[総説] Physiological significance of protease in airway secretion and the control of inflammation in airway tract by protease inhibitor

Physiological significance of protease in airway secretion and the control of inflammation in airway tract by protease inhibitor

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ABSTRACT

We review the action of protease in airway inflammation from the standpoint of the protease-antiprotease system. The tracheobronchial secretion without inflammation has an abundant protease, plasminogen activator (PA), which plays an important role in the continuous opening of the tracheobronchial lumina. Immediately after inflammation occurs in the airway tract, the protease activity is increased in the airway secretion, as compared to the protease activity in other organs. On the other hand, increased protease secretion, plays a strong role in the enlargement and exacerbation of the inflammation. In physiological inflammation, the increased protease activity is mediated and inhibited by protease inhibitor which is concomitantly elevated at the inflammatory locus. In inflammation of the airway tract, therefore, the application and supply of protease inhibitor to the airway tract, should cause the inflammation to subside. We consider that the role of protease inhibitor in limiting the inflammation of the airway tract is of clinical importance. *Ryukyu Med. J., 15(3)121~126, 1995*

Key words: protease, antiprotease, plasminogen activator, tracheobronchial secretion

INTRODUCTION

We have analyzed the process of airway inflammation from the standpoint of the protease-antiprotease system[1~3]. The process of airway inflammation is modified by the relative activities of protease and antiprotease under physiological conditions. The airway tract as part of the respiratory system is always open to the passage of air, oxygen and CO_2. If the airway tract becomes obstructed mechanically or chemically, respiratory function is interfered with and dyspnea appears. It was inferred that a specific and vital mechanism to maintain a certain size of inner diameter of the tract may operate within the airway tract[4]. This would be analogous to the situation in blood vessels, where thrombus formation is disadvantageous to the maintenance of a certain flow of blood and thrombolytic mechanisms act in the blood vessels to prevent thrombus formation. From the above-mentioned considerations, it was anticipated that plasminogen activator, a thrombolytic protease, would occur abundantly in the airway secretion.

The protease activity is elevated under physiological conditions, and examinations of the protease-antiprotease system in the airway secretion without inflammation have shown that the protease activity is already relatively increased before the development of any inflammation. In an attempt to clarify the defense mechanism of the airway tract, the physicochemical properties of proteases in the airway secretion have been analyzed in relation to the control of respiratory diseases. Moreover, disorders of ciliary movement in the airway tract and the physicochemical properties of the airway secretion have been investigated using experimental animals and patients with asthma[5,6]. In particular, the relationship between epithelial damage and the pathogenesis of atopic respiratory disease was observed in human asthma[7,8].

In this paper, we summarize the literature concerning physicochemical analysis of the airway secretion and the protease-antiprotease system in the airway secretion in relation to the control of inflammation in respiratory organs and the respiratory tract.

ELECTROLYTES AND PH OF AIRWAY SECRETION

In the airway secretion with bronchiectasia, chronic bronchitis and lung cancer, the concentration of sodium has been reported to be 90.3 ± 18.4mEq/L, 142 ± 11.4 mEq/L, and 131.8 ± 26.4mEq/L, respectively[9]. In addition, the concentration of potassium was elavated in the airway secretion with pus. In animal experiments using dogs, the airway secretion with experimentally-induced infection revealed an increased concentration of potassium, as in the airway secretion of humans. On the other

hand, stimulation of the autonomic nervous system and smoking increased the concentrations of sodium and chloride, as compared to those in the airway secretion with inflammation. Furthermore, a decrease of potassium and elevation of pH were noted in the airway secretion with stimulation of the autonomic nervous system and smoking. The above findings indicate that the concentration of potassium was increased in the airway secretion with inflammation but decreased in the airway secretion with stimulation of the autonomic nervous system, while the pH was decreased in the airway secretion with inflammation but increased in the airway secretion with stimulation of the autonomic nervous system. It was expected that protease and antiprotease with pH stability under acidic conditions would be activated in the airway secretion with inflammation of the airway tract. In addition, based on animal experiments, it has been clarified that sodium ion and chloride ion in the airway secretion derive from the goblet cells along the airway tract, while potassium ion is derived from the submucosal glands. Thus, an increased concentration of sodium and chloride in the airway secretion may be associated with proliferation of the goblet cells, and an increased concentration of potassium may be associated with hypersecretion of the submucosal glands.

