[Chem. Pharm. Bull. 29(9)2610—2617(1981)]

Studies on Scutellariae Radix. IV.[1] Effects on Lipid Peroxidation in Rat Liver

Yoshiyuki Kimuya,*,[a] Michinori Kubo,[a] Tadato Tani,[a] Shigeru Arichi,[a] and Hiromichi Okuda[b]

The Research Institute of Oriental Medicine, Kinki University,[a] 380 Nishiyama, Sayama-cho, Minamikawachi-gun, Osaka, 589, Japan and 2nd Department of Medical Biochemistry, School of Medicine, Ehime University,[b] Shigenobu-cho, Onsen-gun, Ehime, 791-02, Japan

(Received January 26, 1981)

It was found that the flavonoid components (wogonin, baicalein and baicalin) isolated from Scutellariae Radix (ogon in Japanese) inhibited lipid peroxidation in intact rat liver stimulated by the intraperitoneal administration of $FeCl_2$–ascorbic acid–adenosine 5′-diphosphate (ADP) mixture. Various flavonoids of ogon also inhibited lipid peroxidation stimulated by $FeCl_2$–ascorbic acid and nicotinamide adenine dinucleotide phosphate (NADPH)–ADP mixture in rat liver homogenate. Furthermore, it was found that baicalin, the major flavonoid component of ogon, reduced the increase of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in rats orally given an oxygen-bubbled rapeseed–corn–soybean oil mixture.

Keywords——*Scutellaria baicalensis*; flavonoids; baicalin; lipid peroxidation; lipid metabolism; GOT; GPT; NADPH; ADP

Scutellariae Radix, ogon in Japanese, is a root of *Scutellaria baicalensis* Georgi and has been listed in a Chinese herbal, Shen-nung-pen-ts'ao-ching, as a remedy for suppurative dermatitis, diarrhea and inflammatory diseases.

In our previous paper,[2] we reported that ogon possessed bactericidal activity and that the bactericidal activity was due to 2(*S*), 2′,5,6′,7-tetrahydroxyflavanone and baicalein in ogon. We also reported that ogon affected lipid metabolism in higher animals.[1] Flavone components (wogonin, skullcapflavone II, baicalein and baicalin) of Scutellariae Radix were found to reduce lipid levels of the serum and liver in rats treated with corn oil–cholesterol–sodium cholate mixture.

In higher animals, lipid peroxides are known to injure the liver,[3] kidney[4] and blood vessels.[5] Since the flavonoid components lowered lipid levels in the serum and the liver, we considered that these flavones might reduce the formation of lipid peroxides in higher animals and protect the tissues against injury.

The present paper describes the effects of the extracts and flavone components of ogon on the lipid peroxidation in rat liver stimulated by $FeCl_2$–ascorbic acid–adenosine 5′-diphosphate (ADP) mixture, and in rat liver homogenate stimulated by $FeCl_2$–ascorbic acid and nicotinamide adenine dinucleotide phosphate (NADPH)–ADP mixture. Furthermore, the effects of ogon on rat liver injury caused by oral administration of oxidized rapeseed–corn–soybean oil mixture were also examined.

Materials and Methods

Materials——The water, methanol and ethyl acetate extracts and flavonoid components (oroxylin A, wogonin, skullcapflavone II, chrysin, baicalein, baicalin and wogonin-7-O-D-glucuronide) were prepared from ogon by the methods described previously.[2] In addition to flavonoids of ogon, rutin was obtained from the bud of *Sophora japonica* L. and formononetin, daidzein, daidzin and puerarin were prepared from the roots of *Pueraria lobata* (Willd.) Ohwi. Hesperidin and narigenin were purchased from Sigma Chemical Co., St. Louis, MO. U.S.A. Hesperetin and quercetin were obtained by hydrolysis of hesperidin and rutin, respectively (Fig. 1).

