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Anti-allergic and Anti-inflammatory Actions of *Cimicifuga heracleifolia*: Partial Purification of Active Components

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Abstract—Anti-allergic and anti-inflammatory actions of the water extract from *Cimicifuga heracleifolia* were evaluated in mice and rats. Several criteria were employed to assess the anti-allergic and anti-inflammatory actions of *Cimicifuga heracleifolia*, such as hyaluronidase activity, mediators-induced vascular permeability changes, 48 hour homologous passive cutaneous anaphylaxis (PCA) histamine release from mast cells, and the carrageenan-induced rat paw edema. To further characterize the active components, the water extract was either extracted with organic solvent or fractionated according to molecular weight, and each fraction was tested for some of anti-allergic parameters. Hyaluronidase activities, both in activating and in activated states, were significantly inhibited by the water extract of *Cimicifuga heracleifolia* and by some of its subfractions, molecular weight less than 1,000. The water extracts (50~400 mg/kg) significantly inhibited 48 hr homologous PCA and vascular permeability changes induced by chemical mediators (histamine, serotonin, and leukotriene C_4) in mice. In the case of histamine-induced vascular permeability changes, more extensive studies were conducted; water extract was either fractionated according to molecular weight or extracted with butanol. Anti-histamine actions were observed only from the water layer, and these active components were of the molecular weight less than 1,000. These anti-allergic actions were observed mainly from mice than from rats. On the other hand, anti-inflammatory actions of the water extract from *Cimicifuga heracleifolia* were significant in rats.

Keywords □ *Cimicifuga heracleifolia*, allergy, inflammation, 48-hr-homologous passive cutaneous anaphylaxis, chemical mediators.

Allergy, an immediate hypersensitivity reaction, is initiated with the binding of allergen-induced immunoglobulin E (IgE) on high affinity IgE receptors (FcεRI) (Kinet *et al.*, 1987; Ra *et al.*, 1989; Shimizu *et al.*, 1988). On re-exposure to allergen, cross linkage is made between IgEs bound to the FcεRI receptors, leading to the release of various mediators (Dahlen *et al.*, 1983; Stenson *et al.*, 1983). Allergy is characterized by increase in vascular permeability, vasodilation, and subsequent local inflammation. Allergy and inflammation have common mediators, and it is hard to discuss separately even though the latter might occur in the process of the former.

We reported that water extracts from some of natural products were expected to have anti-allergic activities (Choi *et al.*, 1992). In that screening tests, water extract from *Cimicifuga heracleifolia* was particularly effective as an anti-allergic agent. Thereafter, we extensively investigated anti-allergic and anti-inflammatory actions of the water extract from *Cimicifuga heracleifolia* focusing on the identification of active components.

In this paper, the anti-allergic and anti-inflammatory actions of *Cimicifuga heracleifolia* will be discussed in the light of the physicochemical characteristics of active components and their pharmacological actions.

Materials and Methods

Materials

Male ICR mice weighing 20~35 g, and male Sprague-Dawley rats weighing 180~250 g were used for experiments. Animals were kept at 22±2℃ on a 12 hour light and 12 hour dark schedule, and were freely supplied with food and water. Hyaluronidase type IV-

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S from bovine testis, hyaluronic acid potassium salt from roster comb, histamine, 5-hydroxytryptamine (serotonin), leukotriene C_4, compound 48/80, and carrageenan were obtained from Sigma Chemical Co.. DNP-BSA, DNP-specific antiserum in mice, and DNP-specific mIgE in rat, were kindly provided by Gifu Pharmaceu. Univ., Japan.

