In Vitro and *in Vivo* Antiallergic Effect of the Fructus of *Evodia rutaecarpa* and Its Constituents

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The unripe fruit of *Evodia rutaecarpa* (Juss) Benth (ER, Family Rutaceae) has been used frequently as a traditional medicine against inflammatory diseases in Korea, China and Japan. To evaluate antiallergic effect of ER, we isolated its main constituents, evodiamine and rutaecarpine, and evaluated *in vivo* their inhibitory effects against passive cutaenous anaphylaxis (PCA) reaction induced by IgE-antigen complex and scratching behaviors by compound 48/80. ER and its constituents, evodiamine and rutaecarpine, potently inhibited PCA reaction and scratching behaviors in mice, although ER weakly inhibited scratching behaviors. Evodiamine and rutaecarpine inhibited TNF-α and IL-4 protein expression in RBL-2H3 cells induced by IgE–antigen complex, although these did not inhibit degranulation of RBL-2H3 cells induced by IgE–antigen complex and rat peritoneal mast cells induced by compound 48/80. These findings suggest that ER and its constituents, evodiamine and rutaecarpine, may be effective for IgE-induced allergic diseases such as atopic dermatitis and rhinitis.

Key words *Evodia rutaecarpa*; allergy; evodiamine; rutaecarpine; IgE

Allergic diseases, such as allergic rhinitis, atopic dermatitis, asthma and food allergy, are now rapidly increasing chronic health problem in most countries.[1)] Mast cells and basophils are well-known as a critical participant in various biologic processes of allergic diseases.[2—4)] These cells express on their surface membrane receptors with high affinity and specificity for IgE. The interaction of multivalent antigens with surface-bound IgE releases histamine, prostaglandins, leukotrienes and cytokines.[5,6)] These cytokines activate chemotaxis and phagocytosis of neutrophils and macrophages. Finally cytokine-induced reaction causes tissue inflammation. Therefore, antiallergic agents, such as anti-histamines, steroids and immunosuppressants, have been using against allergic diseases.[7—9)] However, improving these diseases is too difficult. Therefore, herbal medicines have been advanced for allergic diseases, and its effectiveness has received increasing attention.[10,11)]

The unripe fruit of *Evodia rutaecarpa* (Juss) Benth (ER, Family Rutaceae) have been used in Korea, Japan and China as a traditional herbal medicine. ER, which contains quninzoline alkaloids, such as ruatecarpine and evodiamine, as main components, is reported to have many biological properties including stimulation of vasodilation, positive cardiotonic effects, inhibition of pain, and inhibition of prostaglandin production.[12—15)] Nevertheless, its pharmacological studies mainly have been focused on anti-inflammatory activity.

Therefore, to evaluate its antiallergic activity, we isolated main components from the fruit of *Evodia rutaecarpa* and *in vivo* evaluated their inhibitory activities against scratching behaviors and anaphylaxis.

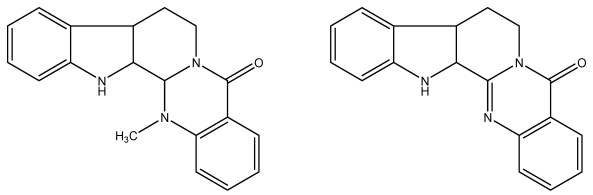
MATERIALS AND METHODS

Materials Dulbecco's modified Eagles medium (DMEM), fetal bovine serum, dinitrophenol-human serum albumin (DNP-HSA), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, cremophor EL, and compound 48/80 were purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.). RNeasy® Mininkit was purchased from Qiagen Co. (Cincinnati, Ohio, U.S.A.).

The unripe fructus of *Evodia rutaecarpa* was extracted with 80% ethanol and rutaecarpine (purity >95%) and evodiamine (purity >95%) (Fig. 1) were isolated from the fruits of *E. rutaecarpa* and structurally identified according to the previous reports.[11,12,16)]

Animals The male ICR mice (20—25 g) and male Sprague-Dawley rats were supplied from Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20—22 °C and 50±10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center, Seoul, Korea) and allowed water *ad libitum*. All procedures relating to animals and their care conformed to the international guidelines 'Principles of Laboratory Animals Care' (NIH publication no. 85-23, revised 1985).

Measurement of Passive Cutaneous Anaphylaxis (PCA) Reaction An IgE-dependent cutaneous reaction was measured according to the previous method of Choo *et al.*[17)] The male ICR mice were injected intradermally with 10 μg of anti-DNP IgE into each of two dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each mouse received an injection of 200 μl of 3% Evans blue PBS containing 200 μg of DNP-HSA *via* the tail vein. The test agents



Evodiamine Rutaecarpine

Fig. 1. Structures of Evodiamine and Rutaecarpine Isolated from the Rhizome of *Evodia rutaecarpa*

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(dissolved in 2% cremophor EL) were orally or intraperitoneally administered 1 h prior to DNP-HSA injection. Thirty min after DNP-HSA injection, the mice were sacrificed and their dorsal skins were removed for measurement of the pigment area. After extraction with 1 ml of KOH 1.0 N and 4 ml of a mixture of acetone and phosphoric acid 0.6 N (13 : 5), the amount of dye was determined colorimetrically (absorbance at 620 nm).