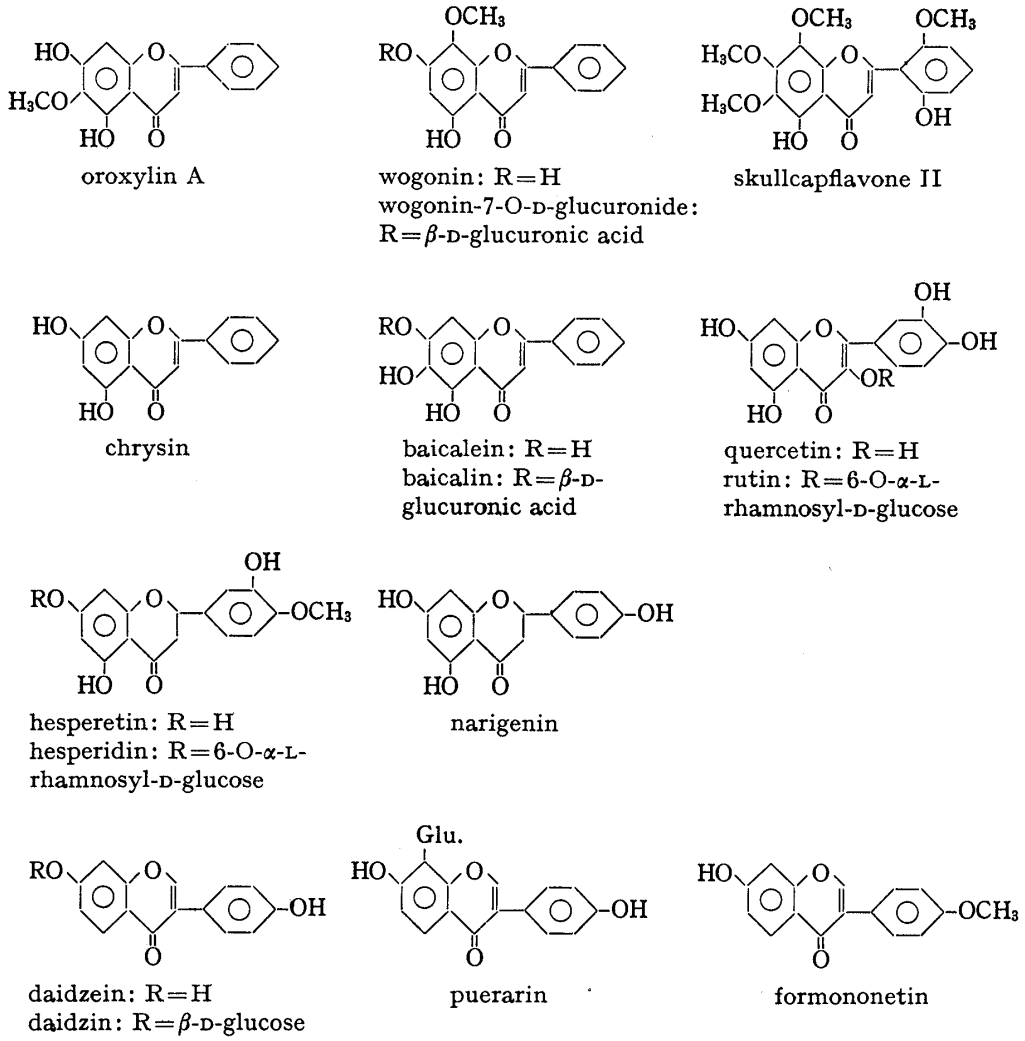

Fig. 1. The Structure of Various Flavonoids

$FeCl_2$, ascorbic acid (Kishida Chemicals Co. Ltd.; the highest grade), DL-α-tocopherol (Nakarai Chemical Co. Ltd.), ADP and NADPH (reduced form) (Sigma Chemical Co.) were used.

Oxidized rapeseed-corn-soybean oil mixture was obtained by heating the oil mixture in an O_2 current at 150°C for 5 h.

Animals——Young male Wistar King strain rats weighing 160 to 200 g were used.

Estimation of Lipids (in Serum and Liver) and Lipid Peroxide (in Liver) of Rats treated with the $FeCl_2$–Ascorbic Acid–ADP Mixture——Peroxidizing reagent admixed with $FeCl_2$ (30 mg/kg body weight), ascorbic acid (176 mg) and ADP (5 mg) was intraperitoneally injected into rats daily for 3 d. The same rats orally received the extracts (water, methanol and ethyl acetate) (1 g/kg), flavones (wogonin, baicalein and baicalin) (100 mg/kg) or DL-α-tocopherol (20 mg/kg) daily 1 h before administration of the peroxidizing reagent.

Rats were killed 24 h after the last injection of the $FeCl_2$–ascorbic acid–ADP mixture. Blood was taken immediately by cardiac puncture, and centrifuged at $1630 \times g$ for 10 min to separate the serum. Cholesterol, triglyceride and phospholipids in the serum were determined by the methods of Richmond,[6] Bucolo and David[7] and Bartlett,[8] respectively.

The total weight of the liver was estimated and then 2 g of the liver tissue was homogenized with 10 ml of 150 mM KCl–Tris-HCl buffer (pH 7.2). The homogenate was then extracted with chloroform–methanol (2:1) mixture, and cholesterol, triglyceride and phospholipids were estimated by the methods of Zak,[9] Fletcher,[10] and Bartlett,[8] respectively.

Lipid peroxide content was estimated by the method of Yoden *et al.*[11] Lipid peroxide value was expressed as nmol of malondialdehyde (MDA) per g of liver tissue.

Estimation of $FeCl_2$–Ascorbic Acid-stimulated Lipid Peroxidation in Rat Liver Homogenate——Rats fed a standard laboratory diet and water *ad lib.* were killed by decapitation, and their liver tissues were quickly removed. A 2 g portion of liver tissue was sliced and then homogenized with 10 ml of 150 mM KCl–Tris-HCl buffer (pH 7.2).

In a glass test tube, 0.5 ml of the liver homogenate (equivalent to 100 mg of liver tissue) was incubated with shaking for 1 h at 37°C in Tris-HCl buffer (pH 7.2) (0.2 ml)–6 mM ascorbic acid (0.2 ml)–4 mM $FeCl_2$ (0.1 ml) solution in the presence of various flavonoid components (oroxylin A, wogonin, skullcapflavone II, chrysin, baicalein, baicalin, wogonin-7-O-D-glucuronide, quercetin, rutin, hesperetin, hesperidin, narigenin, daidzein, daidzin, puerarin and formononetin) (2.5×10^{-4} M or 1.0×10^{-4} M). After incubation, the lipid peroxides were estimated by the methods of Yoden *et al.*[11]

Estimation of NADPH-ADP-stimulated Lipid Peroxidation in Rat Liver Homogenate——In a glass test tube, 0.5 ml of the liver homogenate was incubated with shaking for 1 h at 37°C in Tris-HCl buffer (pH 7.2) (0.2 ml)–4 mM NADPH (0.1 ml)–40 mM ADP (0.1 ml) solution in the presence of various flavonoids (2.5×10^{-4} M or 1.0×10^{-4} M). After incubation, the lipid peroxide level was estimated by the method described above.

Estimation of Serum Glutamic Oxaloacetic Transaminase (GOT) (EC 2.6.1.1) and Glutamic Pyruvic transaminase (GPT) (EC 2.6.1.2) in Rats fed the Oxidized Rapeseed-Corn-Soybean Oil Mixture——Rats were orally administered with the oxygen-bubbled rapeseed–corn–soybean oil mixture (10 ml/kg body weight) (3 times/d) for 3d. The same rats received wogonin, baicalein or baicalin (each 100 mg daily/kg) orally for 3 d together with the oxidized rapeseed–corn–soybean oil mixture. Blood was taken by cardiac puncture 24 h after the last administration of oxidized rapeseed–corn–soybean oil mixture and centrifuged at $1630\times g$ for 10 min to separate the serum. The serum GOT and GPT were determined by the method of Reitman and Frankel.[12]

Results

$FeCl_2$-Ascorbic Acid-ADP-stimulated Lipid Peroxidation (*in Vivo*)

It is well known that administration of Fe^{2+} and ascorbic acid to rats increases the lipid peroxide level in the liver.[13] As shown in Tables I and II, intraperitoneal administration of $FeCl_2$–ascorbic acid–ADP mixture to rats increased the hepatic lipid peroxide, but did not elevate the total cholesterol, triglyceride and phospholipid levels in the serum and liver.

Oral administration of water, methanol, or ethyl acetate extract of ogon, flavone component (wogonin, baicalein or baicalin), or DL-α-tocopherol to the rats treated with $FeCl_2$–ascorbic acid–ADP mixture reduced the lipid peroxide level in the liver. None of the samples changed the serum cholesterol and triglyceride levels, but baicalein and baicalin elevated the serum phospholipid content. The methanol extract of ogon reduced liver total cholesterol and triglyceride content, and the water extract reduced liver triglyceride and phospholipid contents. Wogonin and baicalein decreased triglyceride and phospholipid contents in the liver.

