

Green tea-induced asthma: relationship between immunological reactivity, specific and non-specific bronchial responsiveness

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Summary

Background The relationships between immunological reactivity and bronchial responsiveness to allergen and non-specific bronchial responsiveness are unclear in occupational asthma caused by low molecular weight substances.

Objective We assessed the above relationships in green tea-induced asthma, an occupational asthma of green tea factory workers, in which epigallocatechin gallate (EGCg), a low molecular weight component of green tea leaves, is the causative agent.

Methods Subjects consisted of 21 patients suspected of having green tea-induced asthma, on whom skin test and inhalation challenge with EGCg were performed. The skin sensitivity or end-point titration to EGCg as a measure of immunological reactivity, together with the provocative concentrations causing a 20% or greater fall in forced expiratory volume in 1 s (PC₂₀) of EGCg and methacholine, were determined.

Results We found that 11 patients had green tea-induced asthma, with immediate asthmatic reactions in eight and dual asthmatic reactions in three. We also found that 11 of 13 patients (85%) with immunological reactivity and bronchial hyper-responsiveness to methacholine experienced an asthmatic reaction and that no subject without immunological reactivity reacted. There were significant correlations among skin sensitivity, EGCg PC₂₀ and methacholine PC₂₀. Multiple linear regression analysis showed the relationship: $\log(\text{EGCg PC}_{20}) = 0.42 \log(\text{skin sensitivity}) + 1.17 \log(\text{methacholine PC}_{20}) + 0.93$ ($r = 0.796$, $P < 0.05$).

Conclusion It is concluded that bronchial responsiveness to EGCg can be highly satisfactorily predicted by skin sensitivity to EGCg and bronchial responsiveness to methacholine.

Keywords correlation, inhalation challenge, low molecular weight substances, occupational asthma, skin test

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Introduction

Tiffeneau [1] originally proposed that the dose of allergen that can elicit an asthmatic reaction is a function of non-specific bronchial responsiveness and specific immunological reactivity, and other groups subsequently confirmed this hypothesis [2, 3]. A prediction of bronchial responsiveness to an allergen from skin sensitivity to an allergen and bronchial responsiveness to histamine can be made for non-occupational inhalant allergens [4]. However, these relationships have not been well examined in the case of occupational agents. The reason that some groups found whereas others did not find a relationship between specific and non-specific bronchial responsiveness is that immunological reactivity cannot be assessed properly for low molecular weight agents [5].

Green tea-induced asthma is an occupational asthma caused by inhalation of green tea dust. We have demonstrated that epigallocatechin gallate (EGCg), the major low molecular weight (458 Da) component of green tea leaves, is the causative agent [6, 7]. The purpose of this study was to assess any associations between skin sensitivity and bronchial responsiveness to EGCg, as well as bronchial responsiveness to methacholine.

Materials and methods

Subjects

Subjects consisted of 21 patients suspected of having green tea-induced asthma on the basis of a suggestive clinical history. These patients had worked at different green tea factories and were referred to the Second Department of Internal Medicine, Hamamatsu University School of Medicine or associated hospitals in Shizuoka Prefecture for the

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evaluation of occupational asthma. The patients suspected of having only occupational dermatitis or rhinitis were excluded from this study. The diagnosis of green tea-induced asthma was confirmed if the inhalation challenge with EGCg was positive. No subject had been removed from exposure before diagnosis. All subjects were without medication that might influence the test results and did not report any acute viral infections for at least 1 month before entry into the study. Informed consent was obtained from all subjects prior to these tests. The protocol was approved by the local ethics committee.

Immunological assessment – skin test

EGCg was purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Intradermal skin tests were performed with EGCg in saline solution at concentrations of 1 ng/mL to 1 mg/mL with 10-fold increments. The responses were evaluated after 15 min and the results were considered positive when a weal or erythema was greater than 7 mm in diameter [8]. Skin sensitivity to EGCg was defined as the minimum EGCg concentration that produced a positive reaction.

Inhalation challenge

Inhalation challenge was performed as described previously [6]. EGCg in saline solution was inhaled from the concentration corresponding to the skin sensitivity as a mist using a DeVilbiss Model 646 nebulizer (output, 0.3 mL/min; particle size, 3–5 µm) for 2 min (DeVilbiss, Somerset, PA, USA). The concentration inhaled was increased in 10-fold increments to each threshold concentration or 1 mg/mL. Subjects who had a negative skin reaction to EGCg inhaled the top concentration. Bronchial responsiveness to EGCg was defined as the provocative concentration of EGCg causing a 20% or greater fall in forced expiratory volume in 1 s (FEV₁) in the immediate asthmatic reaction (PC₂₀).

