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Studies on Scutellariae Radix. V.[1] Effects on Ethanol-induced Hyperlipemia and Lipolysis in Isolated Fat Cells

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The effects of oral administration of flavonoid components of Scutellariae Radix on serum and liver lipid levels of rats treated with ethanol were investigated. It was found that wogonin reduced serum triglyceride level, and that baicalein and baicalin, the major components of the drug, decreased total cholesterol, free cholesterol and triglyceride contents in the liver. Baicalein increased high density lipoprotein-cholesterol (HDL-ch) in the serum of the ethanol-treated rats.

In addition to these *in vivo* experiments, the actions of wogonin, baicalein and baicalin on catecholamine–induced lipolysis in isolated fat cells were investigated. It was found that the three flavones inhibited noradrenaline–induced lipolysis in isolated fat cells. The relationship between these *in vivo* and *in vitro* experiments is discussed.

Keywords——*Scutellaria baicalensis*; flavone; baicalin; lipid metabolism; HDL-cholesterol; ethanol-induced hyperlipemia; catecholamine–induced lipolysis; isolated fat cells

Scutellariae Radix, "ogon" in Japanese, is the root of *Scutellaria baicalensis* Georgi and has been used in Chinese medicine, as a remedy for suppurative derimatitis, diarrhea, inflammation and hyperlipemia.

In the previous papers, we showed that the extracts and flavonoid components of Scutellariae Radix ("ogon" in Japanese, "Huang-qin" in Chinese) reduced serum and liver lipid levels of rats fed corn oil-cholesterol–sodium cholate mixture,[2] and that they had inhibitory effects on lipid peroxidation in the liver of rats stimulated by Fe^{2+}-ascorbic acid–adenosine 5′-diphosphate (ADP) and on the elevation of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in rats given oxidized lipid.[1]

It is well known that oral administration or intraperitoneal injection of ethanol causes a fatty liver and hyperlipemia in rats. The present paper deals with the effects of wogonin, baicalein and baicalin, which are major flavonoid components of "ogon," on the lipid metabolism of rats treated with ethanol.

It was suggested that catecholamines are released by the administration of ethanol.[3] In addition to the *in vivo* experiments, the effects of wogonin, baicalein and baicalin on catecholamine–induced lipolysis in fat cells isolated from adipose tissue of rats were therefore investigated.

Materials and Methods

Materials——The flavones (wogonin, baicalein and baicalin) of Scutellariae Radix were isolated by the method described in our previous paper.[4] The flavones were each suspended in 1% sodium carboxymethyl cellulose (CMC-Na) and used the *in vivo* experiments. For *in vitro* experiments, the above flavones were suspended in Krebs–Ringer-phosphate buffer (pH 7.4).

Animals——Young male Wistar King strain rats weighing 180 to 210 g were used the *in vivo* experiments. In *in vitro* experiments, young male Wistar King strain rats, weighing 160—200 g and housed in a room maintained at 25±1°C and 60% relative humidity, were allowed free access to food and water. The room was illuminated for 12 h a day, starting at 7: 00 a.m.

Estimation of Serum and Liver Lipid Levels in Rats treated with Ethanol——Rats were orally administered 60% ethanol (10 ml/kg body weight) and the flavones (wogonin, baicalein and baicalin) (each 100 mg/kg) daily for 8 d, then they were killed 24 h after the last oral administration of ethanol and flavones. Blood was taken by cardiac puncture and centrifuged at $1630 \times g$ for 10 min to separate the serum.

Total cholesterol (TC), free cholesterol (FC), triglyceride (TG), free fatty acids (FFA), low density lipoprotein (LDL), high density lipoprotein-cholesterol (HDL-ch) and phospholipids (PL) in the sera were determined by the methods of Richmond,[5] Bucolo and David,[6] Itaya and Ui,[7] Fried and Hoeflmayr,[8] Ash and Hentschel[9] and Bartlett,[10] respectively.