$FeCl_2$-Ascorbic Acid-stimulated Lipid Peroxidation (*in Vitro*)

It was reported that Fe^{2+} and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria.[14] As shown in Table III, oroxylin A, wogonin, skullcapflavone II,

TABLE I. Effects of Ogon on Serum Lipids (Total Cholesterol, Triglyceride and Phospholipids) in Rats given $FeCl_2$, Ascorbic Acid and ADP intraperitoneally

	Total cholesterol (mg/dl) M±S.E.[b]	Triglyceride (mg/dl) M±S.E.[b]	Phospholipids (mg/dl) M±S.E.[b]
Normal	87.0±4.4	115.0±11.0	167.1±5.8
Control	85.7±3.4	101.3±16.0	171.5±6.9
The ethyl acetate extract (1 g/kg)	79.1±4.0[N.S.]	101.9±15.3[N.S.]	179.1±3.1[N.S.]
The methanol extract (1 g/kg)	87.7±4.1[N.S.]	77.0±6.2[N.S.]	180.8±7.4[N.S.]
The water extract (1 g/kg)	92.2±5.2[N.S.]	74.8±4.7[N.S.]	177.5±7.4[N.S.]
Wogonin (100 mg/kg)	78.0±11.7[N.S.]	91.4±25.5[N.S.]	192.1±13.4[N.S.]
Baicalein (100 mg/kg)	80.5±6.6[N.S.]	77.1±11.8[N.S.]	202.3±12.3[a]
Baicalin (100 mg/kg)	82.5±5.5[N.S.]	83.1±8.4[N.S.]	199.0±5.0[a]
DL-α-Tocopherol (20 mg/kg)	87.1±5.2[N.S.]	93.0±14.4[N.S.]	184.7±13.6[N.S.]

a) $p<$ .02, N.S.: not significant.
b) The data for normal (14 rats), control (12 rats) and the 7 experimental groups (each of 6—7 rats) are means ± standard errors.

TABLE II. Effects of Ogon on Liver Lipids (Total Cholesterol, Triglyceride, Phospholipids and Lipid Peroxides) in Rats given $FeCl_2$, Ascorbic Acid and ADP intraperitoneally

	Total cholesterol (mg/g) M±S.E.[e]	Triglyceride (mg/g) M±S.E.[e]	Phospholipids (mg/g) M±S.E.[e]	Lipid peroxides (nmol/g) M±S.E.[e]
Normal	5.03±0.30	5.03±0.32	39.6±1.45	50.9±3.1
Control	5.29±0.50	5.97±1.23	42.3±1.45	102.9±7.0
The ethyl acetate extract (1 g/kg)	4.21±0.73[N.S.]	3.57±0.43[N.S.]	38.2±2.55[N.S.]	75.0±10.2[a]
The methanol extract (1 g/kg)	3.76±0.31[a]	4.03±0.69[N.S.]	37.1±1.61[b]	66.1±5.5[d]
The water extract (1 g/kg)	4.47±0.44[N.S.]	2.93±0.49[a]	36.1±1.81[c]	60.7±4.9[d]
Wogonin (100 mg/kg)	4.91±0.37[N.S.]	3.25±0.39[N.S.]	36.5±1.41[b]	78.2±4.1[b]
Baicalein (100 mg/kg)	4.15±0.16[N.S.]	2.81±0.25[a]	33.4±1.20[d]	57.0±2.2[d]
Baicalin (100 mg/kg)	4.48±0.26[N.S.]	3.49±0.65[N.S.]	30.0±1.13[d]	61.3±4.0[d]
DL-α-Tocopherol (20 mg/kg)	5.79±0.27[N.S.]	4.03±0.34[N.S.]	41.4±1.72[N.S.]	65.8±2.9[d]

a) $p<0.05$, *b*) $p<0.02$, *c*) $p<0.01$, *d*) $p<0.001$, N.S.: not significant.
e) The data for normal (14 rats), control (12 rats) and the experimental groups (each of 6—7 rats) are means± standard errors.

