Bronchial Responsiveness and Angiotensin-Converting Enzyme Gene Polymorphism in Sarcoidosis Patients*

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Background: Angiotensin-converting enzyme (ACE) inactivates bradykinin and tachykinins, which are potent bronchoconstrictors and mediators of inflammatory reactions. It has recently been shown that an insertion (I)/deletion (D) polymorphism in the ACE gene accounts for variation in serum ACE level. We investigated bronchial responsiveness in patients with sarcoidosis to determine whether it might be associated with ACE gene polymorphism.

Subjects: Bronchial responsiveness was assessed in 21 patients with sarcoidosis, 21 patients with asthma, and 18 healthy control subjects. ACE polymorphism was also examined in the 21 patients with sarcoidosis.

Methods: Bronchial responsiveness was measured by recording respiratory resistance with continuous inhalation of methacholine from 49 to 25,000 μg/mL in concentration. The ACE genotype was determined using the polymerase chain reaction.

Results: We found a significant increase in bronchial responsiveness in sarcoidosis patients as compared with healthy control subjects (p<0.01). In the sarcoidosis group, patients with the II genotype demonstrated significantly more coughing (p<0.05) and a greater bronchial responsiveness (p<0.05) than did those with DI or DD genotypes.

Conclusion: Patients with sarcoidosis have increased bronchial responsiveness to some extent, the degree apparently depending on the ACE genotype.

Key words: angiotensin-converting enzyme; bradykinin; bronchial responsiveness; gene; polymorphism; sarcoidosis; tachykinin

Abbreviations: ACE=angiotensin-converting enzyme; D=deletion; Dmin=minimum dose of methacholine; I=insertion; PCR=polymerase chain reaction

T he angiotensin-converting enzyme (ACE) (kininase II, peptidyl-dipeptidase A; EC 3.4.15.1) is widely used as a parameter in laboratory tests for sarcoidosis. It has been demonstrated that untreated patients with more active clinical disease tend to have higher serum ACE levels.1-3 Recently, it was found that an insertion (I)/deletion (D) polymorphism exists in the ACE gene, and that this polymorphism affects the serum ACE level. There are three genotypes, DD, DI, and II, with the ACE level being highest in DD, intermediate in DI, and lowest in II.4,5 In our previous study, serum ACE values of sarcoidosis patients were reported to increase in the order DD>DI>II.6

ACE inactivates bradykinin, a potent bronchoconstrictor and inflammatory mediator, as well as tachykinins such as substance P and neurokinin A. Tachykinins are inducers of airway smooth muscle constriction, bronchial edema, extravasation of plasma, and mucus hypersecretion, acting as important mediators of neurogenic inflammation.7-10 ACE inhibitors may increase the bronchial responsiveness of patients with asthma.11,12 On the other hand, nonproductive coughing caused by ACE inhibitors and cough reflex with distilled water are more frequent in individuals with the II genotype.13,14 The high frequency of coughing in sarcoidosis patients suggests that the disease is associated with bronchial hyperresponsiveness. Several studies of bronchial responsiveness in pulmonary sarcoidosis have had variable results, however.15-19 This variability could

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be related to a link between bronchial responsiveness and ACE gene polymorphism but, to our knowledge, this has not been studied. Thus, we compared bronchial responsiveness in a series of patients with sarcoidosis and control subjects, and assessed the influence of the ACE genotype.

**Materials and Methods**

**Subjects**

The 21 sarcoidosis patients (18 women, three men) all lived in central Japan. Sarcoidosis was diagnosed based on the clinical presentation and the presence of epithelioid cell granulomas in lung, skin, or lymph node biopsy specimens. The mean age (±SD) was 52.9±14.1 years. All but five had evidence of sarcoidosis on chest radiographs; nine patients had stage I and seven had stage II disease. None of the patients had a history of acute infection of the respiratory tract for 2 or more months, chronic bronchitis, bronchial asthma, or pulmonary emphysema. They were all nonsmokers, and none had undergone steroid therapy. Patients with a history of atopy, an elevated serum IgE level (>170 units/mL), or eosinophilia (>5% peripheral leukocytes) were excluded. None of the subjects had taken any drugs for at least a day before testing.

