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Biological Actions of the Natural Coumarins. I. Changes of Lipids and Glucose Levels in Rat Plasma after Intraperitoneal Administration of Various Linear-Type Furanocoumarins

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Experiments were carried out on the effects of various linear-type furanocoumarins on the concentrations of lipids and glucose in rat plasma. It was found that bergapten and xanthotoxin cause hypolipidemia. In contrast, imperatorin, isoimperatorin, oxypeucedanin and oxypeucedanin hydrate cause hyperlipidemia. However, no effect on any lipid fraction in rat plasma was produced by isopimpinellin, phellopterin, ferulin, byakangelicin or *sec-O*-acetylbyakangelicin. Glucose level in rat plasma was increased by isopimpinellin and psoralen, and decreased by oxypeucedanin. These results suggest that the effects of linear-type furanocoumarins on lipid concentration in rat plasma depend upon whether substitution is present or not at the 5- and 8-positions of the skeleton and upon the kinds of substituents.

Keywords——coumarin; lipid; cholesterol; triglyceride; free fatty acid; glucose; rat plasma

The natural coumarins are widely distributed in the higher plants and 800 such compounds are known. Among these coumarins, furocoumarins occur predominantly in Umbelliferous and Rutaceous plants; for example, they have been isolated from the crude drugs Angelicae Radix, Angelicae dahuricae Radix, Imperatoriae Radix and Rutae Herba.

As regards the biological actions of furanocoumarins, photosensitization and binding to proteins or nucleic acids are well known.[1)]

Further, effects on deoxyribonucleic acid (DNA) template efficiency, protein synthesis, DNA replication, phage and virus functions, tumor development, enzyme activity, skin pigments, lipid peroxide formation, *etc.* have been reported.[1)] Recently, Kimura *et al.* investigated the effects of various furanocoumarins from Angelicae dahuricae Radix in *in vitro* experiments using the fat cells from epididymal adipose tissues of rat and suggested that certain compounds may activate the action of lipolytic hormones and selectively inhibit the effects of antilipolytic hormones.[2)] However, there are few detailed reports relating to other physiological effects in biological systems.

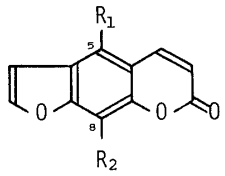
In the present studies, we examined the variation of concentrations of lipids such as cholesterol, triglyceride and free fatty acid and of glucose in rat plasma after administration of various linear-type furanocoumarins *in vivo*.

Materials and Methods

Materials——The twelve furanocoumarins shown in Chart 1 were suspended at a concentration of 10 mg/ml of 5% gummi arabicum.

Animals——Male slc : Wistar/ST rats, 4 weeks of age, were housed in a room at 24 °C, 55% relative humidity, 18 changes of air per hour, and with a 12 h (0600 to 1800 h) light-dark cycle. Both a standard laboratory diet (NMF, Oriental Yeast Co., Ltd., Tokyo) and water were allowed *ad libitum* before the experiment.

Treatment of Animals and Blood Sampling——The rats were divided into 13 groups of 6 animals. Each coumarin, suspended in gummi arabicum, was intraperitoneally administered to the rats at a dose of 100 mg/kg of body weight.



psoralen	$R_1=R_2=H$
bergapten	$R_1=OCH_3$, $R_2=H$
xanthotoxin	$R_1=H$, $R_2=OCH_3$
isopimpinellin	$R_1=R_2=OCH_3$
imperatorin	$R_1=H$, $R_2=OCH_2-CH=C(CH_3)_2$
isoimperatorin	$R_1=OCH_2-CH=C(CH_3)_2$, $R_2=H$
phellopterin	$R_1=OCH_3$, $R_2=OCH_2-CH=C(CH_3)_2$
ferulin	$R_1=OCH_3$, $R_2=OCH_2-CH(O)-C(CH_3)_2$
byakangelicin	$R_1=OCH_3$, $R_2=OCH_2-CH(OH)-C(OH)(CH_3)_2$
sec-O-acetylbyakangelicin	$R_1=OCH_3$, $R_2=OCH_2-CH(OCOCH_3)-C(OH)(CH_3)_2$
oxypeucedanin	$R_1=OCH_2-CH(O)-C(CH_3)_2$, $R_2=H$
oxypeucedanin hydrate	$R_1=OCH_2-CH(OH)-C(OH)(CH_3)_2$, $R_2=H$

Chart 1

TABLE I. Concentrations of Glucose and Lipids in Rat Plasma after Administration of Various Coumarins

