Caffeic Acid Inhibits Compound 48/80-Induced Allergic Symptoms in Mice

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The effect of caffeic acid on scratching behavior and vascular permeability changes induced by compound 48/80 in ICR mice were investigated. An oral dose of 500 mg/kg of caffeic acid significantly inhibited scratching behavior and vascular permeability induced by compound 48/80. The inhibitory effects of daily administration of lower doses of caffeic acid, 100 and 200 mg/kg, were also investigated; and it was found that 200 mg/kg significantly inhibited compound 48/80-induced scratching behavior after the second week of consecutive administration. The effect of 200 mg/kg of caffeic acid on scratching behavior was observed up to the third week of the treatment. The decrease in histamine content induced by compound 48/80 was significantly antagonized by 200 mg/kg. The findings suggest that caffeic acid may be effective for treating itch and edema in allergic dermatitis.

Key words compound 48/80; scratching behavior; vascular permeability; caffeic acid

Caffeic acid (3,4-dihydroxycinnamic acid), one of the moieties of chlorogenic acid, is a phenolic compound widely distributed in plant materials other than coffee, including vegetables and fruits. Some biological effects have been attributed to caffeic acid. Huang et al. have reported the inhibitory effects of chlorogenic and caffeic acid on 12-O-tetradecanoylphorbol-13-acetate-induced tumors in mice. Furthermore, the anti-inflammatory properties of caffeic acid and its derivatives have been proven in vivo and in vitro experiments. In addition, the antioxidant potential with regard to the radical scavenging activity of caffeic acid has also been investigated and demonstrated by Bors et al. Atopic dermatitis is a condition manifesting eczema, serous papules, scaling and crust and in severe cases, erosion of the affected skin. Itch, a sensation causing the urge to scratch, is the most significant outcome of atopic dermatitis, where insistent scratching further aggravates the skin symptoms of the disease. In allergic conditions, histamine contained in skin mast cells, acts as one of the main mediators of itchiness; and it has been proven that antiallergic drugs such as azelastine, oxatomide, terfenadine, epinastine and astemizole exert a relatively potent inhibitory effect on itch induced by compound 48/80 in animal models.

It is now accepted that drugs having an antioxidant effect have an antiallergic effect as well, and that superoxide generation plays an important role in mast cell activation. The release of chemical mediators from tissue mast cells has been centrally implicated in a diversity of allergic and inflammatory disorders. Therefore, in the present study, the effect of caffeic acid on allergic symptoms such as skin scratching behavior and vascular hyperpermeability induced by compound 48/80 were investigated in ICR mice.

MATERIALS AND METHODS

Animals Female ICR mice (6—10 weeks old) were obtained from Japan SLC, Inc. The animals were housed in an air-conditioned room maintained at 24±2 °C with a relative humidity of 55±15%. They were given standard laboratory rodent chow (Oriental Yeast, Tokyo) and water ad libitum. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Drugs The reagents used in the experiments were obtained from the sources shown in parentheses: caffeic acid (Sigma, St. Louis, MO, U.S.A.), compound 48/80 (Sigma), tranilast (Kissei, Matsumoto, Japan). Caffeic acid and tranilast were suspended in 5% arabic gum, while compound 48/80 was dissolved in physiological saline.

Scratching Behavior Caffeic acid was administered to the animals orally, 1 h before starting the experiment. Later, 10 μg/0.02 ml compound 48/80 was intradermally injected into the rostral part of the shaved back of the mice. Immediately after the injection, the animals were put into an observation cage (11 cm in diameter, MicroAct, Neuroscience, Tokyo, Japan), which automatically and objectively detects and evaluates mouse-scratching behavior. Scratching behavior was measured for 1 h.

Skin Histamine Content The mice were sacrificed under ether anesthesia and the rostral part of the back was excised, washed with ice-cold saline, and weighed. The samples were minced and then homogenized in 0.4 n perchloric acid using a Polytron (Kinematica, Lucerne, Switzerland) on ice. After centrifugation at 1500×g for 10 min at 4 °C, the histamine content of the supernatant was measured using an autoanalyzer (Bran Luebbe, Osaka, Japan) and a fluorometric detector (Model U-2000, Hitachi, Tokyo, Japan) as described previously. Compound 48/80 was administered intradermally into the rostral part of the back of the animals 1 h before starting the experiment.

Vascular Permeability of the Skin After intradermal injection of 0.5 μg/0.02 ml compound 48/80 into the rostral part of the back, 2% Evans blue solution was intravenously injected into each animal. The animals were sacrificed 30 min later, and the diameters of the ‘bluing’ area at the compound 48/80 injection site were measured. Caffeic acid
was orally administered 1 h before the experiment.

**Statistical Analysis** All values are expressed as the mean ± standard error of the mean (S.E.M.). Statistical evaluation of the results was performed by one-way analysis of variance (ANOVA) followed by Dunnett’s test. A probability value less than 0.05 was considered statistically significant.

**RESULTS**

**Compound 48/80-Induced Scratching Behavior** Figure 1 shows the effect of a single administration of caffeic acid on compound 48/80-induced scratching behavior. Though caffeic acid showed no inhibitory effect at doses of 100 and 200 mg/kg, 500 mg/kg significantly inhibited compound 48/80-induced scratching behavior. Repeated administration of a lower dose of caffeic acid (200 mg/kg) for 3 weeks resulted in a gradual inhibition of the induced itch symptoms, which was significant after the second week of treatment. Tranilast at a dose of 300 mg/kg significantly diminished the number of scratches induced by compound 48/80 from the first day of treatment (Fig. 2).

