Thrombomodulin in Exercise-Induced Asthma

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Thrombomodulin (TM) is a membrane protein present in the vascular endothelium. It has also been found in human plasma, within which, however, its pathological functions have not been clearly described. In this study, the plasma TM concentrations in 19 asthmatic patients were determined by sandwich enzyme immunoassay using two monoclonal antibodies for human TM. The concentration of plasma TM in exercise-induced asthma (EIA) – positive asthmatic patients was significantly increased by exercise challenge. In addition, for these patients a positive correlation was found between the severity of EIA and the degree of change in plasma TM induced by exercise challenge. These findings suggest that the increase in influx of TM into the plasma in EIA-positive asthmatics may be due to generalized pulmonary endothelial damage following exercise challenge.

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Introduction

Endothelial cells lining blood vessels possess anticoagulant properties, although they can rapidly become procoagulant when injured. The anticoagulant functions of these cells include production of prostacyclin (1), and the secretion of plasminogen activator (2), cell surface heparin-containing proteoglycans that stimulate certain coagulation factor inhibitors (3) and thrombomodulin (TM). TM, the membrane glycoprotein present on the vascular endothelial cell surface, plays an important role as a cofactor in the thrombin-catalyzed activation of protein C (4). TM converts thrombin from a procoagulant protease into an anticoagulant and acts as a regulator of intravascular coagulation.

Exercise-induced asthma (EIA) is a form of symptomatic bronchospasm that develops shortly after strenuous exercise. Most patients with asthma develop EIA when the exercise undertaken is of sufficient duration and intensity. Despite the wide prevalence and clinical significance of EIA, the mechanisms responsible for it have yet to be clearly described. Hyperpnea associated with exercise leads to increased airway water and heat loss, which contributes to the development of EIA (5, 6). Findings related to the participation of inflammatory mediators in either the maintenance or induction of bronchoconstriction evoked by exercise are conflicting. Some studies have reported elevations in plasma histamine in patients with EIA (7), but others have failed to verify these findings (8).

Similarly, the use of selective H₁-receptor antagonists and cyclooxygenase inhibitors has been found to result in either variable or conflicting degrees of attenuation of EIA (9). The author of a recent study concluded that thromboxane play little or no role in the pathogenesis of EIA (10). Recent findings have suggested that leukotrienes may play important roles in the pathogenesis of EIA (11). Evidence has yet to be obtained, however, confirming that airway microcirculation has the potential for playing such a role in the pathophysiology of EIA. The activation of pulmonary mast cells, lymphocytes and macrophages can lead to migration of inflammatory cells to airway microvessels (12), which induces progressive endothelial damage of pulmonary capillaries. It has been reported that TM is also present in human plasma (13); however, neither the physiological nor the pathological significance of circulating TM is known. Recent studies have indicated that TM is increased in the circulating blood of patients with thromboembolism, adult respiratory distress syndrome, chronic renal failure and acute hepatic failure (14). Hence, elevated levels of soluble TM may truly reflect progressive endothelial damage. In the present study, we measured the concentration of thrombomodulin in the plasma of asthmatic patients with or without EIA, and attempted to determine the role played by thrombomodulin in the pathogenesis of EIA.

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Methods

Subjects

Nineteen asthmatic patients, whose clinical characteristics are shown in Table 1, participated in this study. All patients gave their informed consent to this study. Patients who were free of severe asthma exacerbation for at least one month prior to the study, and who had not been undergoing treatment with oral corticosteroids at the time of study were included.

Table 1. Clinical Data of Asthmatic Patients

No.	Sex/Age	FEV _{1.0} (mL)	Atopy	IgE (U/ml)	E _{os} (mm ³)	PC ₂₀ (μg/ml)
EIA (+):						
1	M/54	1500	+	469	1280	313
2	F/50	2350	+	347	517	625
3	M/39	2510	—	281	864	313
4	F/26	2260	+	1208	1470	313
5	F/18	3050	+	4652	704	78
6	M/46	3240	—	61	522	1250
7	F/22	2610	+	1686	1020	313
8	F/43	1520	+	292	675	625
9	F/25	2100	—	126	118	313
EIA (—):						
1	F/66	1200	—	45	160	313
2	F/28	2290	+	680	59	625
3	M/40	2500	+	1400	580	625
4	M/55	1150	—	80	200	1250
5	F/56	1860	+	172	414	313
6	F/29	1850	—	74	1575	1250
7	M/22	1850	+	1747	1386	156
8	F/20	3020	—	150	460	625
9	F/24	3690	—	366	147	625
10	M/21	3480	+	5061	946	156

E_{os}: eosinophil number, FEV_{1.0}: forced expiratory volume in one second, PC₂₀: provocation concentration to cause a 20% fall in FEV_{1.0}.