Twenty-one asthmatic patients (nine women, 12 men) from the same area were also examined. They had a mean age of 42.4±16.4 years. All satisfied the American Thoracic Society criteria for asthma.20 Individuals with cardiogenic disease or pulmonary emphysema were strictly excluded. All asthmatic patients were nonsmokers.

Eighteen unrelated healthy individuals (10 women, eight men) living in the same area of Japan served as healthy control subjects. They had a mean age of 32.5±8.5 years. They had no past history of pulmonary disease and no abnormalities on physical examination, chest radiography, ECG, urinalysis, or routine laboratory blood testing. None was receiving medication at the time of the evaluation. All healthy control subjects were nonsmokers. Informed consent was obtained from all patients and control subjects.

**Measurement of Bronchial Responsiveness**

Bronchial responsiveness was evaluated by measuring of respiratory resistance during the continuous inhalation of methacholine in stepwise increments.21 Respiratory resistance was measured with the dose-response curves drawn by a graphic recorder (Astograph Model TCK-6100H; Chest Corp, Tokyo, Japan) with methacholine hydrochloride solution in isotonic saline; the 10 dilution increments were 49, 98, 195, 390, 781, 1,563, 3,125, 6,250, 12,500, and 25,000 μg/mL. To evaluate the dose-response curve, the minimum dose of methacholine causing bronchoconstriction (Dmin) was calculated as the cumulative dose of methacholine at the point when respiratory resistance started to increase. Dmin was expressed in "units"; one unit equaled 1 min of inhalation of aerosol solution at 1.0 mg/mL (Fig 1). The dose-response curves were evaluated by two independent investigators who did not know whether the data were from patients or control subjects. Ishii et al22 reported a significant correlation between bronchial responsiveness measured by respiratory resistance and bronchial responsiveness measured by a conventional method.

**Figure 1.** Typical dose-response curve of an asthma patient. The dose-response curve of respiratory resistance with methacholine in stepwise increments drawn directly by a graphic recorder is shown. The cumulative dose of methacholine is scaled in units (1 unit equals 1 min of inhalation of aerosol solution of methacholine at 1.0 mg/mL). The Dmin is the cumulative dose of methacholine at the point when respiratory resistance starts to increase. Respiratory resistance in this patient started to increase at a point 4 min after starting inhaling methacholine, ie, after exposure to 781 μg/mL methacholine, with a cumulative dose (Dmin) of 0.732 units.

**Determination of the ACE Genotype**

The ACE genotype of all sarcoidosis patients was determined by polymerase chain reaction (PCR) amplification of the respective fragments for the D and I alleles from intron 16 of the ACE gene and size fractionation by electrophoresis. DNA was extracted from peripheral leukocytes with standard techniques and PCR was performed with 20 picomoles of each primer (sense oligo 5’CTGGAGACCATCCCATTCTTTCT3’and antisense oligo 5‘CATGTGGGCATACATTGCAGAT3’) in a final volume of 25 μL, containing 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.2 mM of each dNTP, and 1.25 units of Taq polymerase (Perkin Elmer-Cetus; Norwalk, Conn).23-24 The DNA was amplified for 30 cycles with denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min (DNA Thermal Cycler 480; Perkin Elmer-Cetus). PCR products were electrophoresed in 2% agarose gels with 5 μg of ethidium bromide/mL. The amplification products of the D and I alleles were identified by 300-nm ultraviolet transillumination as distinct bands (D allele: 191 bp; I allele: 478 base pair).25

**Serum ACE Level Measurement**

Serum ACE levels were measured by a colorimetric method (colorimetric assay kit; Fujirebio; Tokyo, Japan) using p-hydroxy-hippuryl-L-histidyl-L-leucine as the substrate.26