Non-specific bronchial responsiveness

Bronchial challenge tests to methacholine were performed according to the standard method proposed by the Japanese Society of Allergology [9]. A PC₂₀ for methacholine of < 10 mg/mL was defined as bronchial hyper-responsiveness.

Statistical analysis

All data are expressed as means ± SD. Logarithmic transformations of skin sensitivity, PC₂₀ of EGCg and methacholine and total IgE were used for analyses. Comparisons between groups were made with the unpaired *t*-test. Correlations were studied using the Pearson's correlation coefficient. A multiple linear regression analysis was performed to identify if the skin sensitivity to EGCg or the PC₂₀ of methacholine could explain the bronchial responsiveness to EGCg. A *P*-value of < 0.05 was considered significant.

Results

Clinical characteristics

Of the 21 patients enrolled into the study, 11 patients were confirmed as having green tea-induced asthma by skin test and inhalation challenge with EGCg. The clinical characteristics of the patients with green tea-induced asthma (EGCg positive) and the 10 other patients (EGCg negative) are summarized in Table 1. Two of those with negative inhalation challenge had positive skin test to EGCg, thus skin test to EGCg was 100% sensitive and 80% specific. Overall, the mean PC₂₀ of methacholine was significantly lower in EGCg-positive than in EGCg-negative patients (*P* = 0.0409). However, there were no differences in age, smoking habit, duration of exposure to green tea dust, sensitization to common inhalant allergens, total IgE and FEV₁ between the two groups.

Correlations

In the patients with green tea-induced asthma, the skin sensitivity to EGCg had a positive correlation with the EGCg PC₂₀ (*r* = 0.760, *P* = 0.0048), and the methacholine PC₂₀ had a positive correlation with the EGCg PC₂₀ (*r* = 0.717, *P* = 0.0108). Furthermore, the skin sensitivity to EGCg was positively correlated to the methacholine PC₂₀ (*r* = 0.731, *P* = 0.0085) (Fig. 1). The duration of exposure to green tea dust before the onset of symptoms and after the onset to diagnosis did not show any correlation with either skin sensitivity to EGCg, and the methacholine or EGCg PC₂₀. The multiple linear regression analysis showed the following relationship: $\log(\text{EGCg PC}_{20}) = 0.42 \log(\text{skin sensitivity}) + 1.17 \log(\text{methacholine PC}_{20}) + 0.93$ (*r* = 0.796, *P* = 0.018). Using this formula, the EGCg PC₂₀ was accurately predicted in seven, underestimated by a 10-fold concentration in three, and overestimated by a 100-fold concentration in one.

Discussion

Subsequent to Tiffeneau's hypothesis of the relationship between the status of immunological and bronchial reactivities, Cockcroft et al. [3] have clearly shown that the dose of allergen causing an immediate reaction can be predicted accurately from the diameter of immediate weal reaction and the level of methacholine, the latter being inversely related. All subjects included in the studies by Cockcroft et al. had a level of responsiveness to methacholine in the asthmatic range. More recently, Lemi re et al. [10] examined specific reactivity to occupational agents in individuals who still showed skin reactivity to the allergen, but had normal bronchial responsiveness to methacholine. These authors were able to show that the main determinant of responsiveness was the level of specific IgE to the aeroallergen. This conclusion can also be deduced from the present study. Indeed, all subjects, except two with positive skin reactions to EGCg (11/13, 85%), whatever their levels of reactivity to methacholine, developed an asthmatic reaction. Only 11 of 18 subjects (61%) with increased responsiveness to methacholine