Scratching Behavioral Experiments Male BALB/c mice were put into acrylic cages (22×22×24 cm) for about 10 min for acclimation. The behavioral experiments were performed according to the method of Sugimoto *et al.*[18] The rostral part of the skin on the back of mice was clipped, and 50 μg/50 μl of compound 48/80 for each mouse was intradermally injected. The compound 48/80 was dissolved in saline and then used. Control mice received a saline injection in the place of the compound 48/80. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and, for the observation of scratching; their behaviors recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site by the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches for 1 s, and a series of these behaviors was counted as one incident of scratching for 60 min. The test agents (dissolved in 2% cremophor EL) were orally administered 1 h before the scratching agent.

Enzyme-Linked Immunosorbent Assay (ELISA) RBL-2H3 cells (5×10^5 cells) previously cultured in DMEM were treated with 0.5 μg/ml of mouse monoclonal IgE for sensitization of cells. The cells (1.8 ml) were exposed to 0.2 ml of test agents (dissolved in 0.5% dimethyl sulfoxide) for 4 h, followed by the treatment with 0.2 ml of DNP-HSA (1 μg/ml) for 40 min at 37 °C. The supernatant (50 μl) was transferred into 96-well ELISA plates and then IL-4 ad TNF-α concentrations were determined using commercial ELISA Kits (Pierce Biotechnology, Inc., Rockford, IL, U.S.A.).[19]

Assay of Degranulation of Mast Cells Stimulated by IgE–Antigen Complex The inhibitory activity of test agents against the release of β-hexosaminidase from RBL-2H3 cells and histamine of rat peritoneal mast cells was evaluated according to Choo *et al.*[17]

Statistical Analysis All the data were expressed as the mean±standard deviation, and statistical significance was analyzed by one-way ANOVA followed by Student–Newman–Keuls test.

RESULTS AND DISCUSSION

To evaluate antiallergic activity of ER, PCA reaction-inhibitory activity of ER and its main constituents, evodiamine and rutaecarpine, in mice was measured (Table 1). PCA reaction was induced by the injection of IgE and antigen (DNP-HSA) and test agents were administered orally or intraperitoneally 1 h prior to challenge with antigen. Treatment of IgE and antigen potently induced PCA reaction. However, oral administration of ER potently inhibited PCA reaction. Orally administered constituents isolated from ER showed the potent inhibition against PCA reaction. Orally and intraperitoneally administered constituents, evodiamine and rutaecarpine, exhibited the potent inhibition.

Table 1. Effect of ER and Its Constituents on Mouse Passive Cutaneous Anaphylaxis Reaction Induced by IgE–Antigen Complex

	Dose (mg/kg)	Administered route	Inhibition (%)[a]
ER[b]	100	*p.o.*	48±10
	250	*p.o.*	67±6
	5	i.p.	64±5
Evodiamine	5	*p.o*	22±9
	10	*p.o.*	64±20
	50	*p.o.*	81±22
	5	i.p.	64±5
Rutaecarpine	5	*p.o.*	27±11
	10	*p.o.*	87±22
	50	*p.o.*	98±2
Azelastine	5	i.p.	78±11
	10	*p.o.*	80±16
	50	*p.o.*	84±15

a) Amounts of extravasated Evan blue in the dorsal skin (1×1 cm) of control stimulated with IgE–antigen complex and vehicle-treated groups were 27±3 μg and 12±2 μg, respectively. *b*) ER, 80% ethanol extract of fructus of *Evodia rutaecarpa*. Values are expressed as means±S.D. (n=5).

Table 2. Effect of ER and Its Constituents on Scratching Behaviors in Mice Induced by Compound 48/80

Agent	Dose (mg/kg)	Inhibition (%)[a]
ER[b]	100	12±6
	250	19±12
Evodiamine	10	45±15
	50	85±5
Rutaecarpine	10	55±12
	50	92±8
Azelastine	10	89±11

a) Scratching behavior frequency numbers of normal control, which was treated with saline alone, and control group, which was treated with compound 48/80 and saline, for 1 h were 235±22 and 3±2, respectively. *b*) ER, 80% ethanol extract of fructus of *Evodia rutaecarpa*. Values are expressed as means±S.D. (n=5).

The inhibitory activity of ER and its constituents in compound 48/80-induced scratching behavior mice was also investigated (Table 2). The compound 48/80 significantly increased scratching behavior frequency. ER weakly inhibited scratching behaviors. However isolated constituents, evodiamine and rutaecarpine, potently inhibited the scratching behavior frequency and at a dose of 50 mg/kg inhibited the scratching behavior frequency by 85% and 92%, respectively.

