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Effects of Stilbene Components of the Roots of *Polygonum cuspidatum* Sieb. *et* Zucc. on Lipid Metabolism

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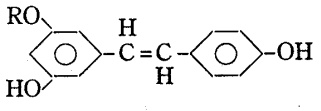
The effects of the roots of *Polygonum cuspidatum* Sieb. *et* Zucc. ("Kojo-kon" or "Itadori-kon" in Japanese) and its stilbene components (resveratrol and piceid) on lipid metabolism in rats and mice (higher animals) were investigated. Resveratrol and piceid inhibited the deposition of triglyceride and cholesterol in the liver of rats fed corn oil–cholesterol–cholic acid mixture. Piceid reduced the serum triglyceride and low density lipoprotein-cholesterol (LDL-ch) levels, and atherogenic index [total cholesterol–high density lipoprotein-cholesterol (HDL-ch)/HDL-ch] in the oil mixture-fed rats. It was found that intraperitoneal or oral administration of resveratrol or piceid reduced triglyceride synthesis from ^{14}C-palmitate in the liver of mice.

In contrast, these stilbene components did not affect hormone-induced lipolysis in fat cells from rat epididymal adipose tissue.

Keywords——*Polygonum cuspidatum*; stilbene; resveratrol; piceid; hyperlipemia; lipid metabolism; atherogenic index; lipogenesis from ^{14}C-palmitate

The dried roots of *Polygonum cuspidatum* Sieb. *et* Zucc. ("Kojo-kon" or "Itadori-kon" in Japanese) have been used for the treatment of suppurative dermatitis, gonorrhoea, favus, athlet's foot and hyperlipemia in Chinese and Japanese traditional medicine.



resveratrol: R=H
piceid: R=D-glucose

Fig. 1

Kubo *et al.*[1] reported that resveratrol possessed antibacterial and antifungal actions. Five anthraquinone components, chrysophanol, physcion, emodin, emodin-8-*O*-D-glucoside and physcion-8-*O*-D-glucoside, and two stilbene com ponents, resveratrol and piceid (resveratrol-3-*O*-D-glucoside), have been isolated from this crude drug.[2]

On the basis of the medical usage of Kojo-kon as a treatment for hyperlipemia, it has been proposed that Kojo-kon might have lipid-lowering activity. The present investigations describe the effects of the major components (resveratrol and piceid) of Kojo-kon on serum and liver lipids in rats fed corn oil–10% cholesterol–1% cholic acid mixture, and the effects of resveratrol and piceid on lipogenesis from ^{14}C-palimitate in mice. In addition to the *in vivo* experiments, the effects of these stilbene components on lipid metabolism in fat cells isolated from epididymal adipose tissue of rats were investigated.

Materials and Methods

Materials——The stilbene components of Kojo-kon (Fig. 1) were isolated by the method described by Nonomura *et al.*[2c]

Animals——Male Wistar-King strain rats weighing 150 g and male ddY strain mice weighing 20—21 g were housed in a room maintained at 25±1°C with 60% relative humidity and given 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 Lipids in the Oil Mixture-fed Rats——The rats of the control groups were given corn oil (10 ml/kg body weight) containing 10% cholesterol and 1% cholic acid (oil mixture) orally

for 7 days, with standard laboratory diet and water *ad lib.* The stilbene components (resveratrol and piceid) were orally administered (50 mg/kg or 100 mg/kg) to the same rats daily 30 min beffre administration of the corn oil mixture. Blood taken by venous puncture 4 h after administration of the corn oil mixture was centrifuged at $1630 \times g$ for 10 min to separate the serum. Total cholesterol (TC), triglyceride (TG), free fatty acids (FFA), phospholipids (PL), high density lipoprotein-cholesterol (HDL-ch) and low density lipoprotein-cholesterol (LDL-ch) in the sera were determined by using Cholesterol B-Test (Wako), Triglyceride B-Test (Wako), NEFA-Test (Wako), Phospholipids-Test (Wako), HDL-cholesterol-Test (Wako) and β-lipoprotein-Test (Wako), respectively. After the liver weight had been estimated, 1 g of liver tissue was homogenized with 10 ml of Krebs-Ringer-phosphate buffer (pH 7.4). Lipids were extracted with $CHCl_3$–MeOH (2: 1) solution (20 ml) and the TC, TG and FFA contents of the extract were estimated as described above.

