[総説] The role of fibrinolytic enzymes in in flammatory process of the upper airway and control of the inflammation by antiplasminic agents

The role of fibrinolytic enzymes in inflammatory process of the upper airway and control of the inflammation by antiplasminic agents

Masanori Sunagawa*, Tadayoshi Kosugi*,**, Mariko Nakamura*,
Kazuhiko Hanashiro* and Kiyohiko Kinjoh*

**First Department of Physiology and **The Research Center of Comprehensive Medicine, Faculty of Medicine, University of the Ryukyus*

(Received on September 20, 1996, accepted on December 6, 1996)

ABSTRACT

We reviewed the characteristics and level of fibrinolytic enzymes in extracts of tissue with inflammatory disease and experimental inflammation in upper airway. In addition, we reviewed the changes of fibrinolytic activity in the circulating blood in both humans and experimental animals with acute inflammation. Tissue-type plasminogen activator (t-PA) existed in tissue extract of paranasal mucous membrane (PMM) with chronic sinusitis but urokinase-type plasminogen activator (u-PA) did not. Both t-PA and u-PA, however, existed in tissue extract of antrochoanal polyp (AP). The fibrinolytic activity in the circulating blood increased at the initial stage of human acute tonsillitis and Arthus tonsillitis of rabbits. It was presented that fibrinolytic enzymes in inflammation have an important role on extension, ending and repair at the local inflammatory sites. Furthermore, it was suggested that the suppression of the elevated activity of fibrinolytic enzyme might control the process of inflammation at the initial stage of acute inflammation. *Ryukyu Med. J., 16(4)157~164, 1996*

Key words: fibrinolytic enzymes, inflammation, upper airway, tissue-type plasminogen activator, urokinase-type plasminogen activator

INTRODUCTION

Inflammation is defined as a defensