

Note

Anti-inflammatory and Anti-allergic Actions by Oral Administration of a Perilla Leaf Extract in Mice

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The anti-inflammatory and anti-allergic activity of perilla leaf extract was investigated. The oral administration of perilla leaf extract to mice inhibited two types of acute inflammatory models, arachidonic acid-induced ear edema and 12-*o*-tetradecanoylphorbol-13-acetate-induced ear edema. Oral administration of perilla leaf extract also inhibited the contact dermatitis model, oxazolone-induced ear edema, by affecting sensitization.

Key words: *Perilla frutescens*; anti-inflammation; anti-allergy

The leaves of *Perilla frutescens* are used as a garnish with raw fish in Japan. It is believed that the original purpose for its use was not only its flavor but also its antidotal property to prevent food poisoning.¹⁾ Dried red perilla leaves are also used as 'soyou' in Chinese herbal medicine and it is one of the components of 'saiboku-to', which is used to treat bronchial asthma.

We have reported that the overproduction of tumor necrosis factor- α (TNF- α) was suppressed by orally administering a perilla leaf extract (PLE).²⁾ TNF- α is a pleiotropic cytokine, originally derived from its antitumor activity,³⁾ and is believed to play a role in many immunologic and inflammatory reactions.^{4,5)} It is also reported that blocking TNF- α can inhibit allergic skin dermatitis. For example, treatment with the anti-TNF- α antibody can inhibit contact hypersensitivity,⁶⁾ arthus reaction⁷⁾ and type I allergy.⁸⁾ It is also reported that PLE inhibits IgE production⁹⁾ and the acidic glycoprotein from *Perilla frutescens* prevents histamine release from mast cells *in vitro*.¹⁰⁾ We therefore believe that the drinking of PLE can improve inflammatory and allergic conditions; however, there is no report that directly confirms such an effect. We examined therefore whether oral administration can also inhibit inflammatory and allergic ear edema.

We first investigated the anti-inflammatory effect of PLE on AA-induced ear edema and TPA-induced ear edema. These models have been accepted as useful phlogistic tools for investigating new anti-inflammatory drugs and it is believed that the symptoms of these conditions resemble human dermatitis.¹¹⁾

Dried leaves of a green type of perilla (*Perilla frutescens* (L.) Britton var. *acuta* Kudo forma *viridis* Makino; 5 g) were soaked in 5 ml of distilled water for 1 h and then homogenized for 10 min with Polytron equipment (Kinematika, Switzerland) at a power setting of 5. The homogenate was filtered through nylon mesh and centrifuged at 7,000 g for 10 min at 4°C. The resulting supernatant was passed through a membrane filter with a 0.45 μ m pore size (Millipore, Tokyo, Japan) and used for the test sample. Male ICR mice (4 weeks old; 18–20 g) were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were orally administered the test sample and 18 h later this was done again. Three hours after the second administration, 250 μ g of AA diluted with 20 μ l of acetone was spread on the right ear of each mouse. Two hours later, ear swelling was measured by weighing pieces of the ears obtained with a 5 mm diameter punch. The experiments were done under the control of the guideline for Animal Experiments in the Law (No. 105) and Notification (No. 6) of the government.

The weight of a cut piece of untreated murine ear was 6.8 ± 0.3 mg and it increased to 8.8 ± 0.5 mg 2 h after topical application of AA to that ear (Fig. 1A). When PLE (400 μ l) was orally administered to mice 3 h before topical application, the weight significantly decreased to 7.9 ± 0.3 mg ($P < 0.001$).

Oral administration of PLE (400 μ l) also inhibited TPA-induced ear edema (Fig. 1B). This inflammatory model is different from that of AA-induced ear edema on chemical mediator involved in. For induction of irritant dermatitis, 200 μ g of TPA in 20 μ l of acetone was spread on the right ear of mice which

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Abbreviations: AA, arachidonic acid; DDW, Deionized distilled water; LC, Langerhans cells; LT, leukotriene; PG, prostaglandin; PLE, perilla leaf extract; TNF- α , tumor necrosis factor- α ; TPA, 12-*o*-tetradecanoylphorbol-13-acetate; Tx, thromboxane

had been orally administered the sample 3 and 21 h earlier. Four hours later, ear swelling was measured. The weight of a cut piece of ear was 13.3 ± 1.2 mg 4 h after topical application of TPA; when PLE (400 μ l) was orally administered to mice 3 h before topical application, it significantly decreased to 11.6 ± 1.0 mg ($P < 0.01$). The flare and itching was also inhibited by oral administration of PLE on both inflammatory models.

