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Studies on the Activities of Tannins and Related Compounds from Medicinal Plants and Drugs. VI.[1] Inhibitory Effects of Caffeoylquinic Acids on Histamine Release from Rat Peritoneal Mast Cells

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The inhibitory effects of caffeoylquinic acids isolated from the leaves of *Artemisia* species on the histamine release from rat mast cells induced by compound 48/80, and on that induced by concanavalin A plus phosphatidylserine were determined. At the concentration of 2.5×10^{-5} M, caffeic acid, chlorogenic acid and methyl chlorogenate exhibited over 50% inhibition of the histamine secretion induced by compound 48/80 from mast cells. The inhibitory effects of caffeic acid and monocaffeoylquinic acids (chlorogenic acid and methyl chlorogenate) were higher than those of dicaffeoylquinic acids on the compound 48/80-induced histamine release from mast cells. On the other hand, caffeic acid, chlorogenic acid and 3,5-di-*O*-caffeoylquinic acid exhibited over 50% inhibition of the histamine secretion induced by concanavalin A plus phosphatidylserine from mast cells at the concentration of 10^{-5} M.

Keywords——tannin; histamine; *Artemisia montana*; *Artemisia princeps*; Compositae; mast cell; compound 48/80; concanavalin A; phosphatidylserine; structure–activity correlation

The dried herbs of *Artemisia* species have been used for the treatments of inflammation, hematemesis, hematuria, hemorrhoids and diarrhea in Chinese, Korean and Japanese traditional medicine. Okuda *et al.*[2] have reported the isolation of chlorogenic acid, methyl chlorogenate, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, and 3,4-di-*O*-caffeoylquinic acid from the leaves of *A. montana*. Among these compounds, which may be called "caffee tannins,"[3] the dicaffeoylquinic acids are main responsible for the tannic activities of these species, as determined[2] by the relative astringency (RA)[4] and relative activity on methylene blue (RMB)[4] methods. The compositions of the mixture of caffeoylquinic acids in these species were found to be markedly different from that in coffee, in that the amount of dicaffeoylquinic acids is larger than that of chlorogenic acid.[2]

In the previous paper,[1] we reported that caffeoylquinic acids and caffeic acid inhibited the lipid peroxidation induced by adenosine 5′-diphosphate (ADP) plus ascorbic acid and by ADP plus reduced nicotinamide adenine dinucleotide phosphate (NADPH) in rat liver mitochondria and microsomes. Among the above compounds, caffeic acid was reported by Murota *et al.*[5] to inhibit the formation of the 5-lipoxygenase product, 5-hydroxy-6,8,11,14-

eicosatetraenoic acid (5-HETE), in arachidonate metabolism in mastocytoma P-815 (MIC).

Thon *et al.*[6] reported that compound 48/80 induced histamine release from mast cells. The release of histamine from mast cells was also caused by the addition of concanavalin A (Con A), phosphatidylserine (PS) and calcium ion.[7] The release of histamine from isolated mast cells is a useful *in vitro* model for studying allergic and inflammatory diseases.[8] Most of the drugs used in the treatment of allergy, asthma and inflammation (for example isoprenaline, theophylline, steroids and indomethacin) are effective inhibitors of *in vitro* histamine release.[8]

In the present study, we attempted to examine the inhibitory effects of various caffeoylquinic acids and caffeic acid on the histamine release from rat peritoneal mast cells induced by compound 48/80 and by Con A plus PS. The correlation between the structures of caffeoylquinic acids and their inhibitory effects is discussed.

