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Studies on Scutellariae Radix. III.[1] Effects on Lipid Metabolism in Serum, Liver and Fat Cells of Rats

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The effects of Scutellariae Radix ("Ogon" in Japanese) and its flavone components on lipid metabolism in the rat were investigated. Wogonin inhibited deposition of liver triglyceride and increased serum HDL-cholesterol level in rats fed on corn oil-cholesterol-sodium cholate mixture. Skullcapflavone II reduced the serum total cholesterol level and liver triglyceride content, and increased serum HDL-cholesterol. Baicalein and baicalin reduced the serum free fatty acid and triglyceride levels and liver triglyceride content.

In addition to the *in vivo* experiments, the effects of flavone components of Ogon on lipid metabolism in fat cells isolated from epididymal adipose tissue of rats were examined. Wogonin and baicalein selectively inhibited adrenaline-induced lipolysis, and baicalein and baicalin selectively activated ACTH-induced lipolysis in fat cells. Skullcapflavone II inhibited adrenaline- and ACTH-induced lipolysis and lipogenesis from glucose in fat cells.

Keywords——*Scutellaria baicalensis*; flavone; baicalin; chlesterolemia; lipid metabolism; HDL-cholesterol; insulin; lipolysis of triglyceride; lipogenesis from glucose; adipose fat cells

Scutellariae Radix, "Huang-qin" in Chinese and "Ogon" in Japanese, is the root of *Scutellaria baicalensis* Georgi and has been listed in Chinese herbals, Shen-nung-pen-ts'ao-ching, as a remedy for suppurative dermatitis, diarrhea and inflammatory diseases. Koda *et al.*[2] reported that baicalin and baicalein, the flavone components of Ogon, possessed anti-allergic action. Kumazaki[3] examined the pharmacological actions of the alcoholic extract of Ogon (containing baicalin, baicalein and wogonin) and observed cholagogic, diuretic and cathartic actions.

On the basis of a historical analysis of the medical usage of Ogon,[4] it has been proposed that Ogon might have lipid-lowering actions. The present investigations describe the effects of Ogon and its flavonoid components on serum and liver lipids in rats fed an oil mixture and their actions on adrenaline- and ACTH-induced lipolysis and on lipogenesis from glucose in fat cells isolated from epididymal adipose tissue of rats.

Materials and Methods

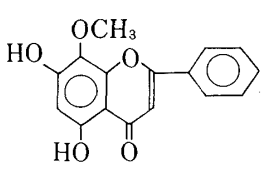
Materials——The powder and water extract of Ogon and flavonoid components isolated from the drug by the method described by Takido *et al.*[5] were used in the present investigations.

Animals——Young male Wistar King strain rats, weighing 180 to 210 g, were given standard laboratory diets and water *ad lib.*

Estimation of Lipids in Serum and Liver——The rats of the control group were given corn oil (10 ml/kg body weight) containing 15% cholesterol and 1% sodium cholate (oil mixture) orally for 3 days, with standard laboratory diet and water *ad lib.*

Rats of the experimental group received the oil mixture orally with powder (2 g/kg), water extract (1 g/kg) or various flavones (100 mg/kg) of Ogon as indicated.

Blood was taken by cardiac puncture 3 h after the last administration of the oil mixture and centrifuged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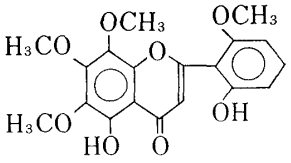
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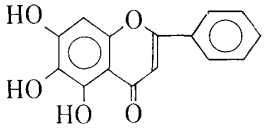
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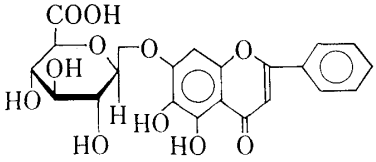
baicalin

at $1630 \times g$ for 10 min to separate the serum. Cholesterol, triglyceride (TG), free fatty acids (FFA) and high density lipoprotein-cholesterol (HDL-ch) in the serum were determined by the methods of Zak,[6] Fletcher,[7] Itaya,[8] and Ash,[9] respectively.

The liver weight was estimated and then 2 g of liver tissue was homogenized with a Teflon homogenizer, and cholesterol and TG in the liver were estimated by the above methods.