Antiasthmatic drugs, if being used for treatment, were not administered during the twelve-hour period preceding the study. Each patient had been previously diagnosed as having bronchial asthma based on symptoms and reversibility of airflow limitation with bronchodilators. Methacholine inhalation challenge testing was performed on all subjects. All challenge tests were performed at approximately 1:00 PM to eliminate the effect of diurnal variation. Following baseline spirometry and inhalation of diluent to establish the stability of forced expiratory volume in one second (FEV_{1.0}), subjects were instructed to take slow inspirations of each set of inhalations. All of the asthmatic patients in this study demonstrated bronchial hyperreactivity to methacholine. Atopy was defined as the presence of a positive cutaneous prick test, and serum levels of total IgE were assessed by immunoenzymatic assay. In all asthmatics studied here, test results for liver and renal functions related to the clearance of thrombomodulin were normal.

Study protocol (Table 2):

Exercise challenge testing was performed on an electrically driven treadmill (Q55xt, Quinton Instrument Co. Japan) for six minutes with a fixed work load adjusted to increase the heart rate to 90% of the maximum predicted for the age (15) of each patient. All subjects breathed unconditioned room air (temperature 22° to 24°). Single lead ECG and pulse oximetry (502-US, CSI, Japan) were monitored continuously. Exclusion criteria were the presence of coronary disease or cardiac arrhythmias. A spirometer (CHESTAC-25, Chest, Japan) was used to obtain spirometric measurements before and after exercise challenge. The higher of two measurements of FEV_{1.0} obtained before exercise challenge was taken as the baseline value. Single measurements of FEV_{1.0} were obtained 1, 3, 5, 7, 10, 15 and 20 min after completion of exercise challenge. The response to exercise challenge was taken to be the ratio of the maximum decrease in FEV_{1.0} after exercise to the baseline value:

Table 2. Protocol of Exercise Provocation Test

$$\% \text{ fall in FEV}_{1.0} = \frac{[\text{FEV}_{1.0} (\text{baseline}) - \text{FEV}_{1.0} (\text{after})]}{\text{FEV}_{1.0} (\text{baseline})} \times 100$$

Those patients whose maximum decrease in $\text{FEV}_{1.0}$ was greater than 20% were considered as EIA-positive asthmatics. The maximum fall in $\text{FEV}_{1.0}$ ($\Delta\text{FEV}_{1.0 \text{ max}}$) and the post-exercise time required for maximum bronchoconstriction (ΔTime) were used as parameters of the severity of EIA (Table 3). EIA-positive groups and EIA-negative groups were matched according to the age and sex of the patients involved.

Thrombomodulin assay

Plasma was obtained from asthmatic subjects before and after exercise challenge. Plasma samples were stored at -40°C until assay. The concentration of TM was determined by

sandwich enzyme immunoassay using two monoclonal antibodies for human thrombomodulin coupled to peroxidase, as previously described (16, 17).

Statistical analysis

Results were expressed as mean $\pm$ SD and group means were compared using Student's *t* test. Correlations were determined using the Spearman correlation test. A *p* value < 0.05 was considered to indicate statistical significance.

Results

The concentration of plasma TM in EIA-positive asthmatics tended to be lower than in EIA-negative asthmatics, although the difference between the two groups was not statistically significant (Fig. 1). The concentration of plasma TM in EIA-positive asthmatics was increased by exercise challenge. On the other hand, the concentration of plasma TM in EIA-negative asthmatics was significantly decreased by exercise challenge (Fig. 2). In EIA-positive asthmatics, there was a positive correlation between the severity of EIA and the degree of change in plasma TM. Furthermore, for these patients the

Table 3. Evaluation of Exercise Provocation Test

Fig. 1. Comparison of plasma thrombomodulin concentration between the EIA-negative group and EIA-positive group.

Fig. 2. Comparison of the degree of change of thrombomodulin concentration following exercise challenge between the EIA-negative group and EIA-positive group.

Fig. 3. Correlation between the maximum change of $FEV_{1.0}$ from baseline ($\Delta FEV_{1.0 \max}$) and the degree of change of plasma thrombomodulin concentration in the EIA-positive group.