TABLE III. Effects of Various Flavonoids on $FeCl_2$–Ascorbic Acid-stimulated Lipid Peroxide Formation in the Liver Homogenate

Additions (/ml reaction mixture)	Lipid peroxides (MDA nmol/g)	Significance
None	73.7±5.36[a]	—
$FeCl_2$ (0.4 mM)+ascorbic acid (1.2 mM)	245.2±12.4	—
$FeCl_2$ (0.4 mM)+oroxylin A (2.5×10^{-4} M)	205.4±10.9	*b*)
(1.0×10^{-4} M)	285.9±14.5	N.S.
$FeCl_2$ (0.4 mM)+wogonin (2.5×10^{-4} M)	190.2±9.60	*c*)
(1.0×10^{-4} M)	264.3±13.4	N.S.
$FeCl_2$ (0.4 mM)+skullcapflavone II		
(2.5×10^{-4} M)	184.7±14.3	*c*)
(1.0×10^{-4} M)	225.1±11.9	N.S.
$FeCl_2$ (0.4 mM)+chrysin (2.5×10^{-4} M)	222.0±22.9	N.S.
(1.0×10^{-4} M)	253.6±15.4	N.S.
$FeCl_2$ (0.4 mM)+baicalein (2.5×10^{-4} M)	128.8±7.86	*d*)
(1.0×10^{-4} M)	148.3±5.01	*d*)
$FeCl_2$ (0.4 mM)+baicalin (2.5×10^{-4} M)	84.1±2.96	*d*)
(1.0×10^{-4} M)	136.0±10.4	*d*)
$FeCl_2$(0.4 mM)+wogonin-7-O-D-glucuronide		
(2.5×10^{-4} M)	114.1±1.79	*d*)
(1.0×10^{-4} M)	222.3±24.9	N.S.

a) The results are means±standard errors for 4—6 replicate experiments.
b) $p<0.02$, *c*) $p<0.005$, *d*) $p<0.001$, N.S.: not significant.

baicalein, baicalin and wogonin-7-O-D-glucuronide inhibited the $FeCl_2$–ascorbic acid-stimulated lipid peroxidation in rat liver homogenate, while chrysin did not affect the generation of lipid peroxides in the homogenate at the concentration of 2.5×10^{-4} M. At the concentration of 1.0×10^{-4} M, baicalein and baicalin inhibited the $FeCl_2$–ascorbic acid-stimulated lipid peroxidation, but the other components of ogon had no effect.

As shown in Table IV, in addition to the flavonoids of ogon, quercetin and hesperetin inhibited the $FeCl_2$–ascorbic acid-stimulated lipid peroxidation, while rutin, hesperidin, narigenin, daidzein, daidzin, puerarin and formononetin had no effect at the concentration of 2.5×10^{-4} M. Quercetin also inhibited the $FeCl_2$–ascorbic acid-stimulated lipid peroxidation at the concentration of 1.0×10^{-4} M.

NADPH-ADP-stimulated Lipid Peroxidation (*in Vitro*)

It is reported that NADPH and ADP stimulated lipid peroxidation in rat liver microsomes.[14] As shown in Table V, all the flavone components of ogon inhibited the NADPH–

Table IV. Effects of Various Flavonoids on $FeCl_2$–Ascorbic Acid-stimulated Lipid Peroxide Formation in the Liver Homogenate

Additions (/ml reaction mixture)	Lipid peroxides (MDA nmol/g)	Significance
None	73.7±5.36[a]	—
$FeCl_2$ (0.4 mM)+ascorbic acid (1.2 mM)	245.2±12.4	—
$FeCl_2$ (0.4 mM)+quercetin (2.5×10^{-4} M)	132.7±13.8	b)
(1.0×10^{-4} M)	140.5±5.58	b)
$FeCl_2$ (0.4 mM)+rutin (2.5×10^{-4} M)	247.8±16.1	N.S.
(1.0×10^{-4} M)	260.6±9.10	N.S.
$FeCl_2$ (0.4 mM)+hesperetin (2.5×10^{-4} M)	171.5±14.3	b)
(1.0×10^{-4} M)	257.4±6.62	N.S.
$FeCl_2$ (0.4 mM)+hesperidin (2.5×10^{-4} M)	222.5±17.3	N.S.
(1.0×10^{-4} M)	272.6±13.1	N.S.
$FeCl_2$ (0.4 mM)+narigenin (2.5×10^{-4} M)	235.5±12.0	N.S.
(1.0×10^{-4} M)	271.0±12.4	N.S.
$FeCl_2$ (0.4 mM)+daidzein (2.5×10^{-4} M)	249.6±7.85	N.S.
(1.0×10^{-4} M)	265.5±15.8	N.S.
$FeCl_2$ (0.4 mM)+daidzin (2.5×10^{-4} M)	240.0±4.52	N.S.
(1.0×10^{-4} M)	262.5±15.0	N.S.
$FeCl_2$ (0.4 mM)+puerarin (2.5×10^{-4} M)	244.6±13.9	N.S.
(1.0×10^{-4} M)	277.0±17.2	N.S.
$FeCl_2$ (0.4 mM)+formononetin (2.5×10^{-4} M)	224.0±15.7	N.S.
(1.0×10^{-4} M)	279.1±15.9	N.S.

a) The results are means±standard errors for 4—6 replicate experiments.
b) $p<0.001$, N.S.: not significant.

