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[Original Article]

EFFECT OF ORAL L-ARGININE ADMINISTRATION ON EXHALED NITRIC OXIDE (NO) CONCENTRATION IN HEALTHY VOLUNTEERS

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Abstract : We previously reported a case of pulmonary hypertension, where the symptoms were improved by oral L-arginine (arginine) administration. Arginine may increase nitric oxide (NO) production in the pulmonary artery. Exhaled NO may reflect pulmonary artery NO production. It has been demonstrated that exhaled NO concentration is higher in patients with allergic diseases, but whether oral arginine administration alters exhaled NO is unknown. Therefore, in this study, we investigated whether oral arginine administration increases exhaled NO among healthy volunteers with and without a history of allergy.

Eleven subjects were given a single oral dose (200 mg/kg) of arginine, and their plasma arginine concentrations and exhaled NO were measured up to 150 minutes. Baseline values of exhaled NO concentration were significantly higher in those with a history of allergy (56.4 ± 20.3 ppb, $n=5$, $P<0.05$) than those without (16.8 ± 4.0 ppb, $n=6$). Oral arginine increased exhaled NO, which peaked at 60 minutes after the administration in those with a history of allergy (85.2 ± 44.8 ppb, $n=5$). However, the increase in exhaled NO was not significant compared to the baseline values. In contrast, plasma arginine concentration was increased significantly by arginine administration ($P<0.01$), regardless of an allergy history. These results suggested that the difference in exhaled NO concentration was not due to a difference in arginine absorption.

Serum IgE level was significantly higher in the group with a history of allergy. Eosinophils and white blood cells were within normal range in all subjects. We conclude that oral arginine administration does not significantly increase exhaled NO, regardless of allergy history. However, as arginine administration has been reported to be effective in patients with pulmonary hypertension, it will be necessary to test exhaled NO in subjects with pulmonary hypertension in the future.

Key words : Pulmonary hypertension, Healthy volunteers, L-Arginine, Exhaled NO, Allergy

INTRODUCTION

Nitric oxide (NO) is produced by nitric oxide synthase (NOS) in vascular endothelial cells and platelets, and has inhibitory effects on platelet aggregation and vasodilation¹⁻³. NOS is also expressed in the trachea and bronchial epithelia, where NO is produced and relaxes the airway smooth muscles to expand the airway⁴. L-arginine

(arginine) is a substrate for NOS to produce NO *in vivo*⁵. We previously reported a case of pulmonary hypertension, in which arginine administration was therapeutically effective⁶. As for the mechanism, there is a possibility that NO production in the pulmonary artery was enhanced by administration of arginine, and the produced NO relaxed the pulmonary artery⁷. The NO inhalation therapy has been applied clinically for pulmonary hypertension of the

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newborn, for which the public health insurance was approved in Japan since 2010^{8,9}. Furthermore, since the breath NO concentration increases significantly in inflammatory airway diseases, including allergic bronchial asthma, the exhaled NO concentration has attracted attention as an indicator of inflammatory airway diseases in recent years^{10,11}.

If NO production in the pulmonary artery is enhanced by arginine, there is a possibility that oral arginine administration will increase exhaled NO concentration. To verify that the pulmonary artery is extended by produced NO, it is necessary to measure the pulmonary arterial pressure. However, the accurate measurement of pulmonary artery pressure requires the insertion of cardiac catheter, which is invasive to the patients. If the exhaled NO concentration can be a measure of the efficacy of arginine, it will be useful and non-invasive.

Therefore, in this study, we administrated arginine orally to healthy volunteers and measured exhaled NO concentrations by chemiluminescence analyzer to investigate whether exhaled NO can be a useful measure to evaluate effectiveness of arginine administration. In addition, we also investigated whether the history of allergy is related to the exhaled NO concentration after arginine administration, because it could affect the exhaled NO concentration. The volunteers were divided into two groups according to the presence or absence of history of allergy by interview. We analyzed the time course of exhaled NO concentration after the oral administration of arginine.