**Statistical Analysis**

Analysis of bronchial responsiveness (Dmin) was performed using the Mann-Whitney U test. The differences in frequency of coughing among sarcoidosis patients with the three genotypes were analyzed with the χ2 test. Differences in serum ACE levels among sarcoidosis patients with the three genotypes were ana-
lyzed with the Kruskal-Wallis test. Correlation coefficients were used to assess the correlation between serum ACE level and log Dmin. A p value <0.05 was considered significant. Values are expressed as means±SD.27

Results

Bronchial Responsiveness

In asthma patients, sarcoidosis patients, and healthy control subjects, the mean Dmin values were 3.9±4.8, 7.7±11.2, and 20.2±16.0, respectively (Table 1). The Dmin was significantly lower in both asthma (p<0.001) and sarcoidosis patients (p<0.01) than in healthy control subjects. The difference between the two patient groups was not significant (p=0.110). There was no significant correlation between Dmin and the radiographic stage of sarcoidosis (data not shown).

Coughing and ACE Polymorphism in Sarcoidosis Patients

Seven sarcoidosis patients were ACE genotype II (33.3%), 10 were type DI (47.6%), and four were type DD (19.1%). Of the 21 sarcoidosis patients, eight (38.1%) complained of persistent or intermittent coughing that had continued for 3 or more months and did not respond to antibiotics; five of the seven type II patients (71%), two of 10 type DI (20%), and one of four type DD (25%) coughed, indicating an association with the II type. Since there were few type DD patients, we grouped the DI and DD cases together for comparison; the combined group demonstrated a significantly lower level of coughing than the II type (p<0.05; Table 2).

Coughing and Bronchial Responsiveness in Sarcoidosis Patients

The mean Dmin of sarcoidosis patients with coughs was 3.5±2.8, and that of patients without coughs was 10.4±13.6. However, the difference was not significant (p=0.19; Table 3).

ACE and Bronchial Responsiveness in Sarcoidosis Patients

The mean serum ACE levels of type II, DI and DD sarcoidosis patients were 18.7±5.9, 21.9±8.8, and 22.5±9.4 IU/L, respectively. Although ACE levels increased according to the order II<ID<DD, no significant differences were found among the three genotypes (p=0.82). The correlation between serum ACE level and log Dmin was significant (r=0.48; p<0.05; Fig 2), but the correlation between serum ACE level and Dmin was not (r=0.36, p=0.11). The mean Dmin values for the type II, DI, and DD patients with sarcoidosis were 2.4±1.5, 11.2±15.0, and 8.5±7.4, respectively. When the DI and DD cases were grouped together, the group’s mean Dmin was significantly different from the value for patients with the II genotype (p<0.05; Table 3).

Discussion

Several authors have reported increased bronchial responsiveness in sarcoidosis patients, although the methods of measurement and assessment varied.15-19 In this study, we also observed a significant increase in sarcoidosis patients compared with healthy subjects. Bronchial hyperresponsiveness has also been shown in other inflammatory diseases of the airway tract,9,20,28 and in sarcoidosis the bronchial wall is often involved as well as the lung parenchyma.15,18 We conclude that inflammatory reactions in the bronchial wall in sarcoidosis patients—e.g., desquamation of epithelium cells, infiltration of inflammatory cells, or release of chemical mediators—might cause their bronchial hyperresponsiveness.

ACE is a widely distributed zinc-metalloproteinase that occurs as a membrane-bound ectoenzyme on the surfaces of vascular endothelial cells and renal epithelial cells and as a circulating enzyme in the plasma. The ACE gene spans 21 kilobases, is located on the 17th chromosome q23, and consists of 26 exons and 25 introns. The polymorphism exists in intron 16. The length of the insertion is 287 base pair, and it is a repetition of a meaningless Alu family configuration.5,29 Why the polymorphism influences the serum ACE level has not been resolved, but some authors have suggested that the insertion/

Table 2—Coughing and the ACE Genotype in Patients with Sarcoidosis

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>Coughing (%)</th>
<th>No Coughing (%)</th>
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<tbody>
<tr>
<td>DD</td>
<td>1 (25)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>DI</td>
<td>2 (20)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>II</td>
<td>5 (71)*</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (38)</td>
<td>13 (62)</td>
</tr>
</tbody>
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*A significantly greater proportion of patients with the II genotype demonstrated coughing compared with the DI and DD genotypes (p<0.05; $\chi^2=4.95; df=1$).