Estimation of Lipogenesis from ^{14}C-Palmitate in the Liver and Adipose Tissue of Mice——The mice of the control groups and experimental groups were given resveratrol (25 mg/kg or 50 mg/kg) and piceid (50 mg/kg or 100 mg/kg) intraperitoneally or orally for 3 d. The same mice were intraperitoneally injected with ^{14}C-palmitate (0.5 μCi/mouse) 2 h after the last injection of the stilbene components (resveratrol and piceid). The mice were killed by decapitation, and their liver and epididymal adipose tissue were quickly removed. The liver weight was estimated and then 1 g of liver tissue was homogenized with 10 ml of Dole's extraction mixture.[3] The homogenate was shaken vigorously for 20 min and then heptane (3 ml) and water (2 ml) were added, and the mixture was shaken again for 10 min. A 3 ml aliquot of the heptane layer was transferred to a stoppered test tube and shaken vigorously with an equal volume of alkaline ethanol (0.05 N NaOH in 50% ethanol) to remove FFA, following the method of Börgstrom.[4] An aliquot of 1 ml of the heptane layer was used for the estimation of radioactivity. Lipogenic activity was expressed as cpm per whole liver tissue. ^{14}C-Triglyceride formation from ^{14}C-palmitate in adipose tissue of mice was also estimated by the same method.

Preparation of Fat Cells——Male Wistar-King strain rats, weighing 150—180 g 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.[5]

Estimation of Adrenaline- and ACTH-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), 0.5 ml of Krebs-Ringer-phosphate buffer (pH 7.4) containing 5% albumin, 0.5 μg of adrenaline and the indicated amount of stibene components were incubated at 37°C for 2 h in a final volume of 1 ml. At the end of the incubation period, the reaction was stopped by adding 5 ml of Dole's extraction mixture,[3] 3 ml of heptane and 2 ml of water, following by shaking for 5 min. The upper heptane layer was transferred to a test tube and subjected to titration with 0.008 N NaOH by the method described by Dole.[3]

ACTH-induced lipolysis was estimated by the same method as described above except that Krebs-Ringer-bicarbonate buffer (pH 7.4) was used instead of Krebs-Ringer-phosphate buffer (pH 7.4). Lipolytic activity was expressed as μEq free fatty acids per 1 g of adipose tissue.

Results

Effects of Resveratrol and Piceid on Serum and Liver Lipid Levels in the Oil Mixture-fed Rats (*in Vivo*)

As shown in Table I-a, serum TG was elevated 2.7-fold by the administration of the oil mixture (control). This level was reduced by 40% in rats orally given piceid (100 mg/kg) as compared to the control animals. Serum LDL-ch was increased 2.4-fold by the administration of the oil mixture. This level was lowered significantly by oral administration of piceid (50 mg/kg or 100 mg/kg). The atherogenic index (TC–HDL-ch/HDL-ch) was reduced by oral administration of piceid (100 mg/kg) as compared to the control animals. However, the lower levels induced by piceid were still much higher than those of normal rats.

Liver TC and TG contents were reduced by oral administration of resveratrol or piceid as compared with those of control rats (Table I-b). On the other hand, liver FFA content was increased by oral administration of piceid (100 mg/kg) as compared with that of control rats.

Effects of Resveratrol and Piceid on Lipogenesis from ^{14}C-Palmitate in Liver and Adipose Tissue of Mice (*in Vivo*)

As shown in Table II, resveratrol and piceid, given intraperitoneally or orally, reduced the lipogenesis from ^{14}C-palmitate to 60—70% of the control value in liver tissue of mice,

Table I-a. Effects of Resveratrol and Piceid on Serum Lipids (Total Cholesterol, Triglyceride, Free Fatty Acids, Phospholipids, High Density Lipoprotein–Cholesterol, Low Density Lipoprotein–Cholesterol, and Atherogenic Index) in Rats fed Corn Oil–10% Cholesterol–1% Cholic Acid Mixture