Preparation of Fat Cells——Young male Wistar King strain rats, weighing 120 to 150 g (6 weeks old), were given standard laboratory diet and water *ad lib.* They were sacrificed by means of a blow on the head, and their epididymal adipose tissue was quickly removed. Fat cells were isolated from the adipose tissue by the method of Rodbell.[10]

Estimation of Adrenaline- and ACTH-induced Lipolysis——In a glass-stoppered test tube, 0.25 ml of fat cell suspension, equivalent to 100 mg of adipose tissue, was incubated with shaking for 2 h at 37°C in 0.25 ml of Krebs-Ringer-phosphate buffer (pH 7.4), and 0.5 ml of Krebs-Ringer-phosphate buffer containing 5% albumin in the presence of adrenaline (1 μg/ml) and various flavones (100 μg/ml) of Ogon. After incubation, FFA were extracted and titrated with NaOH by the method of Dole.[11]

Tris-HCl buffer (25 mM, pH 7.4) containing 5 mM $CaCl_2$ and 5% albumin was used for the estimation of ACTH-induced lipolysis, and other procedures were the same as described above.

Lipolytic activity was expressed as μEq of FFA per g of adipose tissue.

Estimation of Lipogenesis from ^{14}C-glucose in Fat Cells——In a glass-stoppered test tube, 0.25 ml of fat cell suspension was incubated with shaking for 1 h at 37°C in 0.25 ml of Krebs-Ringer-phosphate buffer (pH 7.4), and 0.5 ml of Krebs-Ringer-phosphate buffer (pH 7.4) containing 5% albumin, 5 mM glucose and 0.5 μCi/ml of ^{14}[U]C-glucose in the presence of various flavones (100 μg/ml) of Ogon. After incubation, the reaction was halted by the addition of the Dole's extraction mixture.[11]

The test tube was shaken vigorously for 5 min and then heptane (3 ml) and water (2 ml) were added, and the mixture was shaken again for 5 min. A 3 ml of aliquot of the upper phase (heptane layer) was transferred to a glass-stoppered test tube and shaken vigorously with an equal volume of alkaline ethanol (0.05 M NaOH in 50% EtOH) to remove FFA, following the method of Börgstrom.[12] An aliquot of 1 ml of the heptane layer was used for the estimation of radioactivity.

Lipogenetic activity was expressed as cpm per g of adipose tissue. The radioactivity of the heptane layer was analyzed by thin layer chromatography by the method of Skipski *et al.*[13] More than 95% of the radioactivity was recovered in the TG fraction, and the remainder was mainly in the diglyceride and phosphatidic acid fractions.

Results

Effects of Ogon on Serum Lipids Levels

As shown in Table I, serum cholesterol was found to be reduced in the rats orally given skullcapflavone II as compared to the control animals. Serum FFA was significantly lowered by oral administration of powdered Ogon, baicalin or baicalein. Baicalin and baicalein also reduced the serum TG level.

On the other hand, serum HDL-ch was elevated by oral administration of wogonin, skullcapflavone II or baicalein.

Effects of Ogon on Liver Lipids Levels

Table II showed that liver TG content was reduced by oral administration of powdered Ogon, wogonin, skullcapflavone II, baicalin or baicalein as compared with that of control rats, but no reduction of liver cholesterol level was caused by Ogon and its flavones.

Effects of Ogon on Adrenaline- and ACTH-induced Lipolysis, and Lipogenesis from ^{14}C-Glucose in Fat Cells

As shown in Table III, 100 μg per ml of wogonin, skullcapflavone II, or baicalin inhibited the lipolytic action of adrenaline in fat cells.

TABLE I. Effects of Ogon on Serum Lipid (Cholesterol, Triglyceride, Free fatty Acids and HDL-cholesterol) in Rat orally given the Oil Mixture*

