Association Between the Angiotensin-Converting Enzyme Gene Polymorphisms and Tissue Oxygenation During Exercise in Patients With COPD*

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Study objectives: We have recently determined that the angiotensin-converting enzyme (ACE) DD genotype might be associated with pulmonary hypertension during exercise in patients with COPD. Therefore, this study was designed to determine whether ACE gene polymorphisms adversely affect tissue oxygenation during exercise in patients with COPD.

Design: Cross-sectional analysis.

Setting: University hospital.

Patients: Thirty-nine patients (14 patients with II genotype, 12 patients with ID genotype, and 13 patients with DD genotype).

Interventions: All patients underwent right-heart catheterization and constant-load exercise testing for 5 min on an ergometer.

Measurements and results: The ratio of the change in oxygen delivery ($\Delta D_{O2}$) to the increase in oxygen consumption ($\Delta V_{O2}$) during exercise ($\Delta D_{O2}/\Delta V_{O2}$) was significantly lower in patients with the DD genotype (1.5 ± 0.2) than in those with the II genotype (1.9 ± 0.3, p = 0.0006) and the ID genotype (1.7 ± 0.2, p = 0.037). Mixed venous oxygen tension ($P_{vO2}$) after exercise in patients with the DD genotype (23.5 ± 1.5 mm Hg) was also significantly lower than in patients with the II genotype (26.7 ± 1.6 mm Hg, p = 0.0002) and the ID genotype (25.0 ± 2.0 mm Hg, p = 0.045).

In addition, the change in plasma concentration of lactate during exercise ($\Delta Lactate$) was significantly higher in patients with DD genotype (33.3 ± 4.3 mmol/L) than in those with the II genotype (25.5 ± 3.6 mmol/L, p = 0.0002) and the ID genotype (28.8 ± 4.0 mmol/L, p = 0.029).

The mean pulmonary arterial pressure after exercise was significantly correlated with $\Delta D_{O2}/\Delta V_{O2}$ ($r = -0.423, p = 0.0076$) but not with $P_{vO2}$ after exercise and with $\Delta Lactate$.

Conclusions: The ACE DD genotype may be associated with an impairment in peripheral tissue oxygenation during exercise in patients with COPD.

Key words: angiotensin-converting enzyme; COPD; exercise; pulmonary hypertension; tissue oxygenation

Abbreviations: ACE = angiotensin-converting enzyme; $D_{O2}$ = oxygen delivery; $\Delta D_{O2}/\Delta V_{O2}$ = change in oxygen delivery to oxygen consumption ratio; $P_{vO2}$ = mixed venous oxygen tension; $Q_t$ = cardiac output; $V_{O2}$ = oxygen consumption

The angiotensin converting enzyme (ACE) gene contains a polymorphism based on the presence (insertion [I]) or absence (deletion [D]) within an intron of a 287 base-pair nonsense DNA domain, resulting in three genotypes (DD homozygote, II homozygote, and DI heterozygote).1 The ACE DD genotype is associated with increased circulating and cellular concentrations of ACE2,3 and with the increase in cardiovascular risk. However, the vast majority of the total activity of ACE in the body is found in lung tissue,4 and its activity is further increased by chronic hypoxia.5 Moreover, ACE expression is markedly and consistently increased in the walls of small pulmonary arteries during the development of hypoxia-induced pulmonary hypertension.6 Therefore, ACE in lung tissue may be involved in the pathogenesis of pulmonary hypertension secondary to COPD.

Recently, we determined that the ACE DD genotype might be associated with pulmonary hyperten-
tion with exercise in patients with COPD. Pulmonary hypertension becomes particularly pronounced in patients with COPD when the cardiovascular system is stressed during exercise. This excessive pulmonary hypertension might result in the disturbance of oxygen transport during exercise. However, peripheral tissue oxygenation during exercise in patients with COPD also appears to depend on peripheral factors, including microcirculation and enzymatic capacity of exercising muscles. The purpose of the present study was to determine whether the ACE gene polymorphisms adversely affect peripheral tissue oxygenation during exercise in patients with COPD.

MATERIALS AND METHODS

Thirty-nine patients with COPD (all male patients) from the respiratory outpatient clinic of our institution were enrolled in this study. All had a history of smoking and severe irreversible airflow limitation. All patients satisfied the American Thoracic Society criteria for COPD. Patients with evidence of coronary artery disease, valvular heart disease, systemic hypertension, or primary myocardial disease were excluded from the study. None of the patients had radiologic or clinical evidence of pulmonary congestion or right-heart failure. Concomitant left ventricular dysfunction was excluded in all patients by echocardiography performed prior to the study. All patients with COPD were in clinically stable condition, and none had a history of respiratory infection for at least 4 weeks preceding the study. All patients gave their written informed consent for participation in this study, which was approved by the Ethics Committee of Osaka City University, Japan.