ADP–stimulated lipid peroxidation in the rat liver homogenate at the concentration of 2.5×10^{-4} M. At the concentration of 1.0×10^{-4} M, wogonin, skullcapflavone II, baicalein, baicalin and wogonin-7-O-D-glucuronide inhibited the NADPH–ADP-stimulated lipid peroxidation, while oroxylin A and chrysin had no effect.

In addition to the flavonoid components of ogon, quercetin, rutin, hesperetin, hesperidin, narigenin and formononetin inhibited the NADPH–ADP-stimulated lipid peroxidation, but daidzein, daidzin and puerarin had no effect at the concentration of 2.5×10^{-4} M (Table VI).

Table V. Effects of Various Flavonoids on NADPH–ADP-stimulated Lipid Peroxides Formation in the Liver Homogenate

Additions (/ml reaction mixture)	Lipid peroxides (MDA nmol/g)	Significance
None	77.6±6.46[a]	—
NADPH (0.4 mM)+ADP (4 mM)	131.2±4.32	—
NADPH (0.4 mM)+oroxylin A (2.5×10^{-4} M)	119.6±5.27	b)
(1.0×10^{-4} M)	126.6±3.25	N.S.
NADPH (0.4 mM)+wogonin (2.5×10^{-4} M)	115.2±5.11	e)
(1.0×10^{-4} M)	114.9±6.90	c)
NADPH (0.4 mM)+skullcapflavone II		
(2.5×10^{-4} M)	113.2±6.57	d)
(1.0×10^{-4} M)	113.7±7.49	c)
NADPH (0.4 mM)+chrysin (2.5×10^{-4} M)	116.9±9.07	b)
(1.0×10^{-4} M)	132.9±4.10	N.S.
NADPH (0.4 mM)+baicalein (2.5×10^{-4} M)	95.9±4.86	f)
(1.0×10^{-4} M)	103.4±2.96	f)
NADPH (0.4 mM)+baicalin (2.5×10^{-4} M)	92.0±4.52	f)
(1.0×10^{-4} M)	107.3±4.22	f)
NADPH (0.4 mM)+wogonin-7-O-D-glucuronide		
(2.5×10^{-4} M)	100.2±2.14	f)
(1.0×10^{-4} M)	112.9±2.40	f)

a) The results are means±standard errors for 4—6 replicate experiments.
b) $p<0.05$, c) $p<0.02$, d) $p<0.01$, e) $p<0.005$, f) $p<0.001$, N.S.: not significant.

Table VI. Effects of Various Flavonoids on NADPH-ADP-stimulated Lipid Peroxide Formation in the Liver Homogenate

Additions (/ml reaction mixture)	Lipid peroxides (MDA nmol/g)	Significance
None	77.6±6.46[a]	—
NADPH (0.4 mM)+ADP (4 mM)	131.2±4.32	—
NADPH (0.4 mM)+quercetin (2.5×10^{-4} M)	92.4±0.47	d)
(1.0×10^{-4} M)	103.0±1.95	d)
NADPH (0.4 mM)+rutin (2.5×10^{-4} M)	86.9±4.75	d)
(1.0×10^{-4} M)	120.3±1.22	b)
NADPH (0.4 mM)+ hesperetin (2.5×10^{-4} M)	105.9±2.31	d)
(1.0×10^{-4} M)	116.7±4.33	b)
NADPH (0.4 mM)+hesperidin (2.5×10^{-4} M)	117.6±4.04	b)
(1.0×10^{-4} M)	134.4±5.59	N.S.
NADPH (0.4 mM)+narigenin (2.5×10^{-4} M)	116.7±2.58	c)
(1.0×10^{-4} M)	127.7±2.34	N.S.
NADPH (0.4 mM)+daidzein (2.5×10^{-4} M)	125.3±7.61	N.S.
(1.0×10^{-4} M)	135.2±3.24	N.S.
NADPH (0.4 mM)+daidzin (2.5×10^{-4} M)	126.1±4.40	N.S.
(1.0×10^{-4} M)	134.5±1.90	N.S.
NADPH (0.4 mM)+puerarin (2.5×10^{-4} M)	125.1±4.73	N.S.
(1.0×10^{-4} M)	136.4±2.49	N.S.
NADPH (0.4 mM)+formononetin (2.5×10^{-4} M)	118.2±6.35	b)
(1.0×10^{-4} M)	139.9±6.65	N.S.

