Extract of *Perilla frutescens* Enriched for Rosmarinic Acid, a Polyphenolic Phytochemical, Inhibits Seasonal Allergic Rhinoconjunctivitis in Humans

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Extract of *Perilla frutescens* enriched for rosmarinic acid, a polyphenolic phytochemical, suppresses allergic immunoglobulin responses and inflammation caused by polymorphonuclear leukocytes (PMNL) in mice. However, few placebo-controlled clinical trials have examined the efficacy and safety of polyphenolic phytochemicals for treatment of allergic inflammatory diseases in humans. The present study determined whether oral supplementation with rosmarinic acid is an effective intervention for patients with seasonal allergic rhinoconjunctivitis (SAR). In this 21-day, randomized, double-blind, age-matched, placebo-controlled parallel group study, patients with mild SAR were treated daily with extract of *Perilla frutescens* enriched for rosmarinic acid (200 mg [n = 10] or 50 mg [n = 9]) or placebo (n = 10). Patients recorded symptoms daily in a diary. Profiles of infiltrating cells and concentrations of eotaxin, IL-1β, IL-8, and histamine were measured in nasal lavage fluid. Serum IgE concentrations and routine blood tests were also examined. As compared with placebo supplementation, supplementation with extract of *Perilla frutescens* enriched for rosmarinic acid resulted in a significant increase in responder rates for itchy nose, watery eyes, itchy eyes, and total symptoms ($P < 0.05$). Active treatment significantly decreased the numbers of neutrophils and eosinophils in nasal lavage fluid ($P < 0.05$ vs. placebo). Patients reported no adverse events, and no significant abnormalities were detected in routine blood tests. In conclusion, extract of *Perilla frutescens* enriched for rosmarinic acid can be an effective intervention for mild SAR at least partly through inhibition of PMNL infiltration into the nostrils. Use of this alternative treatment for SAR might reduce treatment costs for allergic diseases. Exp Biol Med 229:247–254, 2004

Key words: rosmarinic acid; seasonal allergic rhinoconjunctivitis; polymorphonuclear leukocytes; alternative medicine; randomized, double-blind, age-matched, placebo-controlled parallel group study; extract of *Perilla frutescens*

During the latter half of the 20th century, the incidence of allergic rhinitis increased worldwide, leading to dramatic escalation in direct and indirect treatment costs (1). In 1996, direct and indirect costs for allergic rhinitis were estimated at $7 billion and $3 billion, respectively. Despite steady advances in conventional therapies for allergy symptoms, phytochemicals and herbal products have been widely used by consumers as alternatives to prescription drugs without definitive clinical evidence (2). In the United States, the prevalence of alternative therapy use for allergies rose from 8.7% in 1990 to 16.6% in 1997 (3).

Among a variety of phytochemicals, polyphenolic phytochemicals have been shown experimentally to inhibit inflammatory and vascular processes (4–7). Furthermore, a randomized, blinded, crossover investigation of the cocoa polyphenol, procyanidin, has shown that it can favorably alter eicosanoid (prostacyclin) synthesis in humans, providing a possible mechanism by which it could inhibit atherogenic disorders (8). In addition, another well-controlled clinical trial has demonstrated that a polyphenol-rich cocoa beverage suppresses ADP- and epinephrine-stimulated platelet activation (9). In contrast, few placebo-controlled clinical trials have examined the efficacy or safety of polyphenolic phytochemicals on allergic inflammatory diseases in humans.

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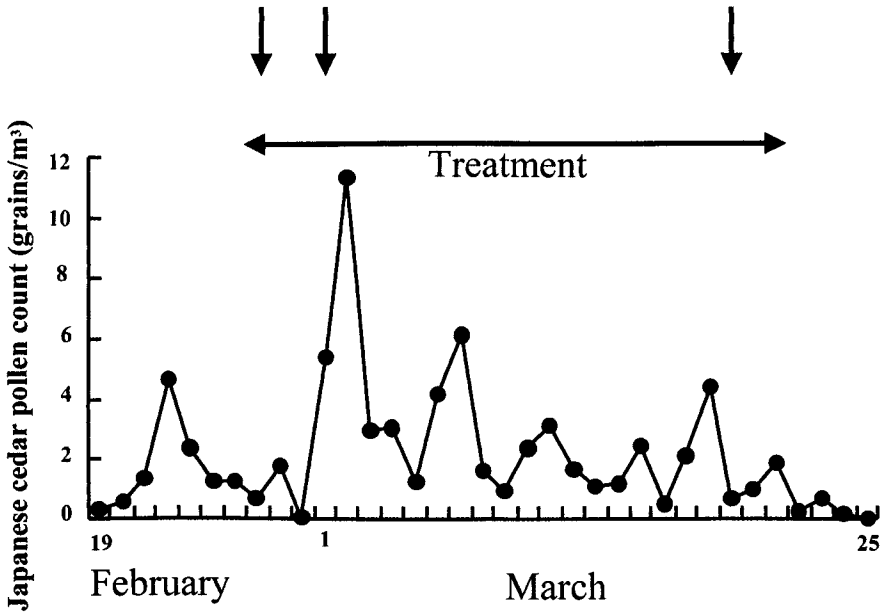