	Total cholesterol (mg/dl) M±S.E.	Triglyceride (mg/dl) M±S.E.	Free fatty acids (mEq/1) M±S.E.	HDL-cholesterol (mg/dl) M±S.E.
Normal	80.2±3.5	190.3±30.6	0.34±0.03	43.3±8.5
Control	98.3±3.5	360.1±37.2	1.25±0.05	29.4±2.3
The powder (2g/kg)	93.5±3.0$^{N.S.}$	300.0±26.7$^{N.S.}$	0.90±0.07$^{c)}$	34.1±1.9$^{N.S.}$
The water ext. (1g/kg)	104.0±3.4$^{N.S.}$	351.2±41.8$^{N.S.}$	1.34±0.16$^{N.S.}$	30.0±1.5$^{N.S.}$
Wogonin (100 mg/kg)	98.9±9.2$^{N.S.}$	281.6±25.1$^{N.S.}$	1.20±0.09$^{N.S.}$	40.2±5.7$^{a)}$
Skullcapflavone II (100 mg/kg)	88.4±3.5$^{a)}$	286.4±32.5$^{N.S.}$	1.09±0.13$^{N.S.}$	37.3±4.2$^{a)}$
Baicalein (100 mg/kg)	94.7±5.2$^{N.S.}$	239.9±22.5$^{b)}$	1.04±0.09$^{b)}$	37.7±2.3$^{b)}$
Baicalin (100 mg/kg)	97.9±6.9$^{N.S.}$	271.5±27.8$^{a)}$	1.01±0.12$^{b)}$	32.8±2.1$^{N.S.}$

a): $p<0.05$, *b*): $p<0.02$, *c*): $p<0.001$, N.S.: not significant.
* The oil mixture was composed of corn oil, 15% cholesterol and 1% sodium cholate. The data for normal (7 rats), control (14 rats) and the 6 experimental groups (each of 10 rats) are indicated as means±standard errors.

TABLE II. Effects of Ogon on Liver Lipids (Total Cholesterol and Triglyceride)in Rats orally given the Oil Mixture

	The cholesterol (mg/g) M±S.E.	Triglyceride (mg/g) M±S.E.
Normal	2.7±0.15	5.4±0.34
Control	4.4±0.32	11.5±0.73
The powder (2 g/kg)	3.9±0.11$^{N.S.}$	8.5±0.46$^{b)}$
The water ext. (1g/kg)	4.3±0.22$^{N.S.}$	10.1±0.37$^{N.S.}$
Wogonin (100 mg/kg)	3.5±0.20$^{N.S.}$	8.8±0.65$^{a)}$
Skullcapflavone II (100 mg/kg)	4.0±0.23$^{N.S.}$	9.0±0.32$^{a)}$
Baicalein (100 mg/kg)	3.5±0.32$^{N.S.}$	7.3±0.33$^{b)}$
Baicalin (100 mg/kg)	4.8±0.32$^{N.S.}$	6.4±0.05$^{b)}$

a): $p<0.02$, *b*): $p<0.001$, N.S.: not significant.
* The oil mixture was composed of corn oil, 15% cholesterol and 1% sodium cholate.
The data for normal (7 rats), control, the powder and the water ext. groups (each of 10 rats) and the wogonin, skullcapflavone II, baicalein and baicalin groups (each of 6 rats) are indicated as means ±standard errors.

TABLE III. Effects of Wogonin, Skullcapflavone II, Baicalein and Baicalin on Adrenaline-induced Lipolysis in Fat Cells

Additions (μg/ml reaction mixture)	Lipolysis (FFA μEq/g) M±S.E.	Significance
None	0.8±0.4	—
Adrenaline (1 μg/ml)	17.2±0.59	—
Adrenaline+wogonin (100 μg/ml)	13.7±1.14	*a)*
Adrenaline+skullcapflavone II (100 μg/ml)	10.3±1.80	*b)*
Adrenaline+baicalein (100 μg/ml)	15.6±1.11	N.S.
Adrenaline+baicalin (100 μg/ml)	12.5±1.08	*b)*
Adrenaline+Insulin (0.1 mI. U./ml)	8.7±1.00	*b)*

a): $p<0.02$, *b*): $p<0.001$, N.S.: not significant.
The results are means of 5 replicate experiments.

Table IV. Effects of Wogonin, Skullcapflavone II, Baicalein and Baicalin on ACTH-induced Lipolysis in Fat Cells

Additions (μg/ml reaction mixture)	Lipolysis (FFA μEq/g) M±S.E.	Significance
None	0.15±0.09	—
ACTH (1 μg/ml)	13.1 ±0.72	—
ACTH+wogonin (100 μg/ml)	11.9 ±0.65	N.S.
ACTH+skullcapflavone II (100 μg/ml)	7.2 ±1.64	c)
ACTH+baicalein (100 μg/ml)	15.6 ±0.48	b)
ACTH+baicalin (100 μg/ml)	15.2 ±0.72	a)
ACTH+Insulin (0.1 mI.U./ml)	8.0 ±0.70	d)

a): $p<0.05$, b): $p<0.02$, c): $p<0.01$, d): $p<0.001$, N.S.: not significant.
The results are means of 5 replicate experiments.