	TC (mg/dl) M±S.E.	TG (mg/dl) M±S.E.	FFA (mEq/l) M±S.E.	PL (mg/dl) M±S.E.	HDL-ch (mg/dl) M±S.E.	LDL-ch (mg/dl) M±S.E.	Atherogenic Index
Normal	91.5± 1.89	122.0± 16.2	0.24± 0.048	141.2± 18.7	32.4± 1.80	53.3± 2.58	1.87± 0.17
Control	180.3± 16.5	456.6± 37.0	0.97± 0.087	205.5± 22.0	26.8± 1.50	129.8± 19.2	5.83± 0.66
Resveratrol (50 mg/kg)	162.1± 6.03$^{N.S}$	508.0± 74.5$^{N.S}$	0.88± 0.084$^{N.S}$	214.2± 15.6$^{N.S}$	27.8± 1.40$^{N.S}$	116.1± 6.96$^{N.S}$	4.88± 0.25$^{N.S}$
Piceid (50 mg/kg)	155.7± 11.4$^{N.S}$	354.9± 50.8$^{N.S}$	0.97± 0.13$^{N.S}$	197.5± 8.97$^{N.S}$	28.8± 2.99$^{N.S}$	103.2± 4.83$^{c)}$	4.98± 1.22$^{N.S}$
(100 mg/kg)	153.9± 7.12$^{N.S}$	283.4± 41.7$^{d)}$	0.90± 0.044$^{N.S}$	196.4± 12.6$^{N.S}$	30.8± 2.45$^{N.S}$	107.6± 5.37$^{b)}$	4.15± 0.48$^{a)}$

The results are means ± standard errors of 6—7 rats of each group.
a) $p<0.05$, *b)* $p<0.02$, *c)* $p<0.01$, *d)* $p<0.001$. N.S., Not significant;
TC, total cholesterol; TG, triglyceride; FFA, free fatty acids; PL, phospholipids; HDL-ch, high density lipoprotein-cholesterol; LDL-ch, low density lipoprotein-cholesterol; Atherogenic Index, TC–HDL-ch/HDL-ch.

Table I-b. Effects of Reseveratrol and Piceid on Liver Lipids (Total Cholesterol, Triglyceride and Free Fatty Acids) in Rats fed Corn Oil–10% Cholesterol–1% Cholic Acid Mixture

	TC(mg/g) M±S.E.	TG(mg/g) M±S.E.	FFA(μEq/g) M±S.E.
Normal	3.62±0.60	9.10±1.96	4.74±0.50
Control	16.2±0.93	36.2±3.71	6.39±0.43
Reseveratrol (50 mg/kg)	11.7±0.37$^{d)}$	24.6±2.15$^{b)}$	6.30±0.29$^{N.S.}$
Piceid (50 mg/kg)	10.4±0.68$^{d)}$	18.0±0.97$^{d)}$	5.47±0.41$^{N.S.}$
(100 mg/kg)	11.6±1.13$^{c)}$	23.5±3.03$^{b)}$	7.30±0.22$^{a)}$

The results are means ± standard errors of 6—7 rats of each group.
a) $p<0.05$, *b)* $p<0.02$, *c)* $p<0.01$, *d)* $p<0.001$. N.S., Not significant; TC, total cholesterol; TG, triglyceride; FFA, free fatty acids.

while piceid did not affect lipogenesis from ^{14}C-palmitate in adipose tissue of mice. There was no clear dose-response relationship in the case of piceid. The oral administration of resveratrol (50 mg/kg) reduced the lipogenesis from ^{14}C-palmitate to 47% of the control value in adipose tissue of mice, while the intraperitoneal administration of resveratrol did not affect it.

Effects of Resveratrol and Piceid on Hormone-induced Lipolysis in Fat Cells (*in Vitro*)

In the control fat cells, lipolysis was not measurable under the conditions used. When adrenaline (0.5 μg/ml) or ACTH (0.5 μg/ml) was added, 10.5 or 8.5 μEq of free fatty acids was released from 1 g of adipose tissue, respectively. Additions of resveratrol and piceid did not affect adrenaline- or ACTH-induced lipolysis in the fat cells.

Discussion

The present investigation demonstrated that resveratrol and piceid, the major stilbene components of the roots of *P. cuspidatum* affected lipid metabolism in higher animals. In the previous paper,[6] we showed that oral administration of an oil mixture (corn oil–cholesterol–sodium cholate) induces both fatty liver and hyperlipemia as compared to normal rats.