A portion (2 g) of the liver tissue was homogenized with Krebs–Ringer–phosphate buffer (10 ml) (pH 7.4). The homogenate was extracted with $CHCl_3$–MeOH (2: 1) solution (20 ml), and the extract was analyzed for TC, FC, and TG by the methods of Zak[11] and Fletcher.[12]

Preparation of Fat Cells——Rats were sacrificed by means of a blow on the head, and their epididymal adipose tissues were quickly removed. Fat cells were isolated by the method of Rodbell.[13]

Estimation of Adrenaline-, Noradrenaline- and Dopamine-induced Lipolysis in Fat Cells——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 (pH 7.4) containing 5% albumin in the presence of adrenaline (1 μg/ml) and 100 μg per ml of the flavones (wogonin, baicalein and baicalin). After incubation, free fatty acids (FFA) were extracted and titrated with 0.008 N NaOH solution by the method of Dole.[14]

Noradrenaline (1 μg/ml)- and dopamine (100 μg/ml)-induced lipolysis were estimated by the same methods as above. Lipolytic activity was expressed as μeq of FFA per gram of the adipose tissue.

Results

Effects of Wogonin, Baicalein and Baicalin on Serum and Liver Lipid Levels of Ethanol-treated Rats

As shown in Tables I and II, wogonin (100 mg/kg) was found to significantly decrease serum TG elevated by ethanol treatment. Baicalein (100 mg/kg) decreased TC, FC, and TG in the liver, and increased HDL-ch in the serum of the ethanol-treated rats. Furthermore, baicalin (100 mg/kg) significantly reduced the FFA level in the serum, and the TC, FC and TG contents in the liver.

The serum TC, FC, PL and LDL levels of the ethanol-treated rats were not affected by the administration of wogonin, baicalein and baicalin.

Table I. Effects of Wogonin, Baicalein and Baicalin on Serum Lipids (Total Cholesterol, Free Cholesterol, Triglyceride, Free Fatty Acids, Low Density Lipoprotein, High Density Lipor protein–cholesterol and Phospholipids) in Rats treated with Ethanol

	TC(mg/dl) M±S.E.[c]	FC(mg/dl) M±S.E.[c]	TG(mg/dl) M±S.E.[c]	FFA (meq/l) M±S.E.[c]	LDL (mg/dl) M±S.E.[c]	HDL-ch (mg/dl) M±S.E.[c]	PL(mg/dl) M±S.E.[c]
Normal	83.5±2.7	11.6±0.8	142.3±12.1	0.22±0.03	184.4±14.2	40.9±2.9	160.8±8.2
Ethanol-treated Control	79.2±7.4	12.5±1.6	194.2±21.0	0.31±0.03	221.2±12.2	44.9±2.8	192.2±12.4
Wogonin (100 mg/kg)	69.2 ±5.9[N.S.]	10.9 ±1.1[N.S.]	127.6 ±9.5[b]	0.30 ±0.05[N.S.]	196.2 ±23.6[N.S.]	45.2 ±3.3[N.S.]	174.6 ±6.7[N.S.]
Baicalein (100 mg/kg)	75.1 ±3.8[N.S.]	12.8 ±0.6[N.S.]	159.7 ±21.9[N.S.]	0.27 ±0.05[N.S.]	263.7 ±25.4[N.S.]	50.9 ±2.1[a]	187.2 ±6.8[N.S.]
Baicalin (100 mg/kg)	74.7 ±4.1[N.S.]	14.3 ±1.5[N.S.]	176.8 ±24.7[N.S.]	0.21 ±0.04[a]	228.1 ±21.1[N.S.]	46.8 ±2.3[N.S.]	190.7 ±5.5[N.S.]

TC, total cholesterol; FC, free cholesterol; TG, triglyceride; FFA, free fatty acids; LDL, low density lipoprotein; HDL-ch, high density lipoprotein-cholesterol; PL, phospholipids.
a) $p<0.05$, *b*) $p<0.01$. N.S.: not significant, *c*) Values are means±standard errors of those in 12 rats.