MATERIALS AND METHODS

This study was conducted with the approval of the Ethics Committee of Fukushima Medical University. (Approval number 1058)

This study was carried out in 11 healthy volunteers (non-smokers) after receiving the written consent. Their age ranged over 22–60 years, and the average age was 29.5 ± 12.6 years (Mean \pm SD). Five of the 11 subjects had allergic history (infantile asthma : 2, hay fever, atopic dermatitis, allergic rhinitis : 1 each). The subjects fasted since the previous evening throughout the end of the study, and all the examinations were conducted in the morning.

L-arginine hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka) was dissolved in water, and a solution of 10 g/100 mL was administered to each subject as a single oral dose of 200 mg/kg. Exhaled NO concentration was measured after arginine administration at the following time points ; before

(0 min), and 30, 60, 120, and 150 minutes after the administration. Chemiluminescence analyzer (Kimoto Electronic Industrial Co., Ltd., Osaka, Japan) was used to measure the exhaled NO concentration¹². Blood pressure and the heart rate were measured immediately before each measurement of exhaled NO. Plasma arginine was measured using a high performance liquid chromatography (LC-2000 JASCO Tokyo, Japan) to detect the concentration changes at each time point after oral administration of arginine¹³. White blood cells, eosinophils, and serum IgE levels were measured in the blood collected before arginine administration.

All the data were expressed as mean \pm SD. The paired *t* test was used for the statistical analysis. Student's *t* test was used for the statistical analysis of white blood cells, eosinophils, and serum IgE levels.

RESULTS

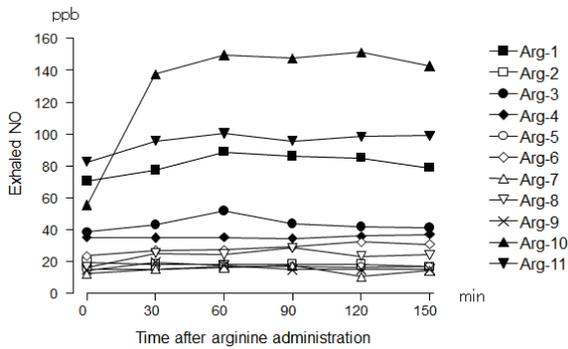
Five of the 11 subjects had a history of allergy, but none of them was under any treatment at the time of this study. In addition, no subject had any symptoms on the day of the test, or the history of treatment during the two weeks before the study.

Figure 1A shows exhaled NO concentration before and after the oral arginine administration over each time point in all subjects.

The exhaled NO concentrations of the five subjects with history of allergy were higher than those of the six subjects without a history of allergy at all time points measured. Figure 1B shows the average values of the exhaled NO concentrations of each group ; with (+) and without (–) history of allergy. The mean concentrations of exhaled NO were significantly higher in the history of allergy (+) group before arginine administration and at all time points after arginine administration. However, in either group at all time points, the exhaled NO concentration did not increase significantly after arginine administration compared to the baseline.

To investigate the correlation between the plasma arginine and exhaled NO concentrations, we measured the plasma concentration of arginine. Figure 2A illustrates changes in the plasma arginine concentration in all subjects. The average values of the plasma arginine concentration in the two groups are shown in Figure 2B. Regardless of a history of allergy, the plasma arginine concentration was significantly elevated by oral arginine administration. Therefore, there was no correlation between exhaled NO and plasma arginine

A



B

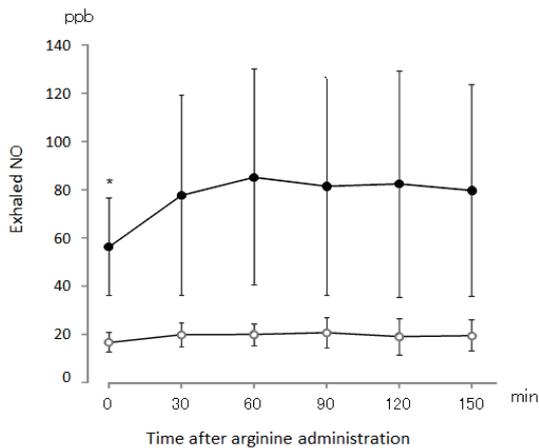


Fig. 1. Exhaled NO concentrations before and after oral administration of arginine.