Extraction and Fractionation of Water Extract from *Cimicifugae heracleifolia*

Well dried roots of *Cimicifuga heracleifolia* were extracted with hot distilled water for 3 hours twice, and then concentrated by rotary vacuum evaporator. The water extract was filtered through 0.22 μm membrane, and fractionated according to molecular weight; fr. I (MW 1,000~10,000), fr. II (MW 10,000~20,000), and fr. III (MW larger than 20,000) by passing through ultrafiltration membranes (Amicon). The filtrate having molecular weight less than 1,000 was further divided into six fractions by sephadex G-15 gel filtration. These six fractions were designated as A to F according to eluted order. Water extract from *Cimicifuga heracleifolia* was also extracted with butanol three times, and each layer was named water layer and butanol layer, respectively.

Hyaluronidase Activity Assay *in vitro*

Hyaluronidase activity was measured according to the method of Leu *et al.* (1990).

Forty Eight Hour Homologous PCA in Mice

DNP-specific antiserum (48 hr homologous PCA titer=1 : 10 was diluted 4 times in saline and intradermally injected into ears (10 μl each) under ether anesthesia. After 48 hrs, mice were challenged with intravenous injection of evans blue saline solution (50 mg/kg) containing DNP-BSA, as an antigen (10 mg/kg). Thirty minutes after the challenge, mice were sacrificed and extravasated dye was quantified from ears (Katayama *et al.*, 1978). The water extract of *Cimicifuga heracleifolia* was dissolved in saline and intraperitoneally administrated 1 hour prior to challenge with antigen.

Measurement of Vascular Permeability Changes Caused by Mediators in Mice

Mediator was injected into both sides of ears under ether anesthesia followed by intravenous injection of evans blue saline solution. After 30 minutes, mice were sacrificed and extravasated dye was measured from ears.

Forty Eight Hour Homologous PCA Reaction and Mediators-induced Vascular Permeability in Rats

The DNP-specific mIgE (48 hr homologous PCA titer=2^7) was 100 times diluted in saline, and intradermally injected into backs of normal rats (0.1 m*l*). After 48 hrs, mediators were intradermally injected at each specific point in the back, and then followed by intravenous injection of evans blue saline solution (20 mg/kg) containing DNP-BSA (4 mg/kg), as an antigen. Thirty minutes after the injection of antigen, the animals were sacrificed and the amount of the dye leaked into the skin was determined (Katayama *et al.*, 1978). Drugs to be tested were intraperitoneally administrated 1 hour prior to challenge with antigen.

Measurement of Histamine Release from Peritoneal Mast Cells in Rats

Peritoneal mast cells was prepared in suspension (Foreman *et al.*, 1972), and were incubated for 10 min with test drugs. DNP-BSA was added as an antigen, then incubated for 10 min. The histamine release was stopped by placing the tubes into the ice bath. Histamine in the supernatant was measured by spectrophotofluorometric method (May *et al.*, 1970).

Carrageenan-induced Rat Paw Edema

Edema was induced by injection of 0.1 m*l* of 1% carrageenan suspension. Paw edema was measured for 8 hours after carrageenan treatment by phlethysmometer (UGO BASILE, Italy).

Statistics

Student's t-tests were used for the statistical treatments.

Results and Discussion

Effects of *Cimicifugae heracleifolia* on Hyaluronidase Activities

Hyaluronidase is one of the mucopolysaccharide-splitting enzymes and it increases the permeability of the vascular systems. Effect on hyaluronidase activity has been used as a parameter for screening anti-allergic and anti-inflammatory agents (Sakamoto *et al.*, 1980; Leu *et al.*, 1990). Generally hyaluronidase exists in an inactive form, but can be activated by various ions. Hyaluronidase can be inhibited both in activating and activated states.

As shown in Table I, the activities of hyaluronidase in activating and activated state were significantly inhibited by water extract from *Cimicifuga heracleifolia* and by its subfractions D, E, and F in a dose-dependent manner. These results suggested the possibility of *Cimicifuga heracleifolia* as an anti-allergic and anti-inflammatory drug.