To understand the antiscratching behavior and anti-PCA reaction activities, inhibitory effect of evodiamine and rutaecarpine on the degranulation of mast cells was investigated (Table 3). Evodiamine and rutaecarpine did not inhibit degranulation of RBL-2H3 cells induced by IgE–antigen complex and rat peritoneal mast cells induced by compound 48/80. Neither these constituents inhibited IgE production in U266 cells nor scavenged superoxide anions (data not shown). However, evodiamine and ruraecarpine inhibited protein expressions of proinflammatory cytokines, TNF-α and IL-4, in RBL-2H3 cells induced by IgE–antigen complex (Fig. 2).

Azelastine, a representative antiallergic drug,[20] is an H1-receptor antagonist and decreases mediator release from mast cells and basophils. Quercetin, a non-steroidal anti-inflammatory flavonoid, scavenges superoxide anion and inhibited

Table 3. Effect of ER and Its Components on the Degranulation of Compound 48/80-Induced Rat Peritoneal Mast Cells and IgE–Antigen Complex-Induced RBL-2H3 Cells

	IC_{50} (μM)[a]	
	Rat peritoneal mast cells	RBL-2H3 cells
ER[b]	>100 (2)	>100 (7)
Evodiamine	>100 (9)[c]	>100 (4)
Rutaecarpine	>100 (6)	>100 (9)
Azelastine	32	26

a) IC_{50} indicates 50% inhibitory concentration. *b*) Concentration of ER is μg/ml. *c*) Values in parenthesis indicate inhibition percents of evodiamine and rutaecarpine at a dose of 100 μM against mast cells and RBL-2H3 cells.

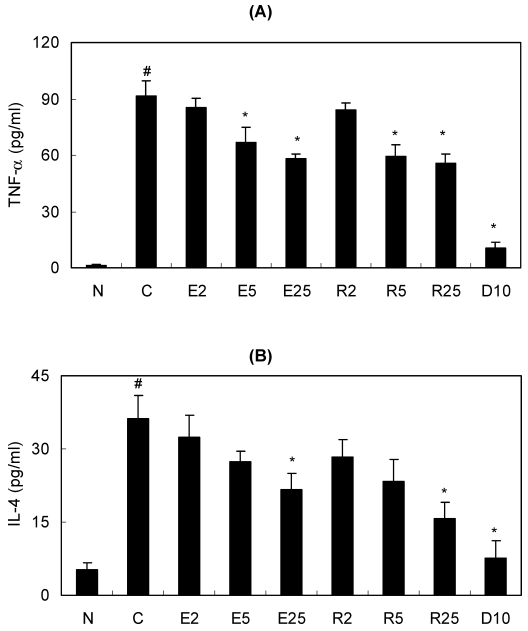

Fig. 2. Effect of Evodiamine and Ruatecarpine on Protein Expression of TNF-α and IL-4 in RBL-2H3 Cells Induced by IgE–Antigen Compex

RBL-2H3 cells (5×10^5 cells) were treated with 0.5 μg/ml of mouse monoclonal IgE, exposed to 2 ml of agents (N, normal; C, vehicle alone; E2, 2 μM evodiamine; E5, 5 μM rutaecarpine; R25 μM rutaecarpine; E2, 2 μM evodiamine; E5, 5 μM evodiamine; E25, evodiamine; E25, 25 μM evodiamine; R2, 2 μM rutaecanpine; R5, 5 μM rutaecanpine; R25, 25 μM rutaecanpine; D10, 10 μM dexamethasone) for 4 h, followed by the treatment with 0.2 ml of dinitrophenol-human serum albumin (DNP-HSA, 1 μg/ml) for 40 min at 37 °C and then ELISA for TNF-α (A) and IL-4 (B) was performed. Normal was treated with vehicle alone instead of agents and IgE-antigen. Values represent the mean±S.D. for duplicate experiments. # Significantly different from the normal control group (# $p<0.05$). * Significantly different from the control group (* $p<0.05$).

PCA reaction.[21] ER and its constituents evodiamine and rutaecarpine inhibited mouse PCA reaction induced by IgE–antigen complex. Evodiamine and rutaecarpine showed potent antiscratching behavioral effect, although ER weakly inhibited scratching behaviors induced by compound 48/80. The previous studies reported that evodiamine inhibited bovine guinea-pig cardiac anaphylaxis induced by bovine serum albumin and rutaecarpine inhibited the contraction of isolated rat aorta induced by histamine.[14,15] These reports suggested that PCA reaction-inhibitory activity of evodiamine and rutaecarpine may be due to their antihistamine actions. However, in the present study, evodiamine and rutaecarpine inhibited biosynthesis of TNF-α and IL-4 in RBL-2H3 cells induced by IgE. This finding suggests that ER and its constituents may regulate the biosynthesis of anaphylaxis-related cytokines in mast cells and basophils, which causes allergic diseases.

Based on these findings, we believe that ER and its constituents, evodiamine and rutaecarpine, can improve IgE-induced allergic diseases such as rhinitis and asthma, and may be effective for patients to exhibit side effects of steroids.

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