PROTEASE AND ANTIPROTEASE IN AIRWAY SECRETION

Masaoka et al, analyzed the protease and antiprotease in airway secretion obtained from the orifice of tracheostomy in human patients[10]. Rudnik et al, studied the protease inhibitor in airway secretion with nonspecific respiratory disease or specific respiratory disease in infants[11]. Rudnik et al, indicated that the concentration of protease inhibitor in the circulating blood and that in the airway secretion in the above-mentioned types of diseases were positively correlated[11]. Subsequently, it was clarified that the protease inhibitor in the airway secretion was derived from the circulating blood by means of transudation and the submucosal gland through hypersecretion. The main protease inhibitors in the airway secretion were α_1-antitrypsin and α_2-macroglobulin from the circulating blood. It has been reported that a decrease in trypsin inhibitor was observed in nasal secretion with acute inflammation[12]. The above findings suggest that consumption of trypsin inhibitor to neutralize the activity of trypsin occurred leading to a decrease in trypsin inhibitor. This inhibitor is supplied by and secreted from the nasal and paranasal mucous membrane.

PLASMINOGEN ACTIVATOR (PA) IN TRACHEOBRONCHIAL SECRETION OF RATS

PA has been reported to exist in the tracheobronchial secretion of rats[13]. In addition, PA of low molecular

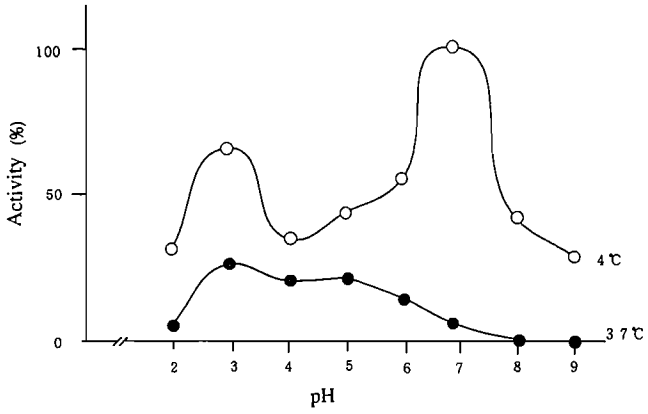

Fig.1 The pH- and thermostability of PA in tracheobronchial secretion[13] Throughout the experiments, the PA activity of pH 6.8 and 4℃ was taken as 100%. pH was adjusted with 0.1 N HCl or 0.1 N NaOH. Ordinate: Activity of PA on standard fibrin plates (%) Abscissa: Change of pH ○ : PA activity at 4℃ ● : PA activity at 37℃

