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Effect of Ascorbic Acid on Bronchoconstriction Induced by Methacholine Inhalation Challenge in Healthy Subjects

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ABSTRACT. We investigated the effect of ascorbic acid (1.0 g orally) on bronchoconstriction induced by methacholine inhalation challenge in five healthy subjects. We also measured the serum concentration of ascorbic acid on the control day and on the ascorbic acid day. The serum ascorbic acid levels in all subjects increased on the ascorbic acid day. The protective effect of ascorbic acid against bronchoconstriction was demonstrated in three subjects. The results suggest an anti-bronchoconstrictive action of high dose ascorbic acid in some cases.

Key words : Ascorbic acid — Methacholine challenge — Bronchoconstriction

Ascorbic acid is a water-soluble vitamin, $C_6H_8O_6$, present in citrus fruits, and in tomatoes, strawberries, and many other fruits and vegetables; it is also made synthetically.[1] Deficiency of ascorbic acid produces scurvy (the name "ascorbic" comes from this reason), and ascorbic acid is called also antiscorbutic vitamin and vitamin C. In the early 13th century, a variety of fruit and vegetables were prescribed as the diet of asthmatics, and in 1803 Reisseissen noted that convulsive asthma occurred in patients with severe scurvy.[2] There is a possibility that ascorbic acid may be useful in the treatment of bronchial asthma, but the role of ascorbic acid in asthma has long been debated. The investigations by Hunt[3] have not shown ascorbic acid to be of any value in the treatment of asthma On the other hand, Anah *et al.*[4] concluded that high dose ascorbic acid is probably a good prophylaxis in some bronchial asthmatics. Recently Ogilvy *et al.*[5] demonstrated the effect of ascorbic acid on bronchoconstriction induced by methacholine aerosol in healthy, nonsmoking men.

The purpose of the present study was to test the effect of ascorbic acid on bronchoconstriction induced by methacholine inhalation challenge in healthy subjects and measure the serum concentration of ascorbic acid with and without the oral intake of ascorbic acid.

MATERIALS AND METHODS

Five healthy, nonsmoking subjects (2 men, 3 women ; mean age, 25.2 years) participated in the study (Table 1). All subjects refrained from taking vitamin C except for food-containing ascorbic acid for one month before the studies

川根博司，副島林造，松島敏春，矢木　晋，原　宏紀，渡辺正俊

TABLE 1. Characteristics of the subjects.

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	%VC (%)	$FEV_{1.0}$% (%)
S.K.	F	25	161	56	139	85
F.K.	F	25	152	46	97	96
S.G.	F	25	165	52	121	93
S.M.	M	25	172	68	122	96
T.S.	M	26	168	66	120	88

began. They were also requested to refrain from having coffee and tea for at least 12 hours before each study day. Informed consent was obtained from each subject.

Forced expiratory volume curves and maximal expiratory flow-volume curves were measured with rolling seal spirometer (PK Morgan), and forced vital capacity (FVC), forced expiratory volume in one second ($FEV_{1.0}$), flow at 50% of FVC ($\dot{V}_{50}$), and flow at 25% of FVC ($\dot{V}_{25}$) were calculated. On the first day (control day), after baseline pulmonary function measurements were performed, subjects were exposed to methacholine aerosol in increasing doses (1.56, 3.13, 6.25, 12.5, 25.0, and 50.0 mg methacholine base/ml saline). The aerosol was produced by a Nissho nebulizer and air compressor set. Subjects inhaled the aerosol at tidal volume breathing for one min. Each subject underwent inhalation challenge with increasing doses of methacholine every 10 min. Forced expiratory maneuvers were performed immediately and at 10 min after each challenge. Inhalation challenge was stopped if the $FEV_{1.0}$ was decreased by 20% from its control value or if the subject developed respiratory symptoms.

On the second day (ascorbic acid day), each subject ingested ascorbic acid (1.0 g) 2 hr prior to methacholine challenge. Pulmonary function was again measured before and after the challenge. Pre-challenge pulmonary function data measured on each day for all subjects appear in Table 2. The dose-response curve of $\dot{V}_{50}$ to methacholine was generated for each subject. The index for airway response to methacholine was expressed as the provocation concentration causing a decrease in $\dot{V}_{50}$ of 25% ($PC_{25}\dot{V}_{50}$). (See Fig. 1.)

Blood samples were taken shortly before the initial methacholine inhalation

TABLE 2. Pre-challenge pulmonary function on each day.