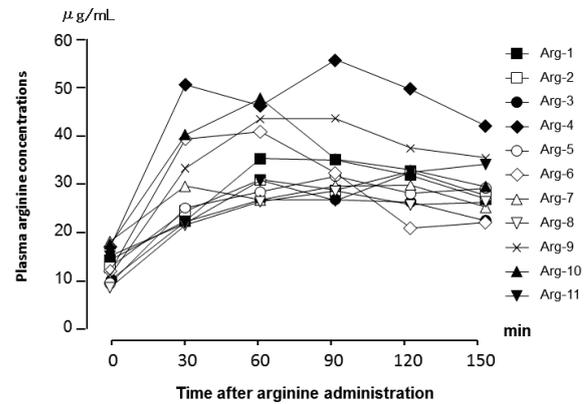
A. Each symbol indicate the data of 11 different subjects. Exhaled NO was measured at 30 minute intervals. Open symbols indicate the data from subjects without history of allergy ($n=6$). Closed symbols and a cross indicate data from subjects with history of allergy ($n=5$). Exhaled NO concentrations are indicated by parts per billion (ppb).

B. The mean NO concentrations of exhaled NO calculated from the data in Fig. 1A for each group. Open symbols indicate the data of subjects without history of allergy ($n=6$). Closed symbols indicate those with history of allergy ($n=5$). Data are indicated as mean \pm SD (* $P < 0.05$, versus history of allergy (-), student's t test).

concentrations. This was also indicated by the different order of the subjects for the plasma arginine (shown in Figure 2A), and for the exhaled NO concentrations (shown in Figure 1A).

The blood pressure and the heart rate of the subjects were also measured, because there was a possibility that arginine administration induced systemic vascular NO production, dilated the blood ves-

A



B

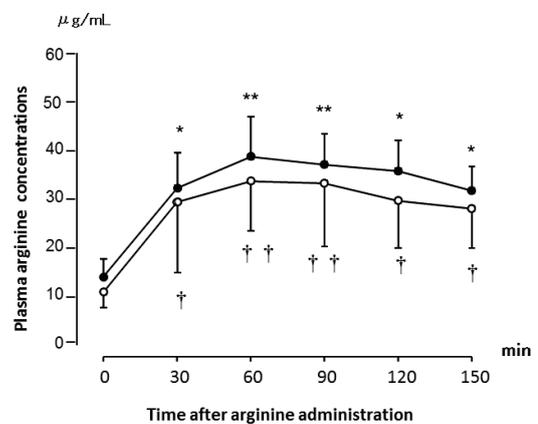


Fig. 2. Plasma arginine concentrations before and after administration of arginine.

A. Each time point value of plasma arginine from all 11 subjects. Symbols are the same as in Fig. 1. Open symbols indicate the data from subjects without history of allergy ($n=6$). Closed symbols and a cross indicate data from subjects with history of allergy ($n=5$). B. Mean plasma arginine concentrations for the two groups. Open symbols indicate the data from subjects without-history of allergy ($n=6$). Closed symbols indicate data from subjects with history of allergy ($n=5$). Data are indicated as mean \pm SD (*, $\dagger P < 0.05$, **, $\dagger\dagger P < 0.01$ versus initial value, paired t test).

sels and lowered the blood pressure. Figure 3A shows the time course of the changes in the blood pressure. After oral administration of arginine, neither systolic or diastolic blood pressure changed significantly. Therefore, we concluded that the arginine administration did not affect systemic blood pressure. In addition, there was no association between the blood pressure and the presence or absence of history of allergy. As shown in Figure 3B, oral arginine did not significantly change the