Table 1—Bronchial Responsiveness of All Subjects

<table>
<thead>
<tr>
<th></th>
<th>Asthma Patients</th>
<th>Sarcoidosis Patients</th>
<th>Healthy Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>21</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Sex, female/male</td>
<td>9/12</td>
<td>18/3</td>
<td>10/8</td>
</tr>
<tr>
<td>Age, yr</td>
<td>42±16.4</td>
<td>52.9±14.1</td>
<td>32.8±8.5</td>
</tr>
<tr>
<td>Dmin, units</td>
<td>3.9±4.8*</td>
<td>7.7±11.2*</td>
<td>20.2±16.0</td>
</tr>
</tbody>
</table>

*p<0.001 vs healthy control subjects.

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deletion may be in linkage disequilibrium with regulatory elements of the ACE gene, or that the insertion itself might modify the splicing process of the ACE precursor messenger RNA by interfering with the lariat formation step.\textsuperscript{4,30} ACE inactivates bradykinin, which causes bronchoconstriction, enhances vascular permeability, leads to mucosal edema, and increases the production of mucus by direct effects or through release of neuropeptides, such as tachykinins, from C fibers.\textsuperscript{7-10} It is well known that ACE inhibitors often cause nonproductive coughing as an adverse effect.\textsuperscript{11,12} In individuals with the II genotype, nonproductive coughing caused by ACE inhibitors is reported to be more frequent than it is in those with the DI or DD types.\textsuperscript{13} Thus our findings are in line with the literature. In addition, an increase in the sensitivity of the cough reflex to stimulation with distilled water has been shown for healthy subjects with the II genotype.\textsuperscript{14} Many investigators have speculated that the effects of bradykinin on the airways might be responsible for the coughing.\textsuperscript{11-14}

In the present series of sarcoidosis patients, a significant increase in bronchial responsiveness was observed in those with the II genotype as compared with the DI/DD genotype. ACE inactivates tachykinins such as substance P and neurokinin A, as well as bradykinin.\textsuperscript{7,8} Tachykinins contract airway smooth muscle and stimulate mucus secretion from submucosal glands; together with bradykinin, they are considered to play important roles in the pathogenesis of asthma.\textsuperscript{9} The II type patients with sarcoidosis may have lower levels of ACE than other genotype patients,\textsuperscript{8} and therefore a comparatively low ability to inactivate bradykinin and tachykinins. This might account for the increased bronchial responsiveness. However, a limitation of this study was the small size of the population. In addition, no information on whether ACE gene polymorphism affects bronchial responsiveness in healthy subjects or in those with other diseases was obtained. Any general association between bronchial responsiveness and ACE must now be investigated in a larger population of patients with sarcoidosis and other bronchial inflammatory diseases.

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REFERENCES


<table>
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<th>Coughing</th>
<th>ACE genotype</th>
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<tbody>
<tr>
<td>Yes (n=8)</td>
<td>DI (n=7) DD (n=4) DI+DD (n=14)</td>
</tr>
<tr>
<td>No (n=13)</td>
<td>11.2±15.0 8.5±7.4 10.4±13.0</td>
</tr>
<tr>
<td>Dmin, units</td>
<td>2.4±1.5*</td>
</tr>
</tbody>
</table>

*p<0.05 as compared with the group of DI/DD genotype patients.

Figure 2. Serum ACE level and bronchial responsiveness in sarcoidosis patients. Serum ACE level positively correlated with log Dmin in sarcoidosis patients (r=0.48; p<0.05).
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