a) The results are means ±standard errors for 4—6 replicate experiments.
b) $p<0.02$, c) $p<0.01$, d) $p<0.001$, N.S.: not significant.

Table VII. Effects of Wogonin, Baicalein and Baicalin on Serum GOT, GPT and Lipid Peroxides in Rats given Oxidized Rapessed-Corn-Soybean Oil Mixture orally

	GOT (Karmen unit) M±S.E.[c]	GPT (Karmen unit) M±S.E.[c]	Lipid peroxides (MDA nmol/ml) M±S.E.[c]
Normal	121.5±8.5	48.0±3.9	6.52±0.45
Control	166.6±11.8	121.0±9.7	7.93±0.89
Wogonin (100 mg/kg)	131.2±7.5[a]	123.5±17.0[N.S.]	7.47±0.90[N.S.]
Baicalein (100 mg/kg)	164.5±14.3[N.S.]	120.8±10.7[N.S.]	7.74±0.56[N.S.]
Baicalin (100 mg/kg)	131.1±5.6[a]	75.7±7.5[b]	7.35±0.98[N.S.]

a) $p<0.01$, b) $p<0.001$, N.S.: not significant.
c) The data for normal (11 rats), control and the 3 experimental groups (each of 10—12 rats) are means±standard errors.

At the concentration of 1.0×10^{-4} M, quercetin, rutin and hesperetin inhibited the NADPH-ADP-stimulated lipid peroxidation, while the other samples had no effect.

The Serum GOT, GPT and Lipid Peroxide Levels in Rats fed the Oxidized Rapeseed-Corn-Soybean Oil Mixture

It was shown that oral administration of lipid peroxide to rats induced liver injury and increased the serum GOT and GPT levels.[3] As shown in Table VII, wogonin reduced serum GOT level and baicalin decreased serum GOT and GPT levels in rats treated with the oxidized oil. Wogonin, baicalein and baicalin did not affect serum lipid peroxide levels in the rats.

Discussion

The present investigation demonstrated that extracts and flavone components of Scutellariae Radix (ogon in Japanese) affect lipid peroxidation in rats (*in vivo* and *in vitro*).

Yasuda and Fujita[13] reported that intraperitoneal administration of $FeCl_2$–ascorbic acid mixture caused elevation of the rat lipid peroxide level in the liver. Placer *et al.*[15] showed that Fe^{2+} caused fatty liver due to triglyceride accumulation in mice.

In the present experiments, intraperitoneal administration of $FeCl_2$–ascorbic acid–ADP mixture induced neither fatty liver nor hyperlipemia in rats. However, the oxidizing mixture elevated the lipid peroxide level in rat liver. Oral administration of the extracts (water, methanol and ethyl acetate) and flavone components (wogonin, baicalein and baicalin) of ogon as well as DL-α-tocopherol inhibited lipid peroxidation in the liver of rats treated with $FeCl_2$–ascorbic acid–ADP mixture.

In order to clarify the mechanism of action of ogon, *in vitro* experiments were undertaken. NADPH and ADP are known to stimulate lipid peroxidation in rat liver microsomes. It was also reported that Fe^{2+} and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria.[14]

The NADPH–ADP-stimulated lipid peroxidation in rat liver homogenate was inhibited by wogonin, skullcapflavone II, baicalein, baicalin and wogonin-7-O-D-glucuronide of ogon at the concentration of 1.0×10^{-4} M, but oroxylin A and chrysin had no effect. In addition to the flavonoid components of ogon, quercetin, rutin and hesperetin showed inhibitory actions at the concentration of 1.0×10^{-4} M.

Baicalein, baicalin and quercetin also inhibited the $FeCl_2$– ascorbic acid-stimulated lipid peroxidation in rat liver homogenate, while the other flavonoid components showed no inhibitory action at the concentration of 1.0×10^{-4} M.