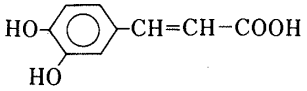
Materials and Methods

Materials——Five caffeoylquinic acids, chlorogenic acid, methyl chlorogenate, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid and 3,4-di-*O*-caffeoylquinic acid, isolated from the leaves of *A. montana* PAMPAN. by Okuda *et al.*,[2] were used in this study (Fig. 1). These compounds were dissolved or suspended in calcium-free Tyrode's solution (137 mM NaCl, 2.7 mM KCl, 0.4 mM $NaH_2PO_4 \cdot 2H_2O$, 1.0 mM $MgCl_2 \cdot 6H_2O$, 5.6 mM Glucose and 20 mM HEPES) (pH 7.4). Compound 48/80 and phosphatidylserine (from bovine brain) were purchased from Sigma Chemical Co. Concanavalin A was purchased from Wako Pure Chemical Industries Ltd. (Japan). Other reagents were of the highest grade available.

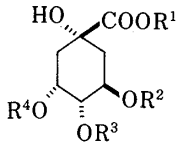
Animals——Adult male Wistar–King strain rats, weighing 250—300 g, were housed in a room at 25±1 °C with 60% relative humidity, and were given free access to food and water. The room was illuminated for 12 h a day starting at 7:00 a.m.

Preparation of Mast Cells——Mast cells were isolated from peritoneal cavity fluid of normal Wistar–King strain rats weighing 250—300 g by using a slight modification of the method described by Chakravarty and Zeuthen.[9] The isolated mast cells were pipetted out, washed three times with 5 ml of calcium-free Tyrode's solution (pH 7.4) and suspended in the same medium at 10^6 cells/ml. The cell suspensions contained 90% or more viable cells, as determined by the toluidine blue (0.1% in 50% ethanol) staining test of Bray and Van Arsdel.[10]

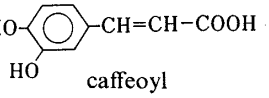
Measurement of Histamine Release from Isolated Mast Cells Induced by Compound 48/80 and by Concanavalin A (Con A) plus Phosphatidylserine (PS)——A mixture of mast cell suspension (0.5 ml, final cell number 5×10^5 cells/ml reaction mixture), calcium-free Tyrode's solution (pH 7.4) containing 7.5 μg/ml of compound 48/80 (0.1 ml, final concentration 0.75 μg/ml), 10 mM $CaCl_2 \cdot 2H_2O$ (0.1 ml, final concentration 1.0 mM) and the indicated amount of



caffeic acid



chlorogenic acid:
$R^1 = R^3 = R^4 = H$,

R^2 = 

caffeoyl

3,5-di-*O*-caffeoylquinic acid:
$R^1 = R^3 = H$, $R^2 = R^4$ = caffeoyl
4,5-di-*O*-caffeoylquinic acid:
$R^1 = R^2 = H$, $R^3 = R^4$ = caffeoyl
3,4-di-*O*-caffeoylquinic acid:
$R^1 = R^4 = H$, $R^2 = R^3$ = caffeoyl
methyl chlorogenate:
$R^1 = CH_3$, $R^3 = R^4 = H$, R^2 = caffeoyl

Fig. 1

various caffeoylquinic acids was incubated at 37 °C for 10 min in a final volume of 1 ml. The reaction was terminated by cooling the mixture in iced water to 0 °C. After centrifugation at $1630 \times g$ for 5 min at 0 °C, histamine in the supernatant fluid was assayed by the method of Shore *et al.*[11] using histamine HCl as a standard. The histamine release from mast cells induced by Con A and PS was determined as described above except that Tyrode's solution (pH 7.4) contained 75 μg/ml of Con A (0.1 ml, final concentration 7.5 μg/ml), 50 μg/ml of PS (0.1 ml, final concentration 5.0 μg/ml) and 10 mM $CaCl_2 \cdot 2H_2O$ (0.1 ml, final concentration 1.0 mM) instead of compound 48/80. The total content of histamine in intact cells which had been frozen and thawed five times. Total content of histamine in control intact cells was taken as 100%. Data are expressed as means ± standard errors of 4 replicate experiments. Statistical analysis was done by the use of Welch's *t*-test.