Effects of *Cimicifugae heracleifolia* on 48 hr Homologous PCA, Skin Reactions, and Histamine Release

PCA utilizes the skin vascular permeability changes in the post-capillary venules followed by antigen and

Table I. Effects of *Cimicifuga heracleifolia* on activities of hyaluronidase in activating and activated state *in vitro*

Drugs	Dose (mg/ml)	Inhibition (%) activating	activated
Water Extract	50	91.2***	97.2***
	10	90.5***	81.2***
	5	86.1***	17.0***
	2.5	32.0***	
fr. I	5	84.2***	10.7**
	2.5	53.5***	
	1	10.1***	
fr. II	5	83.6***	18.3**
	2.5	78.7***	
	1	35.4***	
f. III	5	86.0***	
	2.5	91.7***	26.9
	1	84.1***	
A	5	6.9	0.0
B	5	8.0*	0.0
C	5	53.7***	0.0
	2.5	34.2***	
	1	21.7**	
D	5	95.4***	84.2***
	2.5	94.2***	63.0***
	1	93.7***	25.0***
E	5	91.3***	12.2***
	2.5	85.7***	
	1	45.1***	
F	5	87.2***	30.6***
	2.5	79.1***	27.3***
	1	41.6***	13.5***

Activating state means test solutions are first added followed by activator solutions, and in the reverse order for the activated states. Percent of inhibition represents the mean value of three measurements. The absorbance value of the blank group (hyaluronidase not added) was subtracted from the control and each *Cimicifuga heracleifolia* group, and the percent of inhibition was calculated according to the formula shown below. For the designations of each fraction, please refer to the method part.
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

$$\text{Inhibition } (\%)=\frac{A_{control}-A_{sample}}{A_{control}}\times 100$$

antibody reaction. This local anaphylaxis reaction shares most of aspects with systemic anaphylaxis (Ovary *et al*, 1958a, 1958b), but is easier to study. Allergic mediators, such as histamine and serotonin, play major roles on increment of vascular permeabilities (Chand *et al*, 1985; Takashima *et al*., 1979).

The water extract of *Cimicifuga heracleifolia* inhibited 48 hr homologous PCA in mice (Table II) to a comparable extent to that of ketotifen, an anti-allergic drugs being in use (Martin *et al*., 1981). Smaller but significant effects were also observed in rat at the dose of 400 mg/kg (Table VI).

For skin reactions, effects of the water extract from

Table II. Effects of *Cimicifuga heracleifolia* on 48-hr homologous PCA in the mouse ear

Drugs	Dose (mg/kg)	N	Amount of dye (μg/a pair of ears)	Inhibition (%)
Control		8	12.6± 0.92	
Ketotifen	10	5	3.4± 0.49***	73.0
	1	5	6.0± 0.60***	52.2
Cimicifuga heracleifolia				
	200	5	3.5± 0.47***	72.3
	100	7	6.0± 0.50**	52.0
	50	4	8.5± 0.86*	32.1

The water extract from *Cimicifuga heracleifolia* was intraperitoneally injected 1 hr prior to challenge with antigen. Ketotifen (Martin *et al*, 1981), an anti-allergic drug, was used as a positive control. The amount of dye is shown as the mean± S. E., and N represents the number of animals for each experimental group. The amount of dye is calculated by subtracting the blank value (saline treated).
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Cimicifuga heracleifolia on vascular permeability changes induced by various mediators, such as, histamine, serotonin, LTC_4, and compound 48/80 (a mediator releaser), were tested in rats and mice. In mice, the water extract from *Cimicifuga heracleifolia* significantly inhibited the vascular permeability changes induced by histamine (Table III), LTC_4 (Table IV), and serotonin (Table V) in a dose dependent manner between 25 to 400 mg/kg. Because of the importance in allergic reactions, more extensive studies were conducted for histamine-induced allergic reactions. The water extract was further fractionated according to molecular weight and each fraction was tested for histamine-induced vascular permeability changes. The most profound effects were observed at the doses between 50~100 mg/kg of fraction F in a dose-dependent manner (Table III). To further characterize the active component, the water extract of *Cimicifuga heracleifolia* was extracted with butanol, and each layer was tested for histamine-induced skin reactions. At the dose of 200 mg/kg. The water layer significantly inhibited the histamine-induced skin reactions ($p<0.05$), but butanol layer did not (data not shown). These results suggested that active compounds of *Cimicifuga heracleifolia*, as far as concerned with histamine-induced skin reactions, are highly water soluble and have the molecular weight less than 1,000.