The bud of *Sophora japonica* (which contains quercetin and rutin) has been used for the treatment of inflammatory and hypertensive disease. Recently, it was reported that rutin inhibited the oxidation of ascorbic acid, and caused an increase of permeability in blood vessels.[16]

It is well known that anti-inflammatory drugs such as aspirin and indomethacin inhibit the biosynthesis of prostaglandin.[17] Hassid and Levine[18] reported that quercetin inhibited prostaglandin E 9-ketoreductase. Iijima *et al.*[19] reported that baicalein, wogonin, quercetin and hesperetin inhibited 15-hydroxyprostaglandin dehydrogenase. Thus, the pharmacological effects of quercetin and rutin are similar to the effects of baicalein and baicalin.

Based on the *in vivo* and *in vitro* experimental results, it is suggested that the inhibitory actions of ogon on lipid peroxidation may be due to the effect of flavonoid components on microsomes and mitochondria in rat liver.

Okuda *et al.*[3] reported that oral administration of oxidized oil to rats induced liver injury with elevation of serum GOT and GPT levels. It was found that baicalin reduced the rise of serum GOT and GPT of rats fed the oxidized oil.

Two possible mechanisms can be suggested for the protective action of baicalin against liver injury. One is that baicalin inhibits further production of lipid peroxide in rats fed oxidized oil. The other is that baicalin inhibits the destructive action of lipid peroxide on liver cells. Further work is needed to identify the mechanism that is actually involved.

References and Notes

1) Part III: Y. Kimura, M. Kubo, T. Tani, S. Arichi, H. Ohminami, and H. Okuda, *Chem. Pharm. Bull.*, **29**, 2308 (1981).
2) M. Kubo, Y. Kimura, T. Odani, T. Tani, and K. Namba, *Planta Medica*, in press.
3) *a*) H. Okuda, *Igaku to Yakugaku*, **2**, 411 (1979); *b*) H. Ohminami, H. Okuda, T. Hamai, I. Kitagawa, M. Yoshikawa, S. Arichi, and T. Hayashi, *Eiyo to Shokuryo*, **34**, 105 (1981).
4) T. Shimizu, Y. Matsumura, and S. Morimoto, The Annual Meeting of the Japanese Society of Pharmacy, Tokyo, April 1980, p. 318.
5) H. Izumida, T. Yasugi, T. Shimizu, I. Kobayashi, H. Konno, G. Mizuno, T. Tochohara, S. Tabuchi, and H. Kishi, *The Journal of Japan Atherosclerosis Society*, **8**, 295 (1980).
6) W. Richmond, *Clin. Chem.*, **19**, 350 (1973).

7) G. Bucolo and H. David, *Clin. Chem.*, **19**, 475 (1973).
8) G.R. Bartlett, *J. Biol. Chem.*, **234**, 446 (1957).
9) B. Zak, *Am. J. Clin. Pathol.*, **27**, 583 (1957).
10) M.J. Fletcher, *Clin. Chim. Acta*, **22**, 393 (1968).
11) K. Yoden, Y. Ito, and T. Tabata, *Yakugaku Zasshi*, **100**, 553 (1980).
12) S. Reitman and S. Frankel, *Am. J. Clin. Pathol.*, **28**, 56 (1956).
13) M. Yasuda and T. Fujita, *Igaku no Ayumi*, **99**, 85 (1976).
14) *a*) P. Hochstein and L. Ernster, *Biochem. Biophys. Res. Commun.*, **12**, 388 (1963); *b*) A. Ottolenghi, *Arch. Biochem. Biophys.*, **79**, 355 (1959); *c*) E.H. Thile and J.W. Huff, *Arch. Biochem. Biophys.*, **88**, 203 (1960).
15) Z. Placer, J. Erazimová, R. Polendne, and M. Vidláková, *Experientia*, **27**, 1274 (1971).
16) *a*) R.L. Shanno, *Am. J. Med. Sci.*, **211**, 539 (1946); *b*) B. Mertin, *J. Biol. Chem.*, **171**, 507 (1947).
17) J.R. Vane, *Nature New Biol.*, **231**, 232 (1971).
18) A. Hassid and L. Levine, *Prostaglandins*, **13**, 503 (1977).
19) Y. Iijima, N. Kawakita, and M. Yamazaki, *Japanese Journal of Inflammation*, **1**, 101 (1980).