On the first day of the study, the subjects underwent a progressive incremental exercise test while sitting on an ergometer (EM840; Siemens; Munich, Germany), starting at 0 W for 3 min and adding 10 W every minute until symptom-limited maximum was reached. Expired gas analysis was performed during the exercise test with a respiratory monitor (Respiromonitor RM-300; Minato Medical Science; Osaka, Japan) to continuously measure oxygen consumption (VO$_2$). Heart rate was continuously monitored with standard ECG equipment. The purpose of this incremental exercise test was to determine the maximal exercise capacity. On the day following the test, all subjects underwent right-heart catheterization. All cardiopulmonary medications were withheld for at least 12 h before the study. A balloon-tipped pulmonary arterial catheter was inserted percutaneously into the internal jugular vein and advanced into the pulmonary artery for measurement of pulmonary arterial pressure (PAP) and pulmonary wedge pressure and sampling of mixed venous blood. In addition, a plastic catheter was placed percutaneously into the brachial artery to monitor systemic arterial pressure and to sample systemic arterial blood. Heart rate and rhythm were monitored continuously. PAP was measured using a transducer (UK901; Baxter; Tokyo, Japan) located at the level of the anterior fourth intercostal space, with the patient sitting upright, and was recorded on photographic paper. Pressures were averaged over three respiratory cycles. Mean pressures were obtained by electronic integration. Cardiac output (Qt) was determined by the thermodilution method, using a cardiac output computer (Fukuda Denshi; Tokyo, Japan). Arterial blood gas tensions were measured with a blood gas analyzer (model IL 1312; Instrumentation Laboratory; Tokyo, Japan), and the blood was rapidly deproteinized in iced perchlorate solution and, after centrifugation, was analyzed for lactate concentration by an enzymatic technique. Resting hemodynamic and blood gas data were obtained about 20 min after the patient had been seated comfortably on the ergometer. Each patient then underwent a constant-load exercise test for 5 min on the ergometer at a workload corresponding to 60% of the previously determined maximal workload. Hemodynamic and blood gas measurements were performed during the final minute of constant-load exercise.

Genomic DNA was extracted from peripheral blood leukocytes by standard methods. The ACE genotypes of the subjects were determined by polymerase chain reaction (PCR), using the primers and methods described by Rigat and colleagues. Under some conditions, the ACE D allele amplifies more effectively than the longer I allele, resulting in mistyping of the ID as the DD genotype. Therefore, all DD genotypes were reconfirmed. Briefly, the sense primer used in the PCR was replaced with an insertion-specific primer that leads to selective amplification of the I allele and absence of the D allele in mistyped ID genotypes (Fig 1). No mistyping was identified.

All values are presented as means ± SD. When multiple comparisons were made between groups, significant intergroup variability was first established with the Kruskal-Wallis test. The Mann-Whitney U test was then used for intergroup comparisons. The significance of correlations was evaluated by determining Spearman’s rank correlation coefficients. The significance level was set at p < 0.05.

![ACE genotypes determined by PCR](image)

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![Selective amplification of the I allele](image)

1. Control (I I)
2. Control (I D)
3. Control (DD)
4. Subject 1 (I I)
5. Subject 2 (I D)
6. Subject 3 (DD)

**Figure 1.** Typical pictures of the PCR products in COPD patients with the II, ID, and DD genotypes.
Results

The clinical characteristics for all 39 subjects with COPD are summarized in Table 1. The 14 patients with the II genotype, 12 patients with the ID genotype, and 13 patients with the DD genotype constituted well-matched subgroups with respect to age, baseline lung function, arterial blood gas data at rest or exercise, and hemodynamic data at rest. Additionally, the exercise level at which hemodynamics was definitively performed in each subject was comparable (maximal workload for patients with the II genotype, 69.9 ± 18.5 W; ID genotype, 65.8 ± 10.2 W; and DD genotype, 67.2 ± 15.3 W). However, the mean PAP after exercise was significantly higher in patients with the DD genotype (53.2 ± 3.9 mm Hg) than in those with the II genotype (40.6 ± 4.0 mm Hg, p < 0.0001) and the ID genotype (45.7 ± 5.9 mm Hg, p = 0.003).

The ratio of the change in oxygen delivery (ΔDO2) to the increase in VO2 during a constant-load exercise (ΔDO2/ΔVO2) was significantly lower in patients with the DD genotype (1.5 ± 0.2) than in those with the II genotype (1.9 ± 0.3, p = 0.0006) and the ID genotype (1.7 ± 0.2, p = 0.037) [Fig 2]. Mixed venous oxygen tension (PVO2) at rest did not differ significantly in all three subgroups. However, FV O2 after exercise in patients with the DD genotype (23.5 ± 1.5 mm Hg) was significantly lower than in patients with the II genotype (26.7 ± 1.6 mm Hg, p = 0.0002) and the ID genotype (25.0 ± 2.0 mm Hg, p = 0.045). In addition, the change in plasma concentration of lactate during exercise (ΔLactate) was significantly higher in patients with DD genotype (33.3 ± 4.3 mmol/L) than in those with the II genotype (25.5 ± 3.6 mmol/L, p = 0.0002) and the ID genotype (28.8 ± 4.0 mmol/L, p = 0.029). The mean PAP after exercise was negatively correlated with ΔDO2/ΔVO2 (r = −0.423, p = 0.0076; Fig 3). However, the mean PAP after exercise was not significantly correlated with FV O2 after exercise (r = −0.303, p = 0.515) and with ΔLactate (r = 0.253, p = 0.123).