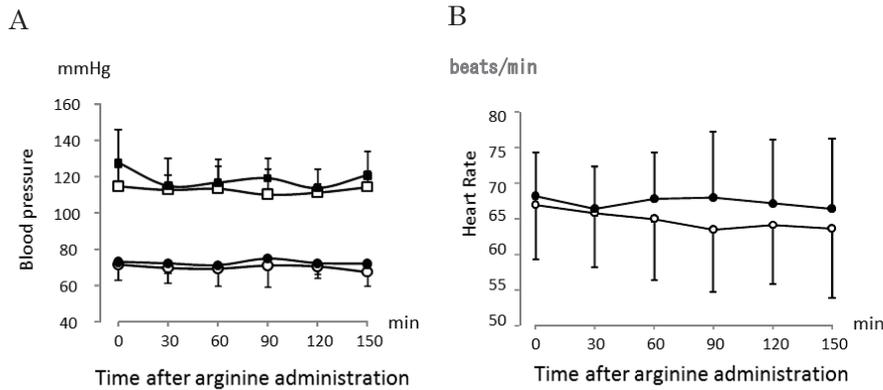


Fig. 3. Mean blood pressure (A) and heart rate (B) before and after administration of arginine. A. The average systolic (squares) and diastolic (circles) blood pressure. Open symbols indicate the data from subjects without history of allergy ($n=6$). Closed symbols indicate data those with history of allergy ($n=5$). B. The average heart rate before and after oral administration of arginine. Open symbols indicate the data from subjects without history of allergy ($n=6$). Closed symbols indicate those with history of allergy ($n=5$).

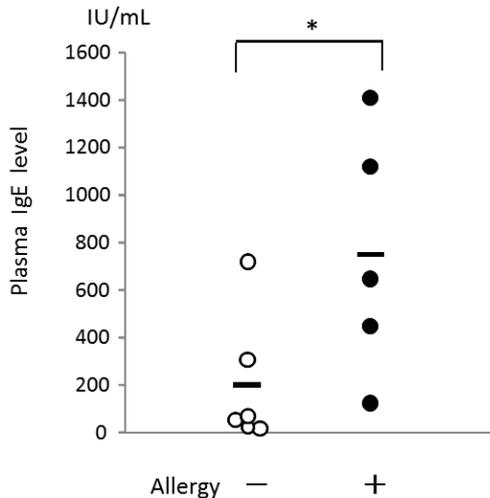


Fig. 4. Comparison of serum IgE levels between the two groups. Open circles indicate the data from those without history of allergy ($n=6$). Closed circles indicate those with history of allergy ($n=5$). Bars indicate the average value. Serum IgE levels are indicated by International Unit (IU). (* $P<0.05$, versus history of allergy (-), student's t test).

heart rate. No association was found between the heart rate and the presence or absence of history of allergy.

Serum IgE levels were measured to objectively evaluate the presence or absence of allergy. Figure 4 shows the serum IgE levels of all subjects measured before taking oral arginine. The mean value of IgE was significantly higher in the subjects with history of allergy than those without.

In addition to IgE, eosinophils and white blood cells were also measured to identify the presence or

absence of inflammation and allergic reactions of the subject on the day of examination. White blood cells (Figure 5A) and eosinophils (Figure 5B) prior to arginine administration were within the normal range in all subjects. These results indicated that there was no inflammation or allergic reaction in the subjects on the day of the examination. No significant differences were found in the numbers of white blood cells and eosinophils between subjects with the presence and absence of history of allergy.

DISCUSSION

In this study, we found that exhaled NO concentration tended to increase after administration of arginine in four of five subjects with a history of allergy (1A Figure). However, the mean exhaled NO concentration did not increase significantly from the initial value in both allergy (-) and (+) group at all measured points after administration of arginine.

Since plasma arginine concentration increased after oral administration in both groups, the absorption capacity of arginine from the intestinal tract must be similar between the two groups (Figure 2). The plasma arginine concentration reached peak at 60 minutes, and then gradually decreased after oral arginine administration. This change in the plasma concentration of arginine is consistent with other reports^{14,15}.