	Glucose (mg/dl)	Lipids		
		Cholesterol (mg/dl)	Triglyceride (mg/dl)	Free fatty acid (μeq/l)
Control	146.9±2.9	61.18±4.16	78.66±6.54	334.3±47.8
Psoralen	156.4±2.3[a]	72.64±3.58	102.32±6.58[a]	461.5±59.6
Bergapten	150.0±4.3	71.57±2.30	58.25±4.48[a]	204.3±30.8[a]
Xanthotoxin	159.4±5.6	71.35±3.21	57.14±3.63[b]	242.1±30.0
Isopimpinellin	166.3±5.0[b]	70.59±7.71	63.70±4.88	417.6±107.8
Imperatorin	150.1±6.0	83.59±4.23[b]	109.57±5.98[b]	784.6±67.3[c]
Isoimperatorin	143.2±5.1	73.43±4.00	138.28±8.46[c]	248.2±54.0
Phellopterin	152.9±2.4	70.72±5.17	95.35±7.57	349.4±33.3
Ferulin	151.0±3.4	69.96±2.13	59.68±8.49	351.9±61.9
Byakangelicin	143.1±3.7	71.68±3.37	71.94±7.03	359.3±44.1
sec-O-Acetylbyakangelicin	142.0±1.8	68.10±2.60	65.93±5.39	273.8±29.6
Oxypeucedanin	137.5±3.4[a]	76.40±3.33[a]	87.58±6.73	1219.8±83.7[c]
Oxypeucedanin hydrate	142.6±4.5	81.88±8.58	83.99±13.17	1173.0±163.0[c]

All values are the means±S.E. of 6 experiments. Significant difference from the control groups: *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$.

Control groups were administered an equal volume of the gummi arabicum alone. The animals were exsanguinated by decapitation at 6 h after the administration and blood samples were collected in heparinized centrifuge tubes. Blood samples were centrifuged at 3000 rpm for 5 min at room temperature. The concentrations of lipids and glucose in the plasma obtained were measured immediately.

Assay of Lipids and Glucose in Plasma——Concentrations of total cholesterol, triglyceride, free fatty acid and glucose in plasma were estimated by enzymatic methods using Iatroset CHOL-E (Iatron Co., Ltd., Tokyo),

Triglyceride C II-Test (Wako Pure Chemicals Co., Ltd., Osaka), NEFA C-Test (Wako Pure Chemicals Co., Ltd., Osaka), respectively.

Results

Concentrations of glucose and lipids such as cholesterol, triglyceride and free fatty acid in rat plasma after the administration of various furanocoumarins (100 mg/kg, *i.p.*) are shown in Table I. Glucose level in the plasma was decreased about 6% by administration of oxypeucedanin and increased about 13 and 6% by administration of isopimpinellin and psoralen as compared with the value of 146.9 ± 2.9 mg/dl in the control group. There was no difference in plasma cholesterol level between the control and the various furanocoumarin groups, except in the groups given imperatorin and oxypeucedanin. Triglyceride level in the plasma was decreased about 25 and 27% by administration of bergapten and xanthotoxin, but was increased about 40, 57 and 30% by imperatorin, isoimperatorin and psoralen, respectively. The free fatty acid concentration in the plasma was decreased or slightly decreased by bergapten and xanthotoxin, but was increased to various extents by imperatorin, oxypeucedanin and oxypeucedanin hydrate.

Discussion

In the present *in vivo* studies using rat plasma, it was observed that the concentration of lipids in rat plasma was altered by the administration of various linear-type furanocoumarins. As shown in Table I, the furanocumarins with a methoxyl group at either C-5 or C-8 (bergapten and xanthotoxin) cause hypotriglycemia. Isopimpinellin, phellopterin, ferulin, byakangelicin and *sec-O*-acetylbyakangelicin, substituted with a methoxyl group at C-5 and a methoxyl group or other group (isopentenyloxy, 2,3-epoxy-3-methybutoxyl, 2,3-dihydroxy-3-methylbutoxyl or 2-acetoxy-3-methylbutoxyl) at C-8 have no effect on any lipid fraction in rat plasma. On the other hand, imperatorin, isoimperatorin, oxypeucedanin and oxypeucedanin hydrate, substituted with an isopentenyloxy group or similar group at either C-5 or C-8, induced hyperlipidemia.

Thus, it appears that the effects of furocoumarins on lipid content in rat plasma can be classified into three types, depending upon whether substituents are present or absent at C-5 and C-8 and upon the kinds of substituents.

These results are difficult to explain since little is known about the mechanism by which the linear-type furanocoumarins affect lipid metabolism and/or mobilization *in vivo*. However, some linear-type furanocoumarins may affect the absorption of lipid from intestine to blood, the secretion as lipoproteins from liver to blood, and the lipolytic and/or antilipolytic mechanisms in adpose tissues.[2)]

In the present studies, the concentrations of lipids and glucose in rat plasma were measured at only 6 h after the administration of various linear-type furanocoumarins at a single dose of 100 mg/kg of body weight. However, the time courses of the concentrations and the effects of different doses were not studied. Thus, elucidation of the exact mechanisms of action of the linear-type furanocoumarins on lipid mobilization will require further experiments.

References

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2) Y. Kimura, H. Ohminami, H. Arichi, H. Okuda, K. Baba, M. Kozawa and S. Arichi, *Planta Medica*, **45**, 183 (1982).