Fig. 4. Correlation between the post-exercise time until maximum bronchoconstriction ($\Delta Time$) and the degree of change in plasma thrombomodulin concentration.

degree of change in plasma TM was positively correlated with maximum change in $FEV_{1.0}$ ($\Delta FEV_{1.0 \max}$) and negatively correlated with the post-exercise time required for maximum bronchoconstriction ($\Delta Time$, Figs. 3, 4).

Discussion

Although the origin of plasma TM remains unclear, there appears to be two possible explanations for the presence of this soluble TM in plasma. One is that plasma TM is produced in unidentified cells and secreted into the circulating blood. It seems likely, however, that TM is not released from endothelial cells under normal physiological conditions, since it is originally a component of the plasma membrane of these cells, and the circadian fluctuation in plasma TM levels is very small (18). The second explanation is that the plasma TM level reflects the degree of proteolysis of endothelial membrane TM occurring in association with endothelial damage. Iwashima et al reported that elevation of TM levels might truly reflect progressive endothelial damage (19). The concentration of TM in EIA-positive asthmatics was low compared with that in EIA-negative asthmatics prior to exercise challenge, although the difference between these two groups was not significant. Recently discovered high spontaneous rates of secretion of inflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF), by unstimulated leukocytes suggest that these cytokines may act locally as pathogenic agents in asthmatic patients (20, 21). These cytokines influence the expression of TM on the endothelial cell surface. Both IL-1 and TNF have been found to down-regulate the expression of TM in cultured human endothelial cells (22, 23). TNF also induces endocytosis and lysosomal degradation of TM, resulting in a reduction of TM activity on the cell surface (23). TNF inhibits the transcription of TM mRNA, and the subsequent decrease in TM protein synthesis may also

contribute to the decline in TM activity.

In the present study, TM levels were increased following exercise challenge in EIA-positive asthmatics, and a significant degree of correlation change of TM existed between values obtained following exercise challenge and the severity of EIA. These findings suggest that the increased influx of TM into the circulating blood in these patients may be caused by pulmonary endothelial damage following exercise challenge. Circulating inflammatory cells have been clearly implicated in the pathogenesis of bronchial asthma (24). Neutrophils stimulated by a variety of agents including TNF and IL-1 have been shown in vitro to damage endothelial cells by the generation of hydrogen peroxide and superoxide radicals.

Neutrophil-derived proteases may also participate in the injury of endothelial cells. In asthmatics, it is possible that proteases released from neutrophils previously adhering to endothelial cells split the surface TM, releasing it into the circulation. We found that TM levels were decreased following exercise challenge in EIA-negative asthmatics. TM is capable of promoting the inactivation of thrombin by antithrombin; it is removed from the circulating blood by binding with thrombin, which inhibits its multiple cell activating functions. Thrombin is the most potent stimulator of platelets. It has previously been reported that participation of platelets in the pathogenetic processes of clinical asthma accompanies acute allergen-induced bronchospasm and exercise-induced bronchospasm (25). A variety of other thrombin activities on cells have been defined, primarily in vitro. Thrombin is chemotactic for monocytes (26), and is mitogenic for lymphocytes and for mesenchymal cells including vascular smooth muscle cells (27). It is also known to have a variety of effects on the vascular endothelium, including stimulation of endothelial production of platelet activating factor (PAF) (28) and endothelins (29).

Thrombin also induces adherence of neutrophils to the

vessel wall by an endothelium-dependent mechanism, probably by inducing the surface expression of GMP-140 (30). Theoretically, these many cell-activating functions of thrombin can be understood as orchestrating the response to vascular injury, potentially mediating not only hemostatic but perhaps also inflammatory and proliferative or reparative responses. Most notably, increased concentrations of circulating eosinophilic granule proteins are present in bronchoalveolar lavage fluid (BALF) obtained from asthmatic patients; these eosinophilic granules are known to impair TM functions (31). Since most immunohistochemical stains for TM concentrate within the endothelium of the lung microvasculature, we suspect that the lung contains high levels of TM. Therefore, a high level of TM in the lung may be a good marker for the presence of pulmonary endothelial damage. Interestingly, it has recently been reported that many types of agents which increase cAMP levels can also enhance the expression of TM (32). Antiasthmatic drugs that increase intracellular cAMP levels, including β -stimulants and theophylline, also increase TM activity and prevent IL-1 and TNF-induced decreases in TM expression. In conclusion, the present findings suggest that the increase in influx of TM into the plasma in EIA-positive asthmatics may be due to generalized pulmonary endothelial damage following exercise challenge.

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