Figure 1. The pollen counts during the study period. At Days 0, 3, and 21 (arrows), daily symptoms, tablet intake, and adverse events were collected and confirmed, and nasal washes were performed. All patients were tested on the same day in the morning at the same location.

A randomized, double-blind, placebo-controlled study of grape seed extract has shown no significant difference between active and placebo groups in rhinitis quality of life assessments, symptom disease scores, or requirements for rescue antihistamine treatment (10).

Rosmarinic acid, a polyphenolic phytochemical, exists in a variety of medicinal species within the plant genus Lamiaceae, such as basil, sage, rosemary, mint, and *Perilla frutescens*, a popular garnish in Japan. Oral supplementation with rosmarinic acid in Perilla decoction reportedly suppresses allergic reaction in mice (11).

The present study was undertaken to investigate whether extract of *Perilla frutescens* enriched for rosmarinic acid is a beneficial intervention for patients with seasonal allergic rhinoconjunctivitis (SAR). Furthermore, we assessed its effects on mediator release and on polymorphonuclear leukocyte (PMNL; neutrophils and eosinophils) infiltration into nasal lavage fluid.

Materials and Methods

Subjects. We enrolled 30 patients aged 21 to 53 years with mild SAR to Japanese cedar (*Cryptomeria japonica*) pollen. All had a known medical history of allergic rhinoconjunctivitis only during the pollen season for at least the previous 2 years and had a positive serum test for Japanese cedar pollen specific for IgE of Class 2 or greater. Patients were excluded if they had received any drugs or had an active respiratory infection within the previous 3 weeks or during the study period. Exclusion criteria also included a history of drug allergies, presence of other medical conditions, an established history of wheezing or asthma, and use of immunotherapy or nasal surgery. The enrolled patients lived within 10 km of the same company and commuted there daily except on weekends. Participants were fully informed regarding the experimental procedures, and written consent was obtained. Safety was monitored by clinical history, physical examinations, and routine blood tests, including hepatic and renal function tests and complete blood counts.

Study Design. The study used a randomized, double-blind, age-matched, placebo-controlled parallel group design. The protocol was approved by the ethics committees of the Kyoto Prefectural University of Medicine. The study was carried out in accordance with the Declaration of Helsinki. The trial was carried out from late February to late March. During the study period, the mean concentration of Japanese cedar pollen was 2.48 million grains/m^3 (SD = 2.41 million; Fig. 1).

Ten patients each were randomized to the rosmarinic acid (200 mg) group, the rosmarinic acid (50) mg group, and to the placebo group. At Day 0, patients were instructed to record their daily symptoms and medication on a diary card. For 21 days, each patient took tablets daily after breakfast. Tablets were indistinguishable between groups and contained rosmarinic acid from *Perilla frutescens* extracts at a daily dose of 200, 50, or 0 mg. At Days 0, 3, and 21, daily symptoms, tablet intake, and adverse events were collected and confirmed, and nasal washes were performed. All patients were tested on the same day in the morning at the same location in the company. Cellular profiles and concentrations of eotaxin, IL-1β, IL-8, and histamine were measured in nasal lavage fluid. Routine laboratory examinations and specific IgE concentrations were measured in blood samples. At Day 21, patient evaluations of global symptoms were assessed using a self-report health-related quality of life questionnaire.