**Changes in Dorsal Skin Histamine Content** The results are shown in Table 1. After the third week of treatment, following an intradermal injection of compound 48/80, the histamine remaining in the skin of the mice was determined. Consecutive administration of 200 mg/kg of caffeic acid and 300 mg/kg of tranilast significantly inhibited *in vivo* histamine release induced by compound 48/80.

**Vascular Permeability Changes in the Skin** Caffeic acid at a dose of 200 mg/kg caused no significant inhibition of the increased skin vascular permeability induced by compound 48/80. However, at 500 mg/kg, it significantly inhibited induced vascular permeability (Table 2).

**DISCUSSION**

Itch in humans and rodents can be triggered through activation of *H*₁ receptors by histamine release from skin mast cells upon mast cell degranulation. Among mast cell degranulators, compound 48/80 has been reported as a potent mast cell histamine releaser in several *in vitro* as well as *in vivo* experiments.

### Table 1. Effect of Caffeic Acid on Skin Histamine Changes Induced by Compound 48/80 in ICR Mice

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>Dose (mg/kg)</th>
<th>Histamine (µg/g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>—</td>
<td>33.0 ± 3.4</td>
</tr>
<tr>
<td>Control (compound 48/80)</td>
<td>10 µg/site</td>
<td>10.7 ± 0.6</td>
</tr>
<tr>
<td>Compound 48/80 + caffeic acid</td>
<td>100 mg/kg</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>14.0 ± 0.8*</td>
</tr>
<tr>
<td>Compound 48/80 + tranilast</td>
<td>300 mg/kg</td>
<td>15.3 ± 0.8**</td>
</tr>
</tbody>
</table>

Caffeic acid was given orally 1 h before compound 48/80 (10 µg/site) injection. Later, the mice were sacrificed and histamine contents measured. Each value represents the mean ± S.E.M. (*n* = 5—10). *, **Significantly different from the control group at *p* < 0.05 and *p* < 0.01, respectively.

### Table 2. Effect of Caffeic Acid on Vascular Permeability Induced by Compound 48/80 in ICR Mice

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (µg/site)</th>
<th>Bluing area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (compound 48/80)</td>
<td>0.5</td>
<td>52.6 ± 3.6</td>
</tr>
<tr>
<td>Compound 48/80 + caffeic acid</td>
<td>200 mg/kg</td>
<td>44.1 ± 5.4</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg</td>
<td>35.7 ± 4.2**</td>
</tr>
</tbody>
</table>

Caffeic acid was given orally 1 h before compound 48/80 (0.5 µg/site) injection. Each value represents the mean ± S.E.M. (*n* = 5—10). **Significantly different from the control group at *p* < 0.01.
In this study, compound 48/80-induced scratching behavior in ICR mice was significantly inhibited by single and consecutive pretreatment with different doses of caffeic acid. Single administration of 500 mg/kg of caffeic acid significantly decreased the number of scratches induced by compound 48/80, however, taking into account that caffeic acid behaves as a stimulant of the central nervous system, lower doses were tested to avoid undesirable effects. Caffeic acid is a metabolite of chlorogenic acid and the major source of chlorogenic acid is coffee. Daily intake of caffeic acid in coffee drinkers is 0.1—1 g, while coffee abstainers ingest less than 100 mg per day of caffeic acid derived from fruits and vegetables. Therefore, we investigated the effects of consecutive administration of lower doses of caffeic acid, and found that 100 mg/kg slightly inhibited the elicited scratching behavior, although not significantly, after the third week of treatment. Furthermore, 200 mg/kg of caffeic acid administered daily for 3 weeks significantly inhibited compound 48/80-induced scratching behavior after the second week of treatment. Interestingly, the group of mice treated with 100 or 200 mg/kg of caffeic acid for 4 weeks showed an increase in scratching behavior during the fourth week (data not shown), an increase that although not equal in intensity to the control group, could be considered as a reverse reaction. On the other hand, tranilast, an antiallergic drug that inhibits the release of chemical mediators from mast cells, clearly blocked the itch response induced by compound 48/80 from the first day until the fourth week of treatment. Regarding coffee and allergy, there is controversy as to whether or not coffee ingestion triggers allergic symptoms. Uenishi et al. have reported that exclusion of offending foods such as coffee, chocolate, cheese and yogurt from the daily diet, brings about a progressive improvement of allergic diseases in sensitive patients; while Pagano et al. and Schwartz et al. argued that coffee and caffeine reduce asthma symptoms. The present results appear to support the findings of Pagano et al. and Schwartz et al.

The specific mechanism of action of caffeic acid in inhibiting itch remains unclear. However, based on the evidence that caffeic acid significantly inhibited compound 48/80-induced histamine release from skin mast cells, we assumed that the inhibitory effects of scratching behavior may be attributable to the inhibition of mast cell degranulation. Recent reports provide evidence that the oxidative state and free radical generation play an important role in mast cell activation and degranulation. The antioxidant effect and free radical generation play an important role in mast cell activation and degranulation. When the effect of caffeic acid on vascular permeability induced by compound 48/80 was investigated, it was found that caffeic acid significantly inhibited leakage induced by the above mentioned compound. Thus, we assumed that this effect resulted from the inhibition of mast cell degranulation.

From the above findings, we can conclude that the inhibitory effects of caffeic acid on scratching behavior and vascular permeability may be due mainly to an inhibition of histamine release from mast cells, probably through prevention of superoxide anion generation.

**REFERENCES**