Table 1. Patient characteristics

Subject no.	Age (years)	Sex	Smoking	Duration of exposure		Sensitization to common inhalant allergens	Inhalation challenge/maximum %fall in FEV ₁											
				Before onset of symptoms (years)	After onset to diagnosis (years)		Total IgE (IU/mL)	Skin sensitivity to EGCg (μg/mL)	EGCg PC ₂₀ (μg/mL)	Asthmatic reaction	Immediate phase (%)	Late Phase (%)	Methacholine PC ₂₀ (mg/mL)	FEV ₁ (%) predicted)				
EGCg positive																		
1	52	M	ex-S	11	3	House dust mites, ragweed, grass pollens	395	10	1000	Immediate	40	6	84.6					
2	60	F	NS	1.7	0.3	Grass pollens	39	100	100	Immediate	50	6	95.1					
3	47	F	NS	7	7	—	40	0.01	10	Immediate	55	2	89.1					
4	37	M	S	3	1	House dust mites	59	0.1	10	Dual	43	30	81.9					
5	44	M	ex-S	9	5	—	310	1	10	Dual	47	40	90.6					
6	56	F	NS	21.2	0.8	—	381	0.1	0.1	Immediate	45	3	85.2					
7	29	F	NS	5	1	—	100	0.01	0.1	Immediate	35	3	71.6					
8	66	M	ex-S	5	2	—	296	1	1	Immediate	37	0	53.2					
9	43	M	ex-S	13	10	—	350	0.01	10	Immediate	25	1	91.1					
10	30	M	S	2	8	Cedar pollen	220	100	1000	Dual	43	27	82.4					
11	26	M	ex-S	4.9	0.1	Cedar pollen	62	1000	1000	Immediate	28	4	97.9					
Mean±SD		45±13		7.5±5.8	3.5±3.5		145±152*	1.0±304.2*	15.2±515.0			4	83.9±12.4					
EGCg negative																		
12	42	M	S	18	2	House dust mites	183	1000	>1000	—	3	0	65.4					
13	42	F	NS	3.7	0.3	House dust mites, cedar pollen, grass pollens	449	—	>1000	—	0	5	102.6					
14	20	F	NS	1.8	0.2	—	176	—	>1000	—	1	0	94.8					
15	58	F	NS	7	16	—	227	—	>1000	—	1	4	93.1					
16	62	M	ex-S	33.7	0.3	Cedar pollen	235	—	>1000	—	4	0	104.9					
17	57	F	NS	18	7	—	237	—	>1000	—	0	4	87.2					
18	28	F	NS	2.9	0.1	House dust mites, cedar pollen	281	—	>1000	—	3	4	92.7					
19	19	F	NS	0	0.1	Cedar pollen	245	—	>1000	—	4	5	78.5					
20	51	M	ex-S	15	1	—	150	—	>1000	—	0	3	89.0					
21	48	F	NS	11	1	Cedar pollen, grass pollens	320	1000	>1000	—	2	0	82.9					
Mean±SD		43±16		11.1±10.4	2.8±5.1		239±78*						89.1±11.6					

EGCg, epigallocatechin gallate; PC₂₀, provocative concentration causing a 20% fall in forced expiratory volume in 1 s (FEV₁); S, smoker; NS, nonsmoker; M, male; F, female.
 *Geometric mean±SD.

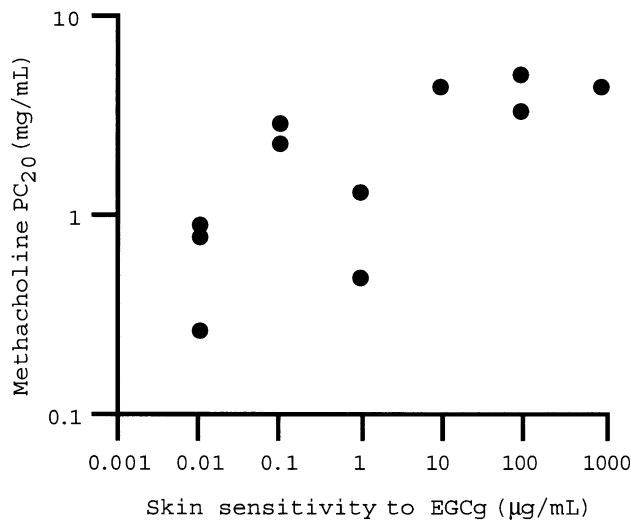


Fig. 1. The correlation between skin sensitivity to EGCg and methacholine PC₂₀ in EGCg-positive patients. There was a significant correlation ($r = 0.731$, $P = 0.0085$).

developed an asthmatic reaction on exposure to EGCg. This again points out that the key element is the status of immunological reactivity.

The two subjects who did not behave according to the rule are subjects 12 and 21 in Table 1. These subjects were still at work and exposed to green tea dust at the time of the study. The maximum falls in FEV₁ at the time of their challenges were 3% and 2%, respectively. We believe that the way we performed specific inhalation challenges in the laboratory yielded to administering a sufficient antigenic load to elicit or exclude an asthmatic reaction.

For high molecular weight agents for which skin tests are possible, both skin sensitivity and methacholine responsiveness are used as a guide to the initial inhaled dose. In this study, even though skin sensitivity and methacholine PC₂₀ predict the EGCg PC₂₀, it is important to be cautious in performing inhalation challenge, since in one patient, the predicted concentration was 100-fold overestimated.

EGCg is the major component of green tea leaves, and 100% purity of which is commercially available. In our earlier report, we confirmed that the patients exhibited positive skin and bronchial responses to EGCg as well as the extract of green tea dust that was collected at the factories [6]. Because of its water-soluble nature, despite low molecular weight, EGCg is suitable for skin test as a screening tool for the

diagnosis. Indeed, the sensitivity of the skin testing with EGCg was 100% in this study.

In conclusion, a prediction of bronchial responsiveness to EGCg from skin sensitivity to EGCg and bronchial responsiveness to methacholine can be highly satisfactorily made for green tea-induced asthma.

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