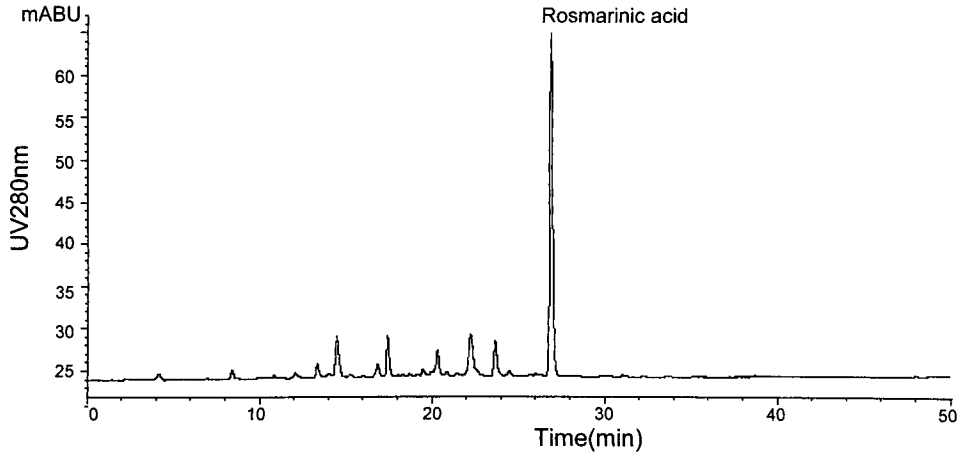

Figure 2. HPLC profile of the tablet containing the extract of *Perilla frutescens*. The concentration of polyphenolic substances including rosmarinic acid was determined by HPLC using a Develosil HG-5 column with solvents A (0.1% v/v trifluoroacetic acid [TFA] in distilled water) and B (0.1% v/v TFA in acetonitrile) under the following conditions: 10%–50% linear gradient of A in B; flow rate, 0.8 ml/min; detection, 280 nm.

Tablets. All test tablets were prepared by Meiji Seika Kaisha, Ltd. In brief, fresh Perilla leaves were extracted with 1.0% w/v citric acid at 90°C for 30 mins. The extract was filtered and freeze-dried. The concentration of polyphenolic substances including rosmarinic acid in this extract was determined by high performance liquid chromatography (HPLC) using a Develosil HG-5 column (Nomura Chemical Co., Ltd., Aichi, Japan) with solvents A (0.1% v/v trifluoroacetic acid [TFA] in distilled water) and B (0.1% v/v TFA in acetonitrile) under the following conditions: 10%–50% linear gradient of A in B; flow rate = 0.8 ml/min; and detection = 280 nm (Fig. 2). The concentration of rosmarinic acid was 20% w/w. The concentrations of luteolin, luteolin-7-O-glucoside, protocatechuic acid, and caffeic acid were below the detection limit. The test tablets were made with this Perilla extract, lactose, and cellulose powder. The placebo tablets were made in the same manner as the test tablets but without Perilla extract. Flavor and pigment were added to the placebo tablets in order to match those added to the test tablets.

Diary Card and Patient Evaluations of Global Symptoms. We used a self-report health-related quality of life questionnaire and a diary card on the basis of the previous reports (12, 13) with modification. Patients recorded their symptoms daily using a score from 0 to 3, where 0 = symptom not present; 1 = symptom present, no discomfort; 2 = some discomfort; and 3 = marked discomfort. This scoring system was used to assess sneezing, rhinorrhea, stuffy nose, itchy nose, watery eyes, itchy eyes, and total symptoms.

A self-report health-related quality of life questionnaire was used to measure patients' assessment of global symptoms. In the questionnaire, patients rated their relief from symptoms on a 3-point scale: no relief or worse (1), partial (2), or complete (3) relief from global symptoms.

Nasal Lavage. Nasal lavages were performed with isotonic sterile saline preheated to 37°C. Specifically, 3 ml of saline was instilled in each nostril. After 15 secs, the lavage fluid was collected. The average volume retrieved was 95% of the instillation volume (6.0 ml); the amounts did not differ by treatment. The lavage fluid was centrifuged at 300 *g* for 10 mins, and the total cell count was determined on a fresh fluid specimen. Differential cell counts were assessed on cytologic preparations stained with Diff-Quik (International Reagent, Kobe, Japan). A total of 500 cells per preparation were counted under oil immersion microscopy (14). The supernatants were stored at –80°C for the measurements of proinflammatory mediators.

Measurement of Proinflammatory Mediators. Eotaxin (Biosource, Camarillo, CA); IL-1β (Genzyme, Minneapolis, MN); IL-8 (Genzyme); and histamine (IBL, Hamburg, Germany) were measured in nasal lavage fluid supernatants using commercially available ELISA kits according to the manufacturer's instructions. Specific IgE concentrations were measured using Pharmacia CAP RAST (Uppsala, Sweden) according to the manufacturer's recommended protocol.