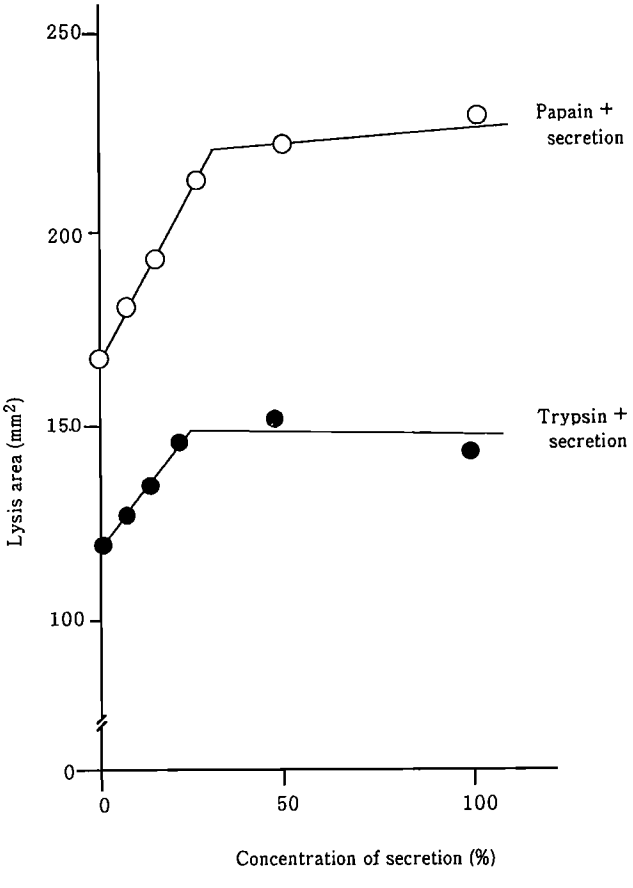

Fig.2 Effect of dilution on the activity of papain and trypsin in tracheobronchial secretion[16]. The secretion was diluted with the phosphate-buffer (0.07M, pH6.8). Ordinate: Lysis area on plasminogen-free fibrin plates (mm^2). Abscissa: Concentration of secretion (%) ○ : Activity of papain in the secretion ● : Activity of trypsin in the secretion

weight has been identified in the tracheobronchial secretion of rats[13]. The chemical properties of this PA with low molecular weight were similar to those of PA with low molecular weight derived from the paranasal mucous membrane[14,15], and these PAs of low molecularweightwere thermolabileatneutralpH[13] (Fig. 1). PA was found to be a latent protease, and the activated protease did not exist in the tracheobronchial secretion of rats without inflammation. In addition, plasmin inhibitor, papain inhibitor and trypsin inhibitor were not observed in the tracheobronchial secretion of rats without inflammation[16,17] (Fig. 2). Based on electrophoretic analysis, glycoprotein could not be identified in the tracheobronchial secretions of rats. α_2-Macroglobulin and α_1-antitrypsin transudating from the circulating blood did not exist in noninflammatory tracheobronchial secretions of rats[18]. PA, as an inactivated protease, which can activate plasminogen, is thus present in the tracheobronchial secretion of rats.

REGULATION OF PA IN TRACHEOBRONCHIAL SECRETION OF RATS

Administration of noradrenaline to rats temporarily increased the blood pressure and the fibrinolytic activity in the circulating blood (Fig. 3). Following the above-mentioned changes, the PA activity becamed elevated in the tracheobronchial secretion of the rats (Fig. 4). On the other hand, administration of atropine sulfate did not affect the blood pressure and fibrinolytic activity in the circulating blood, but the PA activity did decline in the tracheobronchial secretion of the rats[19~21]. The mechanisms of elevation of PA activity in the tracheobronchial secretion following administration of noradrenaline could involve release of PA from target cells in the airway tract and/or transudation of PA from the circulating blood. Although receptor of noradrenaline has been shown to exist on the tracheal smooth muscle, tracheal epithelium and mast cells, it was considered that noradrenaline induced an increase in transudation of PA from the circulating blood into the tracheobronchial secretion. In addition, stimulation of the sympathetic nerves by noradrenaline could lead to relaxation of the bronchial smooth muscle and release of PA into the tracheobronchial secretion.

DEVELOPMENT OF PROTEASE INHIBITOR IN TRACHEOBRONCHIAL SECRETION OF RATS

As mentioned above, activated protease, plasmin inhibitor, papain inhibitor and trypsin inhibitor did not exist in the tracheobronchial secretion of rats without inflammation. However, an increase of protease and the development of protease inhibitor have been reported to occur in the airway secretion with acute inflammation of

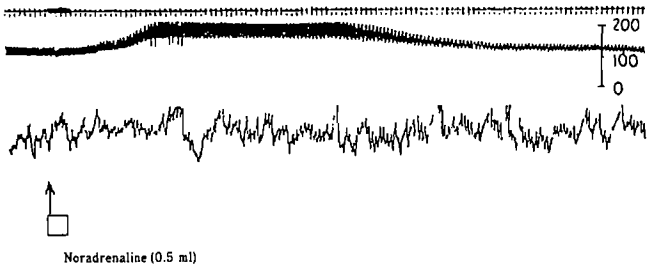

Fig.3 Changes of respiration and arterial blood pressure after administration of noradrenaline[21]. Ordinate: Arterial blood pressure and changes of respiration Abscissa: Time after administration of noradrenaline