Curiously enough, in rats, effects of *Cimicifuga heracleifolia* on PCA was significant but smaller than those in mice at the same dose, and effects on skin reactions was not significant even at the dose of 400 mg/kg (Table VI). Histamine released from rat peritoneal mast cells was not inhibited by the water extract from *Cimi-*

Table III. Effects of *Cimicifuga heracleifolia* on histamine-induced vascular permeability changes in the mouse ear

Test agents	Dose (mg/kg)	N	Amount of dye (μg/a pair of ears)	Inhibition (%)
Control		12	13.4± 0.57	
Cimicifuga	200	5	4.0± 0.35***	70.1
heracleifolia	100	5	5.9± 0.41***	56.0
	50	5	9.8± 0.45	26.9
A	100	6	9.1± 0.91*	32.1
B	100	6	8.6± 0.78**	35.8
C	100	4	11.6± 1.13	13.4
	100	4	11.8± 1.32	11.9
E	100	6	8.5± 0.56***	36.6
	50	5	10.6± 0.67*	20.9
F	100	6	5.2± 0.56***	61.2
	50	5	5.1± 0.52***	62.3
	20	5	8.5± 0.53***	36.6

The water extract from *Cimicifuga heracleifolia* and its subfractions were intraperitoneally injected 1 hr prior to challenge with 10 μl of histamine (10^{-4} g/ml). The amount of dye is calculated by subtracting the blank value (saline treated).
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Table IV. Effects of *Cimicifuga heracleifolia* on LTC_4-induced vascular permeability changes in the mouse ear

	Dose (mg/kg)	N	Amount of dye (μg/a pair of ears)	Inhibition (%)
Control		6	8.7± 0.63	
Cimicifuga				
heracleifolia	400	4	3.8± 1.13*	56.3
	200	5	4.3± 0.61**	50.7
	100	5	4.6± 0.31**	46.9
	50	4	5.3± 0.43*	39.1

The water extract from *Cimicifuga heracleifolia* was intraperitoneally injected 1 hr prior to challenge with 10 μl of LTC_4 (10^{-5} g/ml). The amount of dye is calculated by subtracting the blank value (saline treated).
*: $p<0.05$, **: $p<0.01$.

cifuga heracleifolia at the concentration of 1 mg/ml. Meanwhile, Azelastine, an anti-allergic drug (Katayama *et al.*, 1981), used as a positive control, inhibited the histamine release about 60% at the concentration of 50 μM.

Water Extract from *Cimicifuga heracleifolia* Inhibits Carrageenan-Induced Rat Paw Edema

Carrageenan-induced rat paw edema is a typical model for testing anti-inflammatory actions. Inflammatory processes induced by carrageenan are usually described by three phases; histamine and serotonin work in initial 90 min, kinin in an intermediate phase, followed by prostaglandins acting from 2 hr after carrageenan treatment. In the present experiments, paw edema induced by carrageenan gradually increased following

Table V. Effects of *Cimicifuga heracleifolia* on serotonin induced vascular permeability changes in the mouse ear

Drug	Dose (mg/kg)	N	Amount of dye (μg/a pair of ears)	Inhibition (%)
Control		8	12.9± 0.83	
Ketotifen	10	5	8.0± 1.01**	38.1
	5	4	9.2± 1.13*	28.9
	1	5	11.7± 0.90	9.5
Cimicifuga	400	5	4.6± 0.95***	64.4
heracleifolia	200	4	4.6± 0.46***	64.3
	100	5	6.2± 0.54***	52.5
	50	5	7.7± 0.94**	40.3
	25	5	8.0± 0.78**	37.9