Routine Blood Tests. We estimated complete blood cell counts, hepatic and renal function, and total protein; performed proteinogram analysis; and measured concentrations of electrolytes, lipids, uric acid, and creatine phosphokinase in blood samples.

Statistical Methods. Data were analyzed using SPSS for Windows 7.5.1. (SPSS Japan, Inc., Tokyo, Japan). The responder rate was defined as an improvement of at least two points in the total symptom score and one point in each symptom score between baseline (Day 0) and Days 3 or 21. The chi-square test was used to evaluate differences in improvement between groups. An analysis of covariance (ANCOVA) was used for all other efficacy variables. The number of cells in total nasal lavage fluid was analyzed after log transformation.

Results

The participants in the three groups were well-matched in age, gender, and duration of SAR (Table 1). One patient

Table 1. Baseline Characteristics of Patients[a]

Group	Placebo	Rosmarinic acid 50 mg/day	Rosmarinic acid 200 mg/day
Men	5	5	6
Women	5	4	4
Age (years)	33.0 ± 9.3	32.2 ± 6.6	33.1 ± 6.3
Duration of illness (years)	12.9 ± 9.3	8.7 ± 7.8	6.5 ± 4.6

[a] The value of age and duration of illness represents mean and standard deviation.

in the rosmarinic acid (50 mg) group was excluded from the study because of an acute respiratory infection during the study period. Twenty-nine patients were finally enrolled in the study. The rosmarinic acid (200 mg) group consisted of 10 patients (six men and four women, mean age 33.1 ± 6.3 years); the rosmarinic acid (50 mg) group of nine patients (five men and four women, mean age 32.2 ± 6.6 years); and the placebo group of 10 patients (five men and five women, mean age 33.0 ± 9.3 years). There were no significant differences in baseline total symptom scores between the groups: the rosmarinic acid (200 mg) group, 2.3 ± 3.9; the rosmarinic acid (50 mg) group, 3.8 ± 3.2; and the placebo group, 3.9 ± 7.0. There were also no significant differences in the baseline symptom scores for sneezing, rhinorrhea, stuffy nose, itchy nose, watery eyes, and itchy eyes among the three groups. No rescue medication was required for any patient, and no patient reported any adverse event.

Changes in the symptom scores during the study period were not significantly different among the groups. Patients' evaluations of global symptoms using the health-related quality of life questionnaire, however, showed that only 30% of placebo-supplemented participants improved after treatment (Fig. 3). In contrast, 55.6% and 70% of the patients reported global symptom relief in the rosmarinic acid (50 and 200 mg) groups, respectively ($P = 0.05$; placebo vs. rosmarinic acid at a daily dose of 200 mg).

Compared with placebo supplementation, daily oral supplementation with the extract containing rosmarinic acid at its daily dose of 50 or 200 mg resulted in significant increases in responder rates that were based on symptom score reductions for itchy nose, watery eyes, and itchy eyes on Day 21 ($P < 0.05$; Table 2). The 50-mg dose significantly increased the responder rate on the total symptom score on Day 3 ($P < 0.05$ vs. placebo), and tended to increase it on Day 21. The responder rates for the total symptom score were also greater in the rosmarinic acid (200 mg) group than in the placebo group on Days 3 and 21; however, these increases did not achieve statistical significance.

On Day 3, the numbers of PMNL and neutrophils in nasal lavage fluid were significantly decreased by the extract at both doses of rosmarinic acid ($P < 0.05$ vs. placebo; Table 3). The numbers of eosinophils in nasal lavage fluid were significantly decreased by the daily dose of 200 mg of rosmarinic acid on Day 3 ($P < 0.05$ vs. placebo) and showed a nonsignificant decrease in the rosmarinic acid (50 mg) group. On Day 21, the numbers of these cells were smaller in the rosmarinic acid groups than in the placebo group, but these changes were not statistically significant.

The levels of eotaxin, IL-1β, IL-8, and histamine in nasal lavage fluid supernatants were not significantly different among the three treatment groups (Table 4). There were also no significant differences in serum pollen-specific IgE concentrations among the groups (Table 4).