Results

Dose-Response Relation for Histamine Release from Mast Cells by Compound 48/80 or Con A plus PS

As shown in Fig. 2a, the levels of histamine release from mast cells were 6.6, 23.4, 32.1, 53.7, 64.8 and 67.4% per 5×10^5 cells at doses of 0, 0.1, 0.25, 0.5, 1.0 and 2.0 μg/ml of compound 48/80, respectively. On the other hand, as shown in Fib. 2b, the levels of histamine release from mast cells in the presence of PS (25 μg/ml) were 10.7, 24.9, 33.2, 44.8, 52.5 and 53.7% at doses of 0, 1, 2.5, 5, 10 and 25 μg/ml of Con A, respectively. Furthermore, the histamine release levels from mast cells in the presence of Con A (10 μg/ml) were 10.6, 37.7, 42.0, 52.6, 54.9 and 57.5% on addition of doses of 0, 1, 2.5, 5, 10 and 25 μg/ml of PS, respectively.

Effects of Caffeoylquinic Acids and Caffeic Acid on Compound 48/80-Induced Histamine Release from Mast Cells

The spontaneous release of histamine from mast cells was 1.17 ± 0.284 μg/5×10^5 cells. It increased to 6.17 ± 0.439 μg/5×10^5 cells in the presence of compound 48/80 (0.75 μg/ml). As shown in Table I, 4,5-di-*O*-caffeoylquinic acid and 3,4-di-*O*-caffeoylquinic acid exhibited over 90% inhibition of the histamine release from mast cells induced by compound 48/80 at the dose of 7.5×10^{-5} M. At the dose of 5×10^{-5} M, caffeic acid, chlorogenic acid, methyl

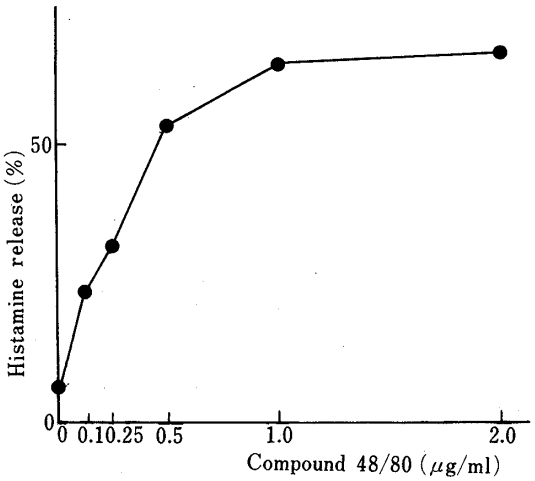


Fig. 2a. Dose-Response Curve for Histamine Release from Mast Cells by Compound 48/80

Mast cell obtained from adult Wistar-King strain rats (250—300 g, weight) were incubated in 1.0 ml of Tyrode's solution (pH 7.4) with calcium ion (1.0 mM) and various concentrations of compound 48/80 for 10 min at 37 °C.

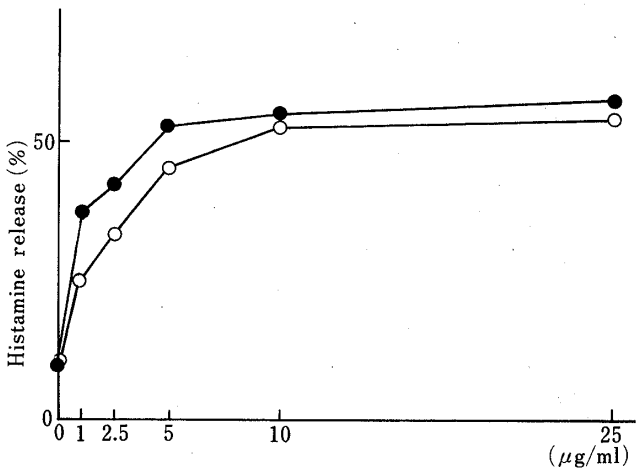


Fig. 2b. Dose Response Curve for Histamine Release from Mast Cells by Con A or Phosphatidylserine

○—○, dose-response curve for histamine release from mast cells by Con A on incubation for 10 min at 37 °C in the presence of phosphatidylserine (25 μg/ml) and 1.0 mM calcium ion. ●—●, dose-response curve for histamine release from mast cells by phosphatidylserine on incubation for 10 min at 37 °C in the presence of Con A (10 μg/ml) and 1.0 mM calcium ion.

