesterol level. The plasma phospholipid level was significantly decreased only by grifolin. The weight of feces and the excretion of cholesterol into the feces were significantly increased by grifolin, but not by orcinol, farnesol or both. Bile acid excretion into the feces was not stimulated by any of the supplements. The excretion of grifolin into the feces in an intact form was estimated to be 9.3±0.5% of the amount of grifolin ingested. However, this does not necessarily indicate that about 90% of the ingested grifolin

Fig. Structures of Grifolin (1), Orcinol (2), and Farnesol (3).
was absorbed, since the extent of hydrolysis of grifolin in the gastro-intestinal tract is not known.

The potency of the hypocholesterolemic action of eritadenine has been shown to be very strong: it significantly decreased the plasma cholesterol level at an addition level of only 0.005% of the diet. On the other hand, the potency of the hypocholesterolemic action of grifolin appears to be far weaker when compared with that of eritadenine, since grifolin had no significant effect when the addition level was decreased from 0.5% to 0.1% (unpublished observation). Nonetheless, it is interesting to know the structure–activity relationship and the mechanism for the hypocholesterolemic action of grifolin, because grifolin may become one of the lead compounds of a novel type to lower the plasma cholesterol level. The results obtained here clearly show that the covalent linkage between orcinol and farnesol is essential for eliciting the hypocholesterolemic action of grifolin, although the effect of modifying and/or substituting the orcinol and farnesyl moiety of grifolin on the cholesterol metabolism remains to be further elucidated.

The results also show that the hypocholesterolemic action of grifolin might be elicited, if not entirely, by the augmented excretion of cholesterol into the feces. Although grifolin also enhanced the excretion of cholesterol into the feces when added to the cholesterol-unsupplemented 25% casein diet at a 0.5% level, it could not significantly decrease the plasma cholesterol level (unpublished observation), indicating that the hypocholesterolemic action of grifolin was largely dependent on the presence of exogenous cholesterol in the diet. The detailed mechanism by which dietary grifolin enhanced the cholesterol excretion into feces remains to be further elucidated.

### References