Subject	FVC (l)		$FEV_{1.0}$ (l)		$\dot{V}_{50}$ (l/sec)		$\dot{V}_{25}$ (l/sec)	
	Control day	Ascorbic acid day	Control day	Ascorbic acid day	Control day	Ascorbic acid day	Control day	Ascorbic acid day
S.K.	4.32	4.40	3.67	3.70	3.72	3.91	1.69	1.58
F.K.	2.84	2.62	2.74	2.56	4.57	5.20	1.93	2.04
S.G.	3.84	3.75	3.56	3.62	4.34	4.30	2.32	2.87
S.M.	5.21	5.00	5.01	4.88	6.79	7.52	3.87	4.01
T.S.	5.00	4.87	4.42	4.45	6.20	6.21	2.36	2.82
mean	4.24	4.13	3.88	3.84	5.12	5.43	2.43	2.66
±S.D.	0.95	0.97	0.87	0.89	1.31	1.47	0.84	0.93

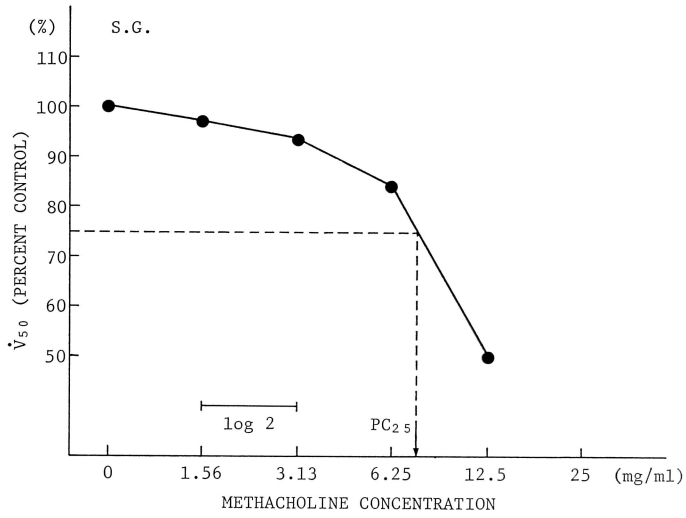

Fig. 1. Dose-response curve to inhaled methacholine in one subject. A best-fit straight line may be drawn and the provocation concentration necessary for a 25% decrease in $\dot{V}_{50}$ (PC_{25} $\dot{V}_{50}$) interpolated.

on each day. Analysis of serum ascorbic acid levels was made using dinitrophenyl hydrazine technique (Mitsubishi Yuka Laboratory of Medical Science).

Furthermore, as for subject S.K., the bronchial hyperresponsiveness by the dose-response curve of respiratory resistance (Rrs) using the direct-writing recorder[6] (Astograph, Chest Co.) was also examined on other two separate days, the placebo (lactose) day and the ascorbic acid day. On the ascorbic acid day ascorbic acid (2.0 g orally) was administered without the subject knowing what it was. The measurements were made at 2 hr after the ingestion of each drug.

RESULTS

Oral administration of ascorbic acid had no significant effect on pre-challenge pulmonary functions (Table 2).

The $PC_{25}\dot{V}_{50}$ of methacholine measured on each day is shown in Table 3. The $PC_{25}\dot{V}_{50}$ on the ascorbic day was higher than that on the control day in

TABLE 3. $PC_{25}\dot{V}_{50}$ (mg/ml) of methacholine.

Subject	Control day	Ascorbic acid day
S.K.	< 1.56	1.8
F.K.	1.8	5.0
S.G.	9.1	8.4
S.M.	21.5	> 50
T.S.	> 50	> 50

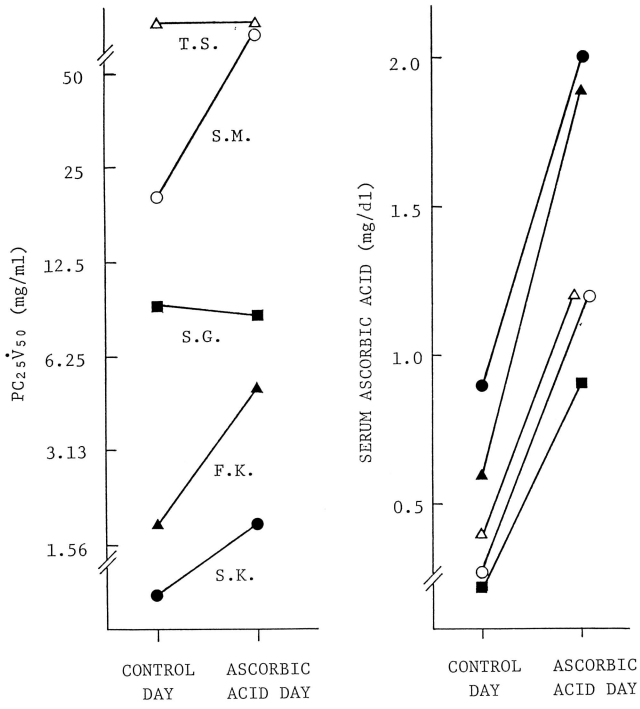

Fig. 2. Comparison of $PC_{25}\dot{V}_{50}$ with serum concentration of ascorbic acid. $PC_{25}\dot{V}_{50}$ increased in three subjects, while there was an increase of serum ascorbic acid in five subjects.