TABLE I. Effects of Various Caffeoylquinic Acids on Compound 48/80-Induced Histamine Release from Rat Peritoneal Mast Cells

Additions (5×10^5 cells/ml reaction mixture)	Histamine ($\mu g/5 \times 10^5$ cells)	Histamine release (%)	(%) Inhibition	Significance
None	1.17 ± 0.284	8.80	—	—
Comp. 48/80 (0.75 μg)	6.17 ± 0.439	46.4	—	—
Comp. 48/80 + caffeic acid (1.0×10^{-4} M)	1.76 ± 0.234	10.4	88.2	b)
(7.5×10^{-5} M)	1.98 ± 0.215	14.9	83.8	b)
(5.0×10^{-5} M)	1.94 ± 0.193	14.6	84.6	b)
(2.5×10^{-5} M)	2.66 ± 0.064	20.0	70.2	b)
(1.0×10^{-5} M)	5.62 ± 0.478	42.3	11.0	N.S.
Comp. 48/80 + chlorogenic acid				
(1.0×10^{-4} M)	2.17 ± 0.148	16.3	80.0	b)
(7.5×10^{-5} M)	1.80 ± 0.119	13.5	87.4	b)
(5.0×10^{-5} M)	1.79 ± 0.241	13.5	87.6	b)
(2.5×10^{-5} M)	3.24 ± 0.151	24.4	58.6	b)
(1.0×10^{-5} M)	5.28 ± 0.646	39.7	17.8	N.S.
Comp. 48/80 + 3,5-di-*O*-caffeoylquinic acid				
(1.0×10^{-4} M)	1.46 ± 0.264	11.0	94.2	b)
(7.5×10^{-5} M)	1.83 ± 0.284	13.8	86.8	b)
(5.0×10^{-5} M)	2.19 ± 0.436	16.5	79.6	b)
(2.5×10^{-5} M)	4.67 ± 0.687	35.1	30.0	N.S.
(1.0×10^{-5} M)	5.72 ± 0.600	43.0	9.0	N.S.
Comp. 48/80 + 4,5-di-*O*-caffeoylquinic acid				
(1.0×10^{-4} M)	1.31 ± 0.255	9.85	97.2	b)
(7.5×10^{-5} M)	1.52 ± 0.347	11.4	93.0	b)
(5.0×10^{-5} M)	2.23 ± 0.276	16.8	78.8	b)
(2.5×10^{-5} M)	4.44 ± 0.592	33.4	34.6	N.S.
(1.0×10^{-5} M)	5.44 ± 0.734	40.9	14.6	N.S.
Comp. 48/80 + 3,4-di-*O*-caffeoylquinic acid				
(1.0×10^{-4} M)	1.31 ± 0.204	9.85	97.2	b)
(7.5×10^{-5} M)	1.21 ± 0.153	9.10	99.2	b)
(5.0×10^{-5} M)	1.40 ± 0.246	10.5	95.4	b)
(2.5×10^{-5} M)	3.98 ± 0.661	29.9	43.8	a)
(1.0×10^{-5} M)	5.18 ± 0.667	38.9	19.8	N.S.
Comp. 48/80 + methyl chlorogenate				
(1.0×10^{-4} M)	1.96 ± 0.211	14.7	84.2	b)
(7.5×10^{-5} M)	1.95 ± 0.163	14.7	84.4	b)
(5.0×10^{-5} M)	1.67 ± 0.141	12.6	90.0	b)
(2.5×10^{-5} M)	2.78 ± 0.067	20.9	67.8	b)
(1.0×10^{-5} M)	4.86 ± 0.433	36.5	26.2	N.S.
Total content of histamine in intact cells	13.3 ± 1.69	100.0	—	a)