On Day 21, no significant abnormalities were detected by routine blood tests including complete blood cell counts, hepatic and renal function tests, total protein and proteino-

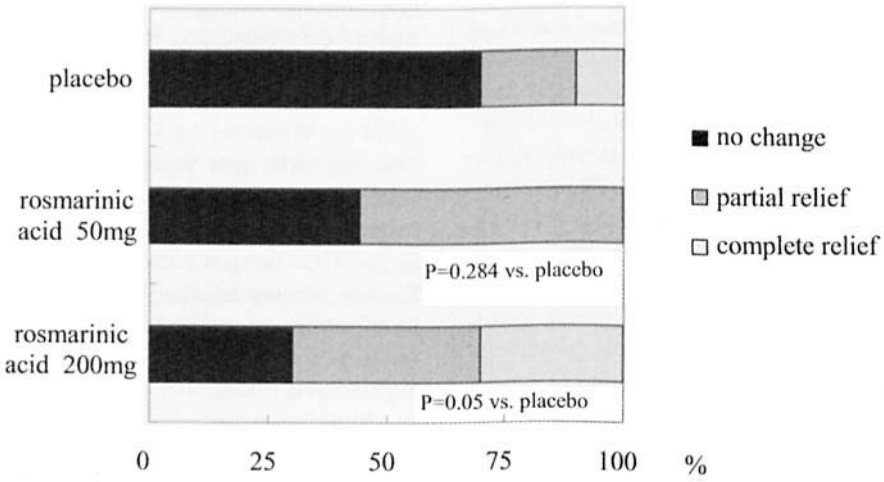

Figure 3. Patients' evaluations of global symptoms using the health-related quality of life questionnaire. Patients' assessments of global symptoms at study endpoint relative to prestudy levels are shown for each group. The chi-square test was used to analyze the difference in variation of quality of life between groups.

Table 2. Responder Rates on Symptom Scores[a]

Symptoms	Placebo (n = 10)		Rosmarinic acid 50 mg (n = 9)			Rosmarinic acid 200 mg (n = 10)		
	No change	Improvement	No change	Improvement	P value	No change	Improvement	P value
Total symptom								
3 days	10	0	6	3	**0.047**	8	2	0.136
21 days	9	1	5	4	0.089	6	4	0.121
Itchy nose								
3 days	9	1	7	2	0.466	8	2	0.531
21 days	10	0	7	2	0.115	6	4	**0.025**
Watery eyes								
3 days	10	0	7	2	0.115	8	2	0.136
21 days	10	0	6	3	**0.047**	8	2	0.136
Itchy eyes								
3 days	8	2	5	4	0.329	5	5	0.160
21 days	10	0	5	4	**0.025**	5	5	**0.010**
Sneezing								
3 days	9	1	6	3	0.213	8	2	0.531
21 days	8	2	7	2	0.906	8	2	1.000
Rhinorrhea								
3 days	9	1	7	2	0.466	8	2	0.531
21 days	9	1	8	1	0.937	6	4	0.121
Stuffy nose								
3 days	9	1	9	0	0.330	8	2	0.531
21 days	8	2	9	0	0.330	9	1	0.531

[a] The chi-square test was used to analyze the difference in variation of symptom scores between groups.

grams, electrolytes, lipids, uric acid, and concentrations of creatine phosphokinase (Table 5).

Discussion

The present randomized, double-blind, age-matched, placebo-controlled parallel group study provides clinical evidence that extract of *Perilla frutescens* enriched for rosmarinic acid is an effective intervention for mild SAR. The effects may be mediated, at least in part, through the inhibition of PMNL infiltration into the nostrils.

In previous experimental studies, polyphenolic phytochemicals including rosmarinic acid have been shown to inhibit IgE responses (11, 15) and inflammation characterized by PMNL infiltration (5, 6, 16, 17). Furthermore,

Table 3. Numbers of Inflammatory Cells in Nasal Lavage Fluid[a]

Inflammatory cells	Placebo (n = 10)	Rosmarinic acid 50 mg (n = 9)	P value	Rosmarinic acid 200 mg (n = 10)	P value
Polymorphonuclear leukocytes in total lavage fluid[b]					
0 day	4.44 ± 0.46	4.32 ± 0.43		4.43 ± 0.14	
3 days	4.72 ± 0.34	3.54 ± 0.44	**0.006**	3.74 ± 0.32	**0.010**
21 days	4.42 ± 0.38	3.74 ± 0.39	0.168	4.04 ± 0.26	0.442
Eosinophils in total lavage fluid[b]					
0 day	3.47 ± 0.40	3.18 ± 0.31		3.68 ± 0.24	
3 days	3.79 ± 0.29	3.05 ± 0.39	0.107	3.17 ± 0.28	**0.029**
21 days	3.94 ± 0.41	3.19 ± 0.40	0.357	3.82 ± 0.33	0.823
Neutrophils in total lavage fluid[b]					
0 day	4.27 ± 0.48	4.24 ± 0.45		3.99 ± 0.23	
3 days	4.54 ± 0.36	3.13 ± 0.54	**0.006**	2.90 ± 0.51	**0.014**
21 days	3.78 ± 0.49	3.04 ± 0.52	0.239	2.76 ± 0.44	0.253

[a] The analysis of covariance was used for difference in variables between groups. Each value represents mean and standard error.
[b] Logarithmically transformed value.