Table II-a. Effects of Resveratrol and Piceid on Lipogenesis from ^{14}C-Palmitate in Liver and Adipose Tissue of Mice

	Lipogenesis (cpm × 10^3/liver tissue)	Significance
Control[saline+^{14}C-palmitate (0.5 μCi/mouse *i.p.*)]	7.92±0.58[a]	—
^{14}C-Palmitate+resveratrol		
(25 mg/kg *i.p.*)	6.10±0.64	$p<0.05$
(50 mg/kg *i.p.*)	4.69±0.39	$p<0.001$
^{14}C-Palmitate+piceid		
(50 mg/kg *i.p.*)	4.82±0.54	$p<0.001$
100 mg/kg *i.p.*)	5.94±0.66	$p<0.05$

	Lipogenesis (cpm × 10^3/adipose tissue)	Significance
Control[saline+^{14}C-palmitate (0.5 μCi/mouse *i.p.*)]	3.80±1.58	—
^{14}C-Palmitate+resveratrol		
(25 mg/kg*i.p.*)	2.72±0.54	N.S.
(50 mg/kg *i.p.*)	4.34±0.53	N.S.
^{14}C-Palmitate+piceid		
(50 mg/kg *i.p.*)	2.82±0.42	N.S.
(100 mg/kg *i.p.*)	2.51±0.81	N.S.

a) The results are means ± standard errors of 6—8 mice.
N.S., not significant; *i.p.*, intraperitoneal injection.

Table II-b. Effects of Resveratrol and Piceid on Lipogenesis from ^{14}C-Palmitate in Liver and Adipose Tissue of Mice

	Lipogenesis (cpm × 10^3/liver tissue)	Significance
Control[^{14}C-palmitate (0.5 μCi/mouse *i.p.*)]	5.24±0.51[a]	—
^{14}C-Palmitate+resveratrol		
(25 mg/kg *p.o.*)	3.87±0.18	$p<0.02$
(50 mg/kg *p.o.*)	3.02±0.28	$p<0.001$
^{14}C-Palmitate+piceid		
(50 mg/kg *p.o.*)	4.19±0.48	N.S.
(100 mg/kg *p.o.*)	3.98±0.36	$p<0.05$

	Lipogenesis (cpm × 10^3/adipose tissue)	Significance
Control[^{14}C-palmitate (0.5 μCi/mouse *i.p.*)]	3.57±0.84[a]	
^{14}C-Palmitate+resveratrol		
(25 mg/kg *p.o.*)	2.45±0.53	N.S.
(50 mg/kg *p.o.*)	1.67±0.36	$p<0.05$
^{14}C-Palmitate+piceid		
(50 mg/kg *p.o.*)	2.33±0.58	N.S.
(100 mg/kg *p.o.*)	3.18±0.68	N.S.

a) The results are means ± standard errors of 8 mice.
N.S., not significant; *i.p.*, intraperitoneal injection; *p.o.*, oral administration.

The serum TC, TG, PL and LDL-ch in rats orally given the oil mixture were significantly increased (Table I). On the other hand, serum HDL-ch level in the oil mixture-fed rats was significantly reduced as compared to that in the normal rats. Furthermore, the administration of resveratrol partly inhibited the accumlation of TC and TG in the liver. Piceid partly inhibited the elevation of serum TG and LDL-ch, and the accumulation of TC and TG in the liver of oil mixture-fed rats.

Resveratrol and piceid reduced the lipogenesis from ^{14}C-palmitate in the liver of mice.

Based on these experimental results, it seems likely that resveratrol and piceid reduce liver TG content in the oil mixture-fed rats by inhibiting lipogenesis in liver tissue. Furthermore, the decrease in serum TG and LDL-ch observed after administration of piceid may also be explained by the inhibitory action on lipogenesis in liver. The other possible mechanism is that resveratrol and piceid may modulate lipid metabolism, *e.g.* by inhibition of lipid absorption and acceleration of lipid utilization in muscles. Lipolysis and lipogenesis in adipose tissue may not be involved in the lipid-lowering effects of these stilbene components.

It has been reported that the elevation of serum HDL-ch is an anti-arteriosclerotic factor.[7] The atherogenic index was reduced in the oil mixture-fed rats by oral administration of piceid.

We have reported that 2,3,5,4′-tetrahydroxy stilbene-2-*O*-D-glucoside isolated from *P. multiflorum* has lipid-lowering actions.[8] The metabolic action of 2,3,5,4′-tetrahydroxy stilbene-2-*O*-D-glucoside was found to reduce serum lipid levels in the oil mixture-fed rats, while this substance had no effect on the lipid levels of liver. Resveratrol and piceid inhibited the accumulation of liver TC and TG. It seems likely that the difference of lipid metabolic action between resveratrol (3,5,4′-trihydroxy stilbene) and piceid (3,5,4′-trihydroxy stilbene-3-*O*-D-glucoside), and 2,3,5,4′-tetrahydroxy stilbene-2-*O*-D-glucoside may be due to the presence or absence of the hydroxy group at the 2-position in the stilbene skeleton.

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