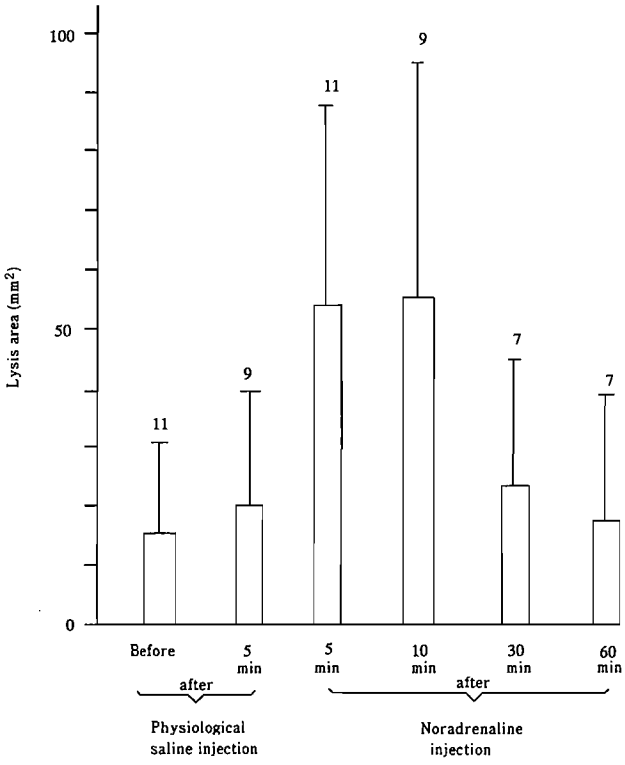

Fig.4 Changes of fibrinolytic activity in the circulating blood after administration of noradrenaline and physiological saline[20]. Data are expressed as the means±SD. The numerals above each set of data indicate the number of rats used in the experiment. Ordinate: Lysis area on standard fibrin plates (mm^2) Abscissa: Time after administration of noradrenaline or physiological saline

the upper respiratory tract in humans[22]. Kosugi et al. examined whether or not protease inhibitor was present in the tracheobronchial secretion of rats following administration of protease to the animals. Tracheal administration of papain to rats induced the development of papain inhibitor in the tracheobronchial secretion of rats without inflammation, although tracheal administration of urokinase and plasmin did not induce urokinase inhibitor and plasmin inhibitor in the tracheobronchial secretion[23]

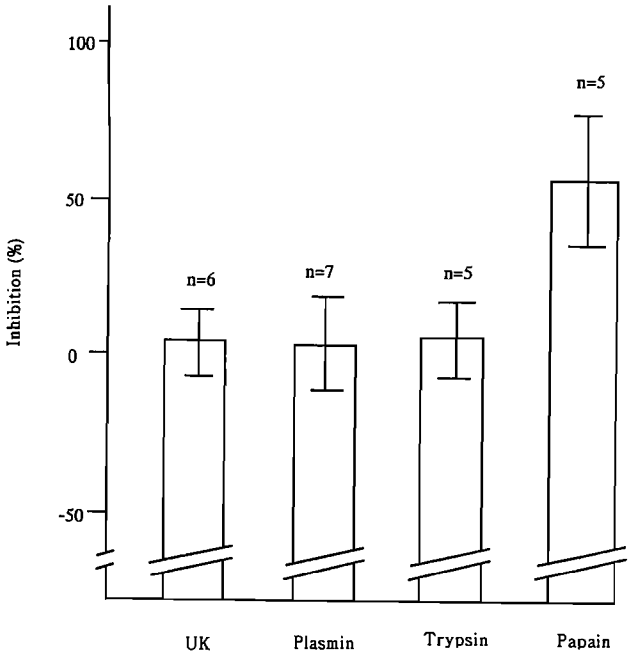

Fig.5 Inhibitory activity in tracheobronchial secretion after administration of various proteases into the trachea[23]. The inhibitory activity before administration of the various proteases into the trachea was taken as 0%. Data are expressed as the means ±SD. Ordinate: Rate of inhibition on plasminogen-rich fibrin plates (%) Abscissa: Various proteases