Table V. Effects of Wogonin, Skullcapflavone II, Baicalein and Baicalin on Lipogenesis from Glucose-^{14}C in Fat Cells

Additions (μg/ml reaction mixture)	Lipogenesis Glucose-^{14}C→TG-^{14}C M±S.E. ($\times 10^3$ cpm/g)	Significance
None	37.3±3.7	—
Wogonin (100 μg/ml)	43.2±7.0	N.S.
Skullcapflavone II (100μg/ml)	3.9±0.3	a)
Baicalein (100 μg/ml)	31.2±3.2	N.S.
Baicalin (100 μg/ml)	37.5±3.2	N.S.

a): $p<0.001$, N.S.: not significant.
The results are means of 5 replicate experiments.

ACTH-induced lipolysis in fat cells was inhibited by the presence of skullcapflavone II and stimulated by baicalin or baicalein.

As shown in Table V, skullcapflavone II reduced TG synthesis from glucose in fat cells.

Discussion

The present investigation showed that the powder, water extract, and flavone components of Scutellariae Radix ("Ogon" in Japanese) affect lipid metabolism in rats.

It is known that a high fat diet causes a fatty liver and a high carbohydrate diet induces hyperlipemia in rats.[14] In the present experiments, it was found that oral administration of an oil mixture (corn oil-cholesterol-sodium cholate) induced both fatty liver and hyperlipemia as compared to normal rats.

In normal rats, serum cholesterol, TG, FFA, and HDL-ch were found to be 80.2 mg/dl, 190.3 mg/dl, 0.34 mEq/l and 43.3 mg/dl, respectively. In contrast, the serum cholesterol, TG and FFA levels in rats orally given the oil mixture were considerably increased. On the other hand, the serum HDL-ch level in the oil mixture-fed rats was reduced as compared to that in normal rats.

In normal rats, liver cholesterol and TG were found to be 2.7 mg/g and 5.4 mg/g, respectively. In the oil mixture-fed rats, liver cholesterol and TG level were increased.

In the oil mixture-fed rats, simultaneous oral administration of wogonin partly inhibited the reduction of serum HDL-ch level and the accumulation liver TG. Skullcapflavone II partly inhibited the elevation of total cholesterol level and the accumulation of liver TG, and the reduction of serum HDL-ch level in the oil mixture-fed rats. Baicalin and baicalein partly inhibited the elevation of serum FFA and TG levels and the accumulation of liver TG.

Baicalein was also found to inhibit the reduction of serum HDL-ch level in the oil mixture-fed rats.

In fat cells, adrenaline-induced lipolysis was inhibited by wogonin, skullcapflavone II and baicalin. Skullcapflavone II reduced lipolysis induced by ACTH and lipogenesis from glucose in fat cells.

Based on the *in vitro* experimental results, it is suggested that the *in vivo* effects of flavone components of Ogon on lipid metabolism in rats might be partly derived from inhibitory actions of the flavones on hormone-induced lipolysis and lipogenesis in adipose tissue.

Baicaline reduced the serum FFA and TG levels and liver TG content in the oil mixtute-fed rats, but did not inhibit adrenaline-induced lipolysis and lipogenesis in isolated fat cells. Furthermore, baicalein increased ACTH-induced lipolysis in fat cells. These experimental results suggested that baicalein affected lipid metabolism *in vivo* by some other mechanism, such as inhibition of lipid absorption and acceleration of lipid utilization in muscles.

It has been reported that the elevation of serum HDL-ch is an anti-arteriosclerotic factor.[15] It was thus of great interest that wogonin, skullcapflavone II, and baicalein increased the serum HDL-ch level in the oil mixture-fed rats.

It is well known that propranolol (β-blocker) has strong anti-lipolytic action toward adrenaline- and ACTH-induced lipolysis.[16] A similar type of inhibitory action was shown by skullcapflavone II in isolated fat cells. On the other hand, wogonin selectively inhibited adrenaline-induced lipolysis and had no effect on ACTH-induced lipolysis in fat cells. In the case of baicalein, ACTH-induced lipolysis was selectively activated. Baicalin was found to reduce adrenaline-induced lipolysis as in the case of wogonin, while it activated ACTH-induced lipolysis in fat cells.

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