TPA and AA provide skin inflammation models suitable to evaluate both topical and systemic anti-inflammatory agents. TPA-induced ear edema is associated with a marked influx of neutrophils and predominant formation of leukotriene B₄ (LTB₄) along with significant changes in levels of thromboxane B₂ (TxB₂).¹²⁾ AA also produced an ear edema response with a rapid onset that was associated with marked increases in levels of prostaglandins (PGs), TxB₂ and LTB₄, with smaller increases in levels of

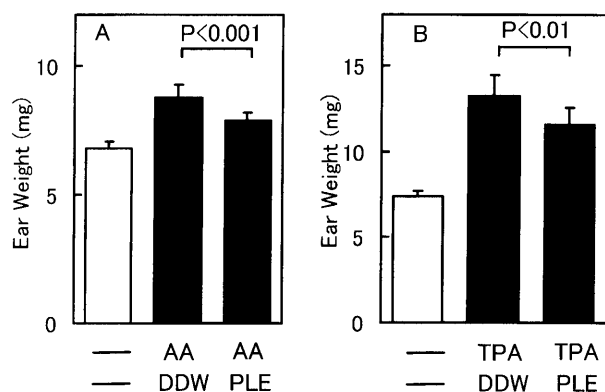


Fig. 1. Inhibition of AA-Induced Ear Edema and TPA-Induced Ear Edema by Orally Administered PLE.

Male ICR mice ($n = 10$) were orally administered with 400 μ l of distilled water or PLE and 18 h later this was orally administered again. Three hours later, 250 μ g of AA (A) or 200 ng of TPA (B) was spread on both sides of the right ear of each mouse (closed bars). Control group were not treated (open bar). Two hours (A) or 4 hours (B) later, ear swelling was measured by weighing the ear pieces obtained with a 5-mm diameter punch. The data are the mean $\pm$ SD. The statistical difference was analyzed by Student's *t*-test.

LTC₄. However, it is suggested that the mediator biosynthetic profile is different between the AA and TPA models, because AA-induced edema is more sensitive to lipoxygenase inhibitors than to cyclooxygenase inhibitors. The TPA-induced inflammatory response is, therefore, thought to be mainly mediated by LTB₄ and the arachidonate model to be regulated by both LTs and PGs. It is suggested that PLE may inhibit TNF- α production while also inhibiting PG and LT metabolism.

Next, we investigated the effect on oxazolone-induced ear edema which is a type IV allergy. The abdominal region of the animals was shaved with an electric clipper and were sensitized by spreading 500 μ g of oxazolone dissolved with 100 μ l of ethanol. After 7 days, the mice were challenged by spreading 100 μ g of oxazolone dissolved with 20 μ l of acetone on ear and ear swelling was determined after 24 hours. We were interested in whether the timing of PLE administration should be changed because of differing conditions between sensitization and challenge. When PLE (400 μ l) was orally administered to mice 3 h before sensitization, the ear edema was significantly decreased (Table 1: Group 2); however, the edema was not affected when administration was done 24 h before sensitization (Table 1: Group 1). When PLE was successively administered between sensitization and challenge, the ear edema was slightly decreased (Table 1: Group 3), but when administered just before challenge, the edema was not affected (data not shown).

Topical application of oxazolone to murine ears induces contact hypersensitivity which is mediated by LTs, PGs, histamine, inflammatory cytokines, and IgE. Our previous study and the anti-inflammatory experiments presented here allow us to conjecture that inhibition of the production of LTs, PGs, TNF- α , and other related cytokines may contribute to the anti-allergic activity. The kinetic study showed that oral administration of PLE inhibits sensitization but not challenge. During sensitization, Langerhans cells (LC) migrate from the epidermis and subsequently accumulate as dendritic cells in draining lymph

Table 1. Inhibition of Oxazolone-Induced Ear Edema by Orally Administered PLE

Group	Sample	Administration (h)*	Weight (mg)	Δ weight (mg)	% of Control	P**
1	—	ND****	7.7 ± 0.3	0 ± 0.3	0 ± 9	—
	DDW	—24	10.9 ± 2.1	3.2 ± 2.1	100 ± 66	NS***
	PLE	—24	11.0 ± 2.2	3.3 ± 2.2	103 ± 69	
2	—	ND****	7.6 ± 0.5	0 ± 0.5	0 ± 24	—
	DDW	—3	9.7 ± 1.8	2.1 ± 1.8	100 ± 86	<0.05
	PLE	—3	8.0 ± 0.9	0.4 ± 0.9	19 ± 42	
3	—	ND****	6.7 ± 0.7	0 ± 0.7	0 ± 27	—
	DDW	+24, 48, 72, 96	9.3 ± 1.8	2.6 ± 1.8	100 ± 69	<0.10
	PLE	+24, 48, 72, 96	8.1 ± 0.6	1.4 ± 0.6	54 ± 23	

Male ICR mice ($n = 10$) were sensitized by applications of oxazolone (500 μ g) to the abdominal region and after 7 days were challenged by spreading oxazolone (100 μ g) on the ear. The control group was not treated. After 24 h, ear swelling was measured by weighing the cut pieces of the ears. Samples (400 μ l) were orally administered at indicated times. The data are the mean $\pm$ SD. *Oral administration period of sample before or after sensitization, **The statistical difference was analyzed by Student's *t*-test. ***NS means not significant. ****ND means not done.

nodes, and it is also reported that the stimulation of LC migration during skin sensitization is dependent upon signals provided by the epidermal TNF- α .¹³⁾ Oral administration of PLE may cause impaired LC migration resulting from the inhibition of TNF- α production.

PLE can improve human atopic dermatitis with a record of about 70%.¹⁴⁾ We believe that the data provided here supports the availability for human inflammatory disease, and that the TNF- α inhibition may contribute to these effects.

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