Discussion

In this study, we evaluated the ΔDO2/ΔVO2 during exercise as an index of convective oxygen transport to the peripheral tissues. We found that the ΔDO2/ΔVO2 was significantly lower in patients with the DD genotype than in those with the II and ID genotypes, and that the mean PAP after exercise was negatively correlated with the ΔDO2/ΔVO2. Pulmonary hypertension with exercise can interfere with convective oxygen transport because of an inappropriate increase in Qt in response to exercise due to unloading of the left ventricle due to relative right ventricular failure caused by the exercise-induced increase in pulmonary afterload. In this study, the magnitude of pulmonary hypertension with exercise was greatest in patients with the DD genotype, with ID genotype patients having less pulmonary hypertension than DD genotype patients, and II genotype patients having the least pulmonary hypertension. Thus, the lower ΔDO2/ΔVO2 during exercise in patients with the DD genotype could be supported. Oxygen diffuses from the alveolar air into the pulmonary circulation and is transported to the muscles. In patients with COPD, the proportion of the total VO2 utilized by the respiratory muscles during exercise is higher than in normal subjects, and the VO2 available to the exercising nonrespiratory muscles would be correspondingly decreased. Therefore, it is conceivable that the disturbance of oxygen transport during exercise has a significant effect on the failure of nonrespiratory tissue oxygenation, in which oxygen transport to the peripheral exercising muscles is already reduced by blood flow diversion to respiratory muscles.

FV O2 during exercise reflects the adequacy of oxygen transport to the peripheral tissues and, thus, the state of tissue oxygenation. In this study, we found that FV O2 after exercise was significantly lower in patients with the DD genotype than in those with the II and ID genotypes. Moreover, ΔLactate was also significantly higher in patients with the DD genotype than in those with the II and ID genotypes. It therefore seems likely that the ACE DD genotype is associated with the disturbance of tissue oxygenation. Local distribution of blood flow to exercising muscles, the partial pressure gradient for oxygen diffusion into tissues, the distance of diffusion to mitochondria, and the capacity of the oxygen utilization process could all theoretically affect tissue oxy-

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<th>Table 1—Clinical Characteristics of Study Patients*</th>
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*p < 0.01 vs DD group.
**p < 0.05 vs ID group.

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generation. Interestingly, we found that the mean PAP after exercise was not significantly correlated with P\textsubscript{v\textsubscript{O}2} after exercise and with ΔLactate. Since tissue oxygenation depends on both the convective and diffusional oxygen transport processes, it is possible that ACE polymorphisms are associated with the disturbance not only of convective oxygen transport, but also of diffusional oxygen transport. The explanation of these findings would suggest the following mechanisms. One mechanism is that the muscle oxygen needs in patients with the DD genotype is significantly greater at any level of exercise than for patients with the II and ID genotypes. Thus, decreased levels of DO\textsubscript{2} for patients with the DD genotypes would result in a relative oxygen deficit for these patients requiring additional oxygen extraction and allowing the development of some larger degree of anaerobiosis. Another mechanism is selective muscle dysfunction with decreased peripheral muscle aerobic efficiency in patients with the DD genotype. Highly inefficient muscles would tend to utilize more oxygen at any level of exercise, and are more likely to cross anaerobic threshold early. Recently, the possibility that ACE may play a role in muscle deconditioning and weakness has been suggested in cardiac patients. However, further research will be required to clarify this issue.

There are several additional possible explanations for the observed association between ACE polymorphisms and disturbance in tissue oxygenation during exercise in COPD patients. Population stratification based on ethnicity or other factors could have contributed to disturbance in tissue oxygenation across genotypes. However, all of the subjects in this study...
were Japanese, so population stratification is less likely. In addition, it is also possible that the deletion polymorphism is associated with tissue oxygenation because this polymorphism is linkage disequilibrium with another causative variant in or near the ACE gene.

In conclusion, though this study had limited sample size, the ACE DD genotype might be associated with disturbance in peripheral tissue oxygenation during exercise in patients with COPD. Further studies are needed to determine whether improved tissue oxygenation with exercise after ACE inhibitors or angiotensin II receptor antagonists is associated with ACE genotype-based variation in response to these therapy in patients with COPD.

REFERENCES


8 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987; 136:225–244