Mast cells obtained from adult Wistar–King strain rats (250—300 g weight) were incubated in 1.0 ml of Tyrode's solution (pH 7.4) with the indicated combinations of compound 48/80 (0.75 μg/ml), calcium ion (1.0 mM) and the indicated amounts of various caffeoylquinic acids and related compounds for 10 min at 37 °C.

The results are expressed as means ± standard errors of 4 replicate experiments.

Total content of histamine in control intact cells is taken as 100%.

Significantly different from compound 48/80-induced histamine release from mast cells (Welch's *t*-test); *a*) $p < 0.05$, *b*) $p < 0.01$; N.S., not significant.

chlorogenate and 3,4-di-*O*-caffeoylquinic acid exhibited over 80% inhibition of the histamine release induced by compound 48/80. Caffeic acid, chlorogenic acid and methyl chlorogenate exhibited over 50% inhibition at the dose of 2.5×10^{-5} M. Two dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid) had no effect at the dose of 2.5×10^{-5} M or 1.0×10^{-5} M.

TABLE II. Effects of Various Caffeoylquinic Acids on Concanavalin A plus Phosphatidylserine-Induced Histamine Release from Rat Peritoneal Mast Cells

Additions (5×10^5 cells/ml reaction mixture)	Histamine ($\mu g/5 \times 10^5$ cells)	Histamine release (%)	(%) Inhibition	Significance
None	1.06 ± 0.049	7.74	—	—
Con A (7.5 μg) + PS (5 μg)	7.60 ± 0.191	55.5	—	—
Con A, PS + caffeic acid (1.0×10^{-4} M)	1.04 ± 0.142	7.59	>100.0	*b)*
(7.5×10^{-5} M)	0.955 ± 0.050	6.97	>100.0	*b)*
(5.0×10^{-5} M)	1.13 ± 0.056	8.25	98.9	*b)*
(2.5×10^{-5} M)	1.60 ± 0.086	11.7	91.7	*b)*
(1.0×10^{-5} M)	4.29 ± 0.340	31.3	50.6	*b)*
Con A, PS + chlorogenic acid				
(1.0×10^{-4} M)	2.03 ± 0.080	14.8	85.2	*b)*
(7.5×10^{-5} M)	1.80 ± 0.180	13.1	88.7	*b)*
(5.0×10^{-5} M)	1.87 ± 0.158	13.6	87.6	*b)*
(2.5×10^{-5} M)	2.68 ± 0.369	19.6	75.2	*b)*
(1.0×10^{-5} M)	3.96 ± 0.288	28.9	55.7	*b)*
Con A, PS + 3,5-di-*O*-caffeoylquinic acid				
(1.0×10^{-4} M)	2.03 ± 0.122	14.8	85.2	*b)*
(7.5×10^{-5} M)	1.88 ± 0.122	13.7	87.5	*b)*
(5.0×10^{-5} M)	2.11 ± 0.229	15.4	83.0	*b)*
(2.5×10^{-5} M)	2.64 ± 0.289	19.3	75.8	*b)*
(1.0×10^{-5} M)	3.93 ± 0.803	28.7	56.1	*a)*
Con A, PS + 4,5-di-*O*-caffeoylquinic acid				
(1.0×10^{-4} M)	1.83 ± 0.149	13.4	88.2	*b)*
(7.5×10^{-5} M)	1.61 ± 0.056	11.8	91.6	*b)*
(5.0×10^{-5} M)	2.38 ± 0.508	17.4	79.8	*b)*
(2.5×10^{-5} M)	3.18 ± 0.415	23.2	67.6	*b)*
(1.0×10^{-5} M)	4.98 ± 0.559	36.4	40.1	*a)*
Con A, PS + 3,4-di-*O*-caffeoylquinic acid				
(1.0×10^{-4} M)	1.70 ± 0.080	12.4	90.2	*b)*
(7.5×10^{-5} M)	1.75 ± 0.061	12.8	89.4	*b)*
(5.0×10^{-5} M)	2.65 ± 0.666	19.3	75.7	*b)*
(2.5×10^{-5} M)	3.82 ± 0.355	27.9	57.8	*b)*
(1.0×10^{-5} M)	5.06 ± 0.552	36.9	38.8	*a)*
Con A, PS + methyl chlorogenate				
(1.0×10^{-4} M)	1.51 ± 0.065	11.0	93.1	*b)*
(7.5×10^{-5} M)	1.71 ± 0.126	12.5	90.1	*b)*
(5.0×10^{-5} M)	1.94 ± 0.073	14.2	86.5	*b)*
(2.5×10^{-5} M)	2.70 ± 0.466	19.7	74.9	*b)*
(1.0×10^{-5} M)	4.38 ± 0.799	32.0	49.2	*a)*
Total content of histamine in intact cells	13.7 ± 0.355	100.0	—	—