Therefore, there is a possibility that the expression of airway epithelial NOS is higher in the subjects with history of allergy. This might be a reason for the different concentration of exhaled NO depending on the presence or absence of personal history of allergy. There are 3 types of nitric oxide

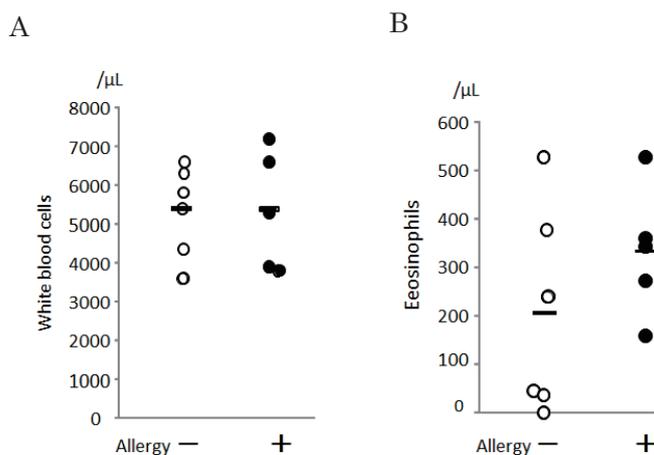


Fig. 5. White blood cells (A) and eosinophils (B) in the peripheral blood obtained immediately before administration of arginine. Open circles indicate the data from subjects without history of allergy ($n=6$). Closed circles indicate the data from those with history of allergy ($n=5$). Bars indicate the mean value.

synthase (NOS)^{16,17}; i.e. nNOS, eNOS, and iNOS. eNOS is expressed in vascular endothelium, and nNOS in the nervous system. iNOS expression is induced by inflammation and allergic reactions^{18,19}.

In the present study, there was no inflammation that causes an increase in white blood cells, because the numbers of white blood cells of all subjects were within the normal range (Figure 5A). In addition, the subjects had a little possibility of allergic reactions, since the numbers of eosinophils were within the normal range in all subjects (Figure 5B). However, the average values of exhaled NO and plasma IgE were significantly higher in the group with a history of allergy (Figure 1B, 4). Thus, there is a possibility that the five subjects with allergy history have potential allergic diseases. It has already been reported that the concentration of exhaled NO increases in patients with allergic bronchial asthma^{18,20,21}.

Subject # 11, who showed the highest exhaled NO concentration, had a history of asthma during childhood. This subject also had the highest number of eosinophils and IgE in the present study.

After administration of arginine, the blood pressure and the heart rate did not change significantly regardless of the presence or absence of allergy history. Therefore, it can be suggested that arginine administration does not alter systemic circulation in healthy subjects. The subjects did not develop any adverse physical manifestations, such as gastrointestinal symptoms. Therefore, we think that the oral administration of arginine is safe.

Since NO relaxes the airway smooth muscle, oral administration and inhalation of arginine has been tested for a treatment of bronchial asthma, and

an increase in the concentration of exhaled NO and the improvement of forced expiratory volume have been reported^{22,23}. Furthermore, in cystic fibrosis patients with airway obstruction, inhalation and intravenous administration of arginine significantly increased exhaled NO concentration compared to placebo, and the respiratory function was improved^{19,24}.

Efficacy of oral administration of 0.5 g/10 kg arginine has been suggested not only for allergic airway obstructive disease, but also for pulmonary hypertension due to occlusion of the pulmonary artery⁷.

In the present study, oral arginine administration did not significantly increase exhaled NO concentration in healthy subjects. However, the reports on the effectiveness of arginine for patients with pulmonary hypertension^{6,7,25}, suggest that the production of NO by arginine administration in the airways and the pulmonary artery may be enhanced in patients with pulmonary hypertension. It is necessary to examine whether oral arginine increases exhaled NO in patients with pulmonary hypertension in the future.

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