Table II. Effects of Wogonin, Baicalein and Baicalin on Liver Lipids (Total Cholesterol, Free Cholesterol, and Triglyceride) in Rats treated with Ethanol

	TC(mg/g) M±S.E.[e]	Fc(mg/g) M±S.E.[e]	TG(mg/g) M±S.E.[e]
Normal	4.7±0.3	2.4±0.2	5.1±0.6
Ethanol-treated Control	6.3±0.6	3.4±0.4	9.6±1.7
Wogonin (100 mg/kg)	5.5±0.4[N.S.]	3.1±0.2[N.S.]	8.5±1.3[N.S.]
Baicalein (100 mg/kg)	4.7±0.3[b]	2.5±0.2[b]	5.6±0.4[b]
Baicalin(100 mg/kg)	5.0±0.3[a]	2.5±0.1[c]	4.8±0.3[d]

TC, total cholesterol; FC, free cholesterol; TG, triglyceride.
a) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.005$. N.S.: not significant.
e) Values are means±standard errors of those in 12 rats.

Table III. Effects of Wogonin, Baicalein and Baicalin on Catecholamine-induced Lipolysis in Fat Cells

Additions (μg/ml reaction mixture)	Lipolysis (FFA μeq/g) M±S.E.[a]	Significance
None	0.8±0.4	—
Adrenaline (1 μg/ml)	17.2±0.59	—
Adrenaline+wogonin (100 μg/ml)	13.7±1.14	$p<0.02$
Adrenaline+baicalein (100 μg/ml)	15.6±1.11	N.S.
Adrenaline+baicalin (100 μg/ml)	8.7±1.00	$p<0.001$
None	0.0±0.0	—
Noradrenaline (1 μg/ml)	21.0±0.95	—
Noradrenaline+wogonin (100 μg/ml)	17.7±0.58	$p<0.02$
Noradrenaline+baicalein (100 μg/ml)	17.2±0.96	$p<0.02$
Noradrenaline+baicalin (100 μg/ml)	14.0±0.71	$p<0.001$
None	0.0±0.0	—
Dopamine (100 μg/ml)	10.0±0.71	—
Dopamine+wogonin (100 μg/ml)	8.5±0.45	N.S.
Dopamine+baicalein (100 μg/ml)	12.0±1.38	N.S.
Dopamine+baicalin (100 μg/ml)	7.5±0.85	$p<0.05$

a) The results are means of 5 replicate experiments.
N.S.: not significant.

Effects of Wogonin, Baicalein and Baicalin on Adrenaline-, Noradrenaline- and Dopamine-induced Lipolysis in Fat Cells

Table III shows that wogonin and baicalin inhibited adrenaline-induced lipolysis in isolated fat cells, but that baicalein did not affect adrenaline-induced lipolysis. Wogonin, baicalein and baicalin inhibited noradrenaline-induced lipolysis in fat cells. Dopamine-induced lipolysis in fat cells was also inhibited by baicalin.

Discussion

The present investigation showed that the major flavone components (wogonin, baicalein and baicalin) of Scutellariae Radix ("ogon" in Japanese) affect lipid metabolism in ethanol-treated rats.

Mallov *et al.*[15] reported that a single large dose of ethanol given to rats by stomach tube or intraperitoneal injection, provoked significant accumulation of triglycerides in the liver within 12—16 h. In the present experiments, it was found that oral administration of ethanol to rats for 8 d caused a fatty liver and hyperlipemia as compared to normal rats. The oral administration of wogonin reduced TG content in the serum of the ethanol-treated rats.

Baicalein reduced TC, FC and TG contents in the liver, and increased HDL-ch level in the serum. Baicalin also reduced the serum FFA, and TC, FC and TG contents in the liver.

As regards the mechanism of metabolic actions resulting from the administration of ethanol, Feigelson *et al.*[3] reported that the release of catecholamines was particularly implicated because of their recognized ability to promote FFA release from the adipose tissue and TG accumulation in the liver. In fat cells, adrenaline- and noradrenaline-induced lipolysis was inhibited by wogonin and baicalin. Baicalein also inhibited noradrenaline-induced lipolysis. Dopamine-induced lipolysis was also inhibited by baicalin.

Based on the *in vitro* experimental results, it is suggested that the *in vivo* effects of flavone components of ogon on lipid metabolism in ethanol-treated rats might be partly due to the inhibitory actions of the flavones on catecholamine-induced lipolysis in adipose tissue.

The results suggest that Scutellariae Radix may be an effective crude drug for the treatment of hyperlipemia and fatty liver caused by ethanol.

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