Mast cells obtained from adult Wistar–King strain rats (250—300 g weight) were incubated in 1.0 ml of Tyrode's solution (pH 7.4) with the indicated combinations of concanavalin A (Con A, 7.5 μg/ml), phosphatidylserine (PS, 5 μg/ml), calcium ion (1.0 mM) and the indicated amounts of various caffeoylquinic acids and related compounds for 10 min at 37 °C.

The results are expressed as means ± standard errors of 4 replicate experiments.

Total content of histamine in control intact cells is taken as 100%.

Significantly different from concanavalin A plus phosphatidylserine-induced histamine release from mast cells (Welch's *t*-test); *a)* $p<0.05$, *b)* $p<0.01$.

Effects of Caffeoylquinic Acids and Caffeic Acid on Con A plus PS-Induced Histamine Release from Mast Cells

The spontaneous release of histamine from mast cells was 1.06 ± 0.049 $\mu g/5 \times 10^5$ cells. Upon stimulation by Con A (7.5 μg/ml), PS (5 μg/ml) and calcium ion (1.0 mM), it increased to

7.60 ± 0.191 μg/5×10^5 cells. As shown in Table II, caffeic acid exhibited over 90% inhibition of the histamine release from mast cells induced by Con A plus PS at the dose of 2.5×10^{-5} M. At the dose of 1.0×10^{-5} M, caffeic acid, chlorogenic acid and 3,5-di-*O*-caffeoylquinic acid exhibited over 50% inhibition. 4,5-Di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and methyl chlorogenate showed 40.1, 38.8 and 49.2% inhibitions of the Con A plus PS-induced histamine release from mast cells at the dose of 1.0×10^{-5} M, respectively.

Discussion

It is well known that a primary of allergic reaction and inflammatory reaction is the release of histamine from the tissues. Histamine release-preventing drugs are mainly used for the treatments of allergic and inflammatory diseases. It is well known that compound 48/80 causes histamine release from mast cells by degranulation.[6] The action of compound 48/80 (which does not act through an immunological mechanism) does not require the presence of extracellular calcium ion, and is not enhanced by the addition of phosphatidylserine.[7a] On the other hand, the mechanism of Con A-induced histamine secretion from mast cells is dependent on the calcium concentration of the medium and the extent of Con A-induced histamine secretion is enhanced by the addition of phosphatidylserine in the presence of calcium ion.[7b,c] Furthermore, it was reported that phosphatidylserine selectively enhances the histamine release from mast cells induced by Ig E-dependent agents.[12,13]