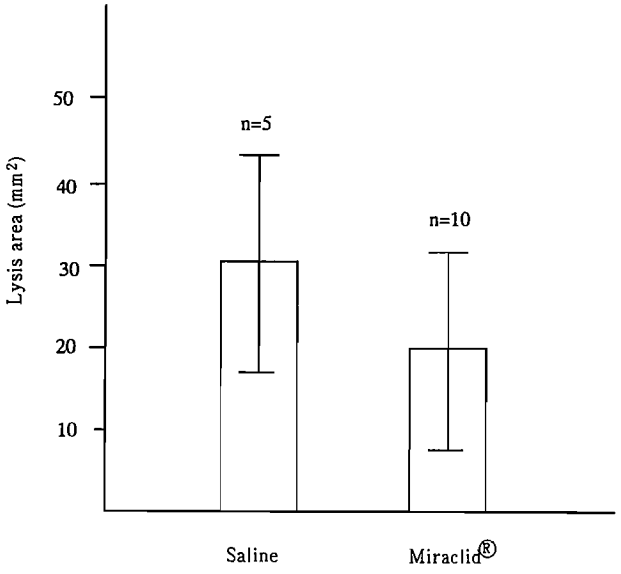

Fig.6 Changes of fibrinolytic activity in tracheobronchial secretion after administration of Miraclid® by nebulization[25]. Data are expressed as the means ±SD. Ordinate: Lysis area on fibrin plates (mm^2) Abscissa: Miraclid® (n = 10) and saline (n = 5)

(Fig. 5). In rats, thiol protease inhibitor was induced but serine protease inhibitor was not present in the tracheobronchial secretion after the administration of various proteases. It was inferred that increased protease levels induced thiol protease inhibitor in the tracheobronchial secretion but did not induce the release of polyvalent protease inhibitor, α_2-macroglobulin and α_1-antitrypsin originating from the circulating blood.

INHALATION OF PROTEASE INHIBITOR BY RATS

Inhalation of polyvalent protease inhibitor (Miraclid)® nebulized with an ultrasonic nebulizer inhibited the activity of PA in the tracheobronchial secretion of rats without inflammation[24,25] (Fig. 6). On the other hand, protease inhibitor of low molecular weight has been shown to exist in the sputum of patients with purulent bronchitis[26]. It has been reported that in acute inflammation of the human upper respiratory tract, acid-stable inhibitor of protease was elevated in the airway secretion[27] and this inhibitor could inhibit leukocyte protease[28]. Increase and development of protease inhibitor in the airway secretion inhibited the increased activity of protease in the airway secretion, and were considered to control the increased protease in a physiological manner. Miraclid®, a polyvalent protease inhibitor derived from human urine, is an acid-stable inhibitor and administration of this inhibitor to rats was found to inhibit the activity of PA in the tracheobronchial secretion. The results of our studies using rats suggest that administration of acid-stable inhibitor should cause the inflammation in the airway tract to subside by means of inhibition of the PA activity. In acute inflammation of the human upper respiratory tract, supply of acid-stable protease inhibitor should control the development and enlargement of the inflammation.

CONCLUSION

PA which converts plasminogen to plasmin exists in the circulating blood. The PA in the circulating blood derives from the endothelium of the blood vessels. PA can be classified into two groups based on its immunological characteristics, viz., tissue-type PA (t-PA) and urokinase-type PA (u-PA)[29,30]. In addition, PA exists in saliva[31,32]. It has been suggested that the PA in saliva, circulating blood and airway secretion may play an important role in the continuous opening of the salivary ducts, blood vessels and airway tract. In other words, the PA in these organs provide a defense which inhibits obstruction and stenosis of the various tracts. In particular, if the tract of the tracheobronchus does not continue to remain open, respiratory function cannot be maintained physiologically. However, although our previous studies have confirmed that PA in the airway tract is important

for continuous opening, the immunological characteristics of the PA in the tracheobronchial secretion of rats remain to be clarified. In a noninflammatory condition of the tracheobronchial secretion, the activity of protease is stronger than that of protease inhibitor. If inflammation should occur in the airway tract, an abrupt increase in protease appears in the airway secretion and protease inhibitor decreases relatively through consumption in order to neutralize the increased protease. It is inferred therefore that supply of protease inhibitor to the airway tract could be useful for controlling the inflammation clinically.

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