Table 4. Inflammatory Mediators in Nasal Lavage Fluid or Serum[a]

Mediators	Placebo (n = 10)	Rosmarinic acid 50 mg (n = 9)	P value	Rosmarinic acid 200 mg (n = 10)	P value
Eotaxin (pg/total lavage fluid)					
0 day	44.20 ± 21.31	22.53 ± 14.52		24.59 ± 9.72	
3 days	53.86 ± 15.35	47.46 ± 35.02	0.980	22.59 ± 11.63	0.477
21 days	61.71 ± 16.97	101.25 ± 25.28	0.118	54.00 ± 14.77	0.942
Histamine (ng/total lavage fluid)					
0 day	41.73 ± 10.83	33.73 ± 7.28		36.81 ± 9.21	
3 days	34.23 ± 9.57	32.22 ± 6.17	0.806	46.41 ± 12.78	0.172
21 days	30.20 ± 6.11	41.30 ± 9.63	0.212	43.12 ± 8.19	0.193
IL-1 (pg/total lavage fluid)					
0 day	8.05 ± 1.46	12.94 ± 5.13		5.00 ± 1.42	
3 days	11.79 ± 4.80	7.29 ± 1.83	0.382	7.62 ± 2.99	0.490
21 days	6.46 ± 1.78	7.75 ± 3.04	0.680	4.96 ± 1.45	0.209
IL-8 (pg/total lavage fluid)					
0 day	377.49 ± 143.11	174.52 ± 49.13		97.34 ± 26.65	
3 days	266.92 ± 71.48	145.26 ± 63.03	0.289	113.24 ± 42.55	0.728
21 days	133.78 ± 32.90	192.26 ± 61.93	0.099	71.40 ± 18.40	0.763
Specific IgE (Ua/ml serum)					
0 day	14.6 ± 4.5	13.0 ± 3.6		15.5 ± 2.3	
3 days	14.7 ± 4.4	13.1 ± 3.5	0.995	15.9 ± 2.4	0.761
21 days	28.7 ± 9.2	23.3 ± 3.4	0.599	26.4 ± 3.8	0.639

[a] Each value represents mean and standard error. The analysis of covariance (ANCOVA) was used for difference in variables between groups.

Makino *et al.* (11) have reported that Perilla decoction suppresses the PCA reaction in mice. Since the degree of inhibition by Perilla decoction and rosmarinic acid were nearly equal, they postulated that the anti-allergic effect of Perilla decoction is primarily due to rosmarinic acid (11). In another report, polyphenol strictinin isolated from tea leaves has inhibited IgE production in B cells (15). Green tea polyphenols have also been shown to attenuate bowel inflammation caused by autoimmunity as demonstrated by lower histologic scores and wet colon weights (5). Finally, it has been reported that virgin olive oil with a higher content of polyphenolic compounds protects experimental animals from carrageenan-induced inflammation and adjuvant arthritis (6). However, there have been no clinical reports that oral supplementation with polyphenolic phytochemicals is an effective intervention for SAR. To our knowledge, the current study is the first report demonstrating the efficacy of phytochemicals for patients with SAR.

Although a previous randomized, double-blind, placebo-controlled clinical trial suggests that a Chinese herbal formulation is partially effective in treatment of perennial allergic rhinitis (18), the mode of action has not been elucidated. In our investigation, amelioration of total symptoms on Day 3 showed consistent correlation with decreased numbers of PMNL in nasal lavage fluid. Since allergic rhinitis is inflammation characterized by PMNL such as eosinophils and neutrophils, the beneficial effects of extract of *Perilla frutescens* enriched for rosmarinic acid on SAR may depend, at least in part, on the inhibition of PMNL infiltration into nostrils.