The water extract from *Cimicifuga heracleifolia* was intraperitonelly administered 1 hr prior to challenge with 10 μl of serotonin (5×10^{-6} g/ml). The amount of dye is calculated by subtracting the blank value (saline treated).
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

Table VI. Effects of *Cimicifuga heracleifolia* on PCA and skin reactions in rat dorsal skin

	Amount of dye (μg/a spot)			
	PCA	histamine	serotonin	compound 48/80
Control	99.7± 6.65	20.9± 4.01	20.9± 3.17	31.6± 2.43
Cimicifuga				
heracleifolia	68.7± 8.85*	16.6± 2.43	22.6± 4.94	28.1± 1.51

The water extract from *cimicifugae heracleifolia* was intraperitoneally injected 1 hr prior to challenge at dose of 400 mg/kg. Each amount of dye represents the mean± S. E. of values from 5 animals. The amount of dye is calculated by subtracting the blank value (saline treated).
*: $p<0.05$.

the injection, reaching a plateau between 4 to 5 hours, then slowly subsided. Carrageenan-induced rat paw edema was remarkably inhibited by the water extract from *Cimicifuga heracleifolia* at dose of 400 mg/kg (Fig. 1).

***Cimicifuga heracleifolia* as an Anti-allergic and Anti-inflammatory drug**

It was reported that the ether and butanol fractions from *Cimicifugae Rhizoma* showed some anti-inflammatory, antipyretic, and analgesic effects. Also water extract seemed to inhibit edema (Shibata *et al.*, 1975, 1977). However, anti-allergic actions of *Cimicifuga heracleifolia* have not been reported yet.

With these reasons, we were more interested in the anti-allergic actions of *Cimicifuga heracleifolia* than its anti-inflammatory actions. Even though it is not easy to discuss the anti-inflammatory and anti-allergic actions separately, anti-inflammatory actions (judged by inhibitory effects on edema) were in good agreement

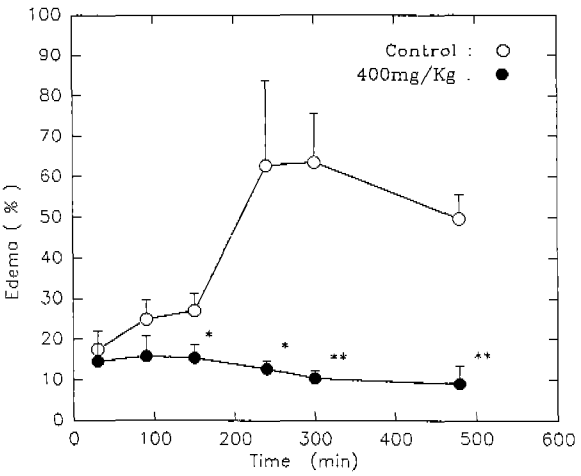

Fig. 1. Effects of *Cimicifuga heracleifolia* on carrageenan-induced rat paw edema. Each point represents the mean+S.E from 5 experimental animals. *: $p<0.05$, **: $p<0.01$.

with other studies. Anti-allergic actions of *Cimicifuga heracleifolia*, however, were observed mainly in the mice but were trivial in rats, furthermore, histamine release was not affected at all.

Nevertheless, it seems to be very clear that active components for anti-allergic and anti-inflammatory actions are distinct, because only anti-inflammatory actions were predominantly observed but not anti-allergic actions in rats. These facts indirectly suggested that anti-allergic actions in mice were genuine, in other words, anti-allergic actions were produced by distinct components from those produced anti-inflammatory actions in rats. Differences in physiological characteristics such as drug metabolism could be speculated for these differences in anti-allergic actions between rats and mice, but we do not have any clear answer for that at this point.

Based on these results, we concluded that *Cimicifuga heracleifolia* showed some possibilities as an anti-inflammatory and anti-allergic drug, and its active component is highly water soluble with molecular weight less than 1,000 as far as concerned with anti-histamine actions.

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