The present investigations showed that various caffeoylquinic acids isolated from the leaves of *Artemisia* species affect the histamine release from mast cells induced by compound 48/80 or by Con A plus phosphatidylserine. It was found that caffeic acid, chlorogenic acid and methyl chlorogenate showed over 50% inhibition of the compound 48/80-induced histamine release from mast cells at the dose of 2.5×10^{-5} M. However, dicaffeoylquinic acids such as 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid had no effect at the dose of 2.5×10^{-5} M. Among dicaffeoylquinic acids, 3,4-di-*O*-caffeoylquinic acid showed weak inhibition at the dose of 2.5×10^{-5} M. It is noteworthy that the inhibitory effects on the compound 48/80-induced histamine release of the small-molecular polyphenols such as caffeic acid and monocaffeoylquinic acids (chlorogenic acid and methyl chlorogenate) are greater than the inhibitory effects of dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid and 3,4-di-*O*-caffeoylquinic acid). These results indicate that the inhibitory actions of caffeic acid and caffeoylquinic acids on compound 48/80-induced histamine release from mast cells may require the two phenolic hydroxyl groups in the molecules of the above compounds.

On the other hand, it was found that caffeic acid, chlorogenic acid and 3,5-di-*O*-caffeoylquinic acid showed over 50% inhibition of the Con A plus phosphatidylserine-induced histamine release from mast cells. It was also observed that the inhibitory effects of caffeic acid or monocaffeoylquinic acids were stronger than those of the dicaffeoylquinic acids. Therefore, the two phenolic hydroxyl groups in the molecule appear to be important in this case as well. Furthermore, the order of the strength of the inhibitory effects on the Con A plus phosphatidylserine-induced histamine release among dicaffeoylquinic acids with the same skeleton and different locations of the caffeoyl groups (3,5-di-*O*-caffeoylquinic acid > 4,5-di-*O*-caffeoylquinic acid > 3,4-di-*O*-caffeoylquinic acid) is different from that observed for the compound 48/80-induced histamine release (3,4-di-*O*-caffeoylquinic acid > 4,5-di-*O*-caffeoylquinic acid > 3,5-di-*O*-caffeoylquinic acid). The order of the strength of tannic activities of dicaffeoylquinic acids (RA and RMB) was 3,5-di-*O*-caffeoylquinic acid (RA 0.18; RMB 0.25) > 4,5-di-*O*-caffeoylquinic acid (RA 0.11; RMB 0.15) > 3,4-di-*O*-caffeoylquinic acid (RA 0.09; RMB 0.25). Therefore, the strengths of the inhibitory actions of dicaffeoyl quinic acids seems to be unrelated to the levels of the tannic activities. In general, the

inhibitory effects of caffeoylquinic acids and related compounds on the Con A plus phosphatidylserine-induced histamine release in the cells are stronger than those on the compound 48/80-induced histamine release.

In the previous paper,[1] we reported that the inhibitory effects of dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid) on the lipid peroxidation induced by ADP plus NADPH in liver microsomes and on that induced by ADP plus ascorbic acid in liver mitochondria were higher than those of monocaffeoylquinic acids (chlorogenic acid and methyl chlorogenate) or caffeic acid. The order of the strength of the inhibitory effects on the above lipid peroxidation does not seem to be the same as that observed for the compound 48/80- or Con A plus phosphatidylserine-induced histamine release, or the tannic acitivities, and also does not seem to be related to the molecular weight.

In the previous paper,[1] it was reported that caffeoylquinic acids and caffeic acid have no effect on cyclooxygenase and 12-lipoxygenase activity in arachidonate metabolism in platelets. Experiments are in progress to study the effects of these caffeoylquinic acids on 5-lipoxygenase activity in arachidonate metabolism in rat polymorphonuclear leukocytes. It seems likely that the above-mentioned six compounds may be effective as anti-allergic or anti-inflammatory drugs.

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