It is well recognized that PMNL are activated by proinflammatory cytokines and chemokines including IL-1β, IL-8, and eotaxin. IL-8 and eotaxin are chemotactic for neutrophils and eosinophils, respectively. In fact, our recent experimental study has shown that extract of *Perilla frutescens* enriched for rosmarinic acid inhibits murine airway inflammation induced by diesel exhaust particles possibly through inhibition of the enhanced local expression of proinflammatory cytokines and chemokines such as IL-1β and IL-8 (17). In the present clinical study, however, the levels of the proinflammatory cytokines and chemokines were not proportional to the numbers of PMNL in the nasal lavage fluid. In general, allergic inflammation consists of multiple steps, including immediate and late allergic responses where a variety of proinflammatory cytokines and chemokines as well as chemical mediators are locally expressed at different times after allergen exposure and play important roles in the pathogenesis. Thus, the timing and the dose of allergen exposure can affect the local expression of these proinflammatory mediators. In the present study, however, the enrolled patients' daily exposure (timing and the dose of allergen) was not strictly matched. Future time-course studies using controlled provocation with known allergens at an identical dose could further elucidate the role of these mediators in the beneficial molecular actions of rosmarinic acid. Similarly, future measurement of these

Table 5. Routine Laboratory Tests[a]

Laboratory tests	Placebo (n = 10)	Rosmarinic acid 50 mg (n = 9)	Rosmarinic acid 200 mg (n = 10)
T-protein (g/dl)			
0 day	7.5 ± 0.2	7.6 ± 0.2	7.7 ± 0.1
21 days	7.2 ± 0.2	7.5 ± 0.2	7.2 ± 0.1
Alb (g/dl)			
0 day	4.8 ± 0.1	4.7 ± 0.1	4.8 ± 0.1
21 days	4.6 ± 0.1	4.6 ± 0.2	4.4 ± 0.1
A/G			
0 day	1.8 ± 0.1	1.7 ± 0.0	1.6 ± 0.1
21 days	1.8 ± 0.1	1.6 ± 0.1	1.6 ± 0.1
GOT (U/L)			
0 day	22.2 ± 1.7	24.8 ± 4.3	23.8 ± 2.5
21 days	19.8 ± 1.3	20.4 ± 4.3	20.6 ± 1.8
GPT (U/L)			
0 day	19.7 ± 3.4	33.7 ± 12.3	27.5 ± 5.9
21 days	16.1 ± 2.6	31.7 ± 12.2	19.0 ± 3.5
LDH (u/L)			
0 day	299.9 ± 19.5	331.9 ± 22.0	299.9 ± 10.7
21 days	278.2 ± 18.4	317.5 ± 29.2	264.4 ± 10.5
ALP (U/L)			
0 day	203.8 ± 17.9	206.2 ± 20.8	218.5 ± 16.8
21 days	200.9 ± 17.5	203.2 ± 19.3	201.1 ± 14.2
gamma-GTP (U/L)			
0 day	21.7 ± 3.3	30.4 ± 8.4	35.8 ± 8.0
21 days	18.6 ± 2.0	33.2 ± 10.0	32.8 ± 6.7
LAP (U/L)			
0 day	48.8 ± 3.5	116.1 ± 73.5	55.3 ± 2.6
21 days	46.8 ± 3.1	51.8 ± 4.8	52.8 ± 3.1
ChE (U/L)			
0 day	4648.7 ± 259.3	5243.9 ± 318.9	5074.8 ± 206.5
21 days	4388.2 ± 204.9	5147.2 ± 435.1	4718.3 ± 235.5
T-Bil (mg/dl)			
0 day	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.1
21 days	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
Cre (mg/dl)			
0 day	1.0 ± 0.0	1.0 ± 0.1	0.9 ± 0.0
21 days	1.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.0
BUN (mg/dl)			
0 day	15.2 ± 1.3	14.1 ± 1.1	14.3 ± 1.4
21 days	14.3 ± 1.1	14.4 ± 1.3	14.0 ± 1.4
Uric acid (mg/dl)			
0 day	5.3 ± 0.4	5.0 ± 0.5	5.1 ± 0.5
21 days	4.9 ± 0.4	5.1 ± 0.5	4.8 ± 0.5
CPK (U/L)			
0 day	127.0 ± 20.0	110.5 ± 10.6	93.3 ± 10.1
21 days	162.7 ± 41.8	107.7 ± 20.5	104.5 ± 18.3
T-Chol (mg/dl)			
0 day	190.8 ± 9.4	182.2 ± 7.8	196.5 ± 8.5
21 days	182.9 ± 10.8	190.7 ± 11.5	186.7 ± 5.8
Free Chol (mg/dl)			
0 day	47.8 ± 2.7	45.9 ± 1.8	49.3 ± 1.7
21 days	45.1 ± 3.0	47.2 ± 2.7	46.4 ± 1.4
HDL-Chol (mg/dl)			
0 day	69.3 ± 4.0	56.3 ± 4.6	62.8 ± 5.7
21 days	68.8 ± 4.2	56.8 ± 5.2	59.8 ± 5.9
TG (mg/dl)			
0 day	82.3 ± 10.6	97.5 ± 12.9	119.8 ± 23.3
21 days	86.3 ± 9.1	109.4 ± 17.6	136.6 ± 36.4
TL (mg/dl)			
0 day	590.6 ± 23.0	573.5 ± 19.8	640.4 ± 35.3
21 days	576.4 ± 28.0	604.3 ± 36.0	629.6 ± 42.4
FFA (mg/dl)			
0 day	0.3 ± 0.1	0.2 ± 0.0	0.3 ± 0.0
21 days	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0
PL (mg/dl)			
0 day	221.8 ± 7.3	202.5 ± 7.8	225.5 ± 8.9
21 days	215.5 ± 9.1	208.5 ± 11.0	212.7 ± 6.4
Na (mEq/L)			
0 day	144.2 ± 1.8	140.7 ± 0.5	143.9 ± 1.8
21 days	144.7 ± 2.2	142.3 ± 2.4	139.5 ± 0.8
K (mEq/L)			
0 day	4.5 ± 0.2	4.2 ± 0.1	4.4 ± 0.1
21 days	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.1
Cl (mEq/L)			
0 day	106.3 ± 1.3	103.7 ± 0.7	101.6 ± 1.0
21 days	107.8 ± 1.9	105.3 ± 1.4	103.8 ± 0.7
Ca (mEq/L)			
0 day	4.8 ± 0.1	4.7 ± 0.0	4.8 ± 0.1
21 days	4.7 ± 0.1	4.6 ± 0.1	4.6 ± 0.1
IP (mg/dl)			
0 day	3.4 ± 0.2	3.3 ± 0.2	3.7 ± 0.2
21 days	3.4 ± 0.1	3.2 ± 0.2	3.5 ± 0.2
Mg (mg/dl)			
0 day	2.2 ± 0.1	2.1 ± 0.0	2.2 ± 0.0
21 days	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.0
Fe (mg/ml)			
0 day	86.7 ± 14.1	97.0 ± 11.4	102.9 ± 16.8
21 days	88.3 ± 16.2	108.9 ± 13.2	86.2 ± 12.4
Cu (mg/ml)			
0 day	107.7 ± 5.1	101.4 ± 2.0	111.9 ± 4.6
21 days	101.0 ± 5.7	103.2 ± 4.6	109.2 ± 5.1