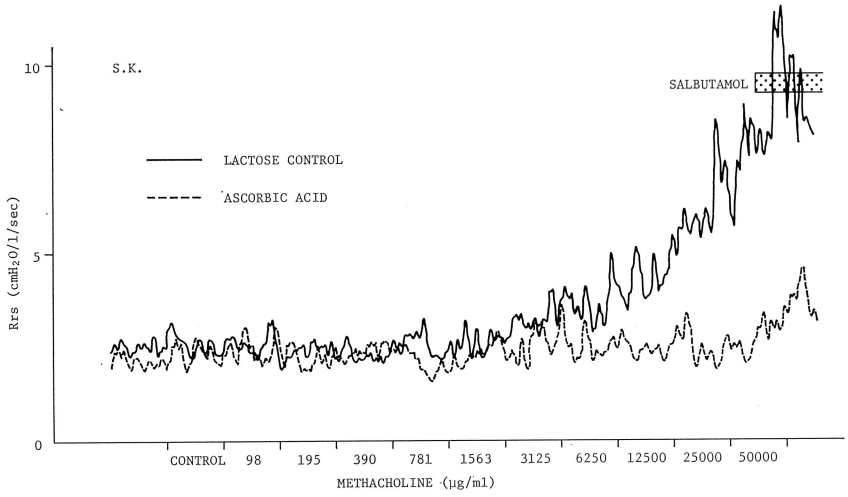

Fig. 3. Dose-response curves of subject S.K. using Astograph. With inhalation of incremental challenge of methacholine, respiratory resistance (Rrs) for lactose control increased curvilinearly.

3 subjects. Figure 2 displays the comparison between the changes of $PC_{25}\dot{V}_{50}$ and serum concentration of ascorbic acid. The serum ascorbic acid levels in all subjects were increased on the ascorbic acid day. And the ascorbic acid levels

in subjects S.K. and F.K. became above the normal values (0.3–1.4 mg/dl).

Figure 3 shows the dose-response curves of subject S.K. measured by Astograph. With inhalation of incremental challenge of methacholine, Rrs increased curvilinearly on the placebo day, while there was little increase on the ascorbic acid day.

DISCUSSION

Our results suggest that ascorbic acid modifies airway responsiveness to methacholine aerosol in some persons. Ascorbic acid administration significantly increased the serum concentration in all subjects, but the reduction of responsiveness to methacholine was demonstrated in 3 subjects. Two of them seemed to have airway hyperresponsiveness though they were non-asthmatics. In subject S.K. the effect of ascorbic acid on bronchoconstriction was demonstrated again by Rrs using Astograph.

Zuskin *et al.*[7] studied the effect of ascorbic acid (500 mg) on histamine-induced airway constriction in healthy subjects and concluded that ascorbic acid probably has a direct effect on airway smooth muscle. Recently Ogilvy *et al.*[5] demonstrated that both the duration and intensity of the bronchoconstriction induced by methacholine aerosol were reduced by prior administration of ascorbic acid (1.0 g) in healthy subjects. Since this ameliorating action of ascorbic acid was blocked by ingestion of indomethacin, they suggested that ascorbic acid exerted its effects by altering the production of a bronchodilator prostaglandin. More recently Mohsenin and coworkers[8] from the same laboratory reported the effect of ascorbic acid (1.0 g) in mild asthmatic subjects undergoing bronchial challenge with methacholine. Administration of 50 mg of indomethacin, orally, reversed the effect of ascorbic acid, and their results also suggest that ascorbic acid exerts its effect via alteration of arachidonic acid metabolism.

Schachter and Schlesinger[9] demonstrated a mild antibronchospastic action of ascorbic acid in subjects with exercise-induced bronchospasm. Four general mechanisms have been invoked to explain ascorbic acid's effects on smooth muscle contractile states.[9] These include (1) the effect of vitamin C in accelerating the metabolism of histamine, (2) the direct effect of vitamin C on smooth muscle, (3) vitamin C's effect on cyclic AMP metabolism and (4) the modulation of prostaglandin production by vitamin C. The last explanation is consistent with the observations by Ogilvy *et al.*[5] and Mohsenin *et al.*[8] The role of prostaglandins (PGs) and leukotrienes (LTs) in airway function has been the subject of intense investigation. The metabolites of arachidonic acid show very complicated actions, e.g. PGs vs. LTs, prostacyclin (PG I_2) vs. thromboxane A_2, and PG E_2 vs. PG $F_{2\alpha}$. The probable relationship of ascorbic acid to arachidonic acid metabolism may explain both the relatively small effect of ascorbic acid and the negative results of other studies. Kreisman *et al.*[10] failed to show the effect of ascorbic acid and suggested that the protective action of ascorbic acid may be temporary, occurring shortly after single high doses.

The oral administration of 1.0 g of ascorbic acid was not enough to elevate the serum concentration of ascorbic acid over the normal values in 3 of 5 subjects. This may indicate that some persons should take higher dose of

ascorbic acid in order to demonstrate the protective effect of ascorbic acid.

In summary, our study adds further support that ascorbic acid has the effect against bronchoconstriction. And ascorbic acid may be useful for the treatment of bronchial asthma in some cases.

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