[a] Each value represents mean and standard error.

mediators in eye wash fluid or tears might provide further information about how rosmarinic acid acts, since this extract predominantly inhibited eye, rather than nose, symptoms. Furthermore, the role of the other mediators such as complements, platelet activating factor, and leuko-

trienes, which are reportedly chemotactic for PMNL, should be examined.

In our study, extract of *Perilla frutescens* enriched for rosmarinic acid did not affect pollen-specific IgE concentrations in the serum, which is not consistent with the findings of a previous animal study (11). The discrepancy might be explained in part by differences in species, allergen, or period of rosmarinic acid administration. Our patients received the extract after past sensitization with pollen, whereas the experimental animals received it before sensitization with ovalbumin. Our data suggest that the beneficial action of this extract in SAR patients is mediated by inhibition of PMNL-dependent inflammation, rather than by an effect on pollen-specific IgE production. Concentration of the pollen-specific IgE present in the nose or eye, which can directly induce the release of proinflammatory mediators in the local site, might be used to elucidate the mechanism of rosmarinic acid action.

In the present study, routine laboratory tests including complete blood cell counts, hepatic and renal function tests, and creatine phosphokinase showed safety of rosmarinic acid at a daily dose of 200 mg for 21 days.

In conclusion, extract of *Perilla frutescens* enriched for rosmarinic acid can be an effective intervention for patients with mild SAR that may act through inhibition of PMNL-dependent inflammation. The present preliminary study provides clinical evidence that this alternative treatment for SAR might reduce treatment costs for allergic diseases. A more detailed study involving a larger number of patients treated for a longer period of time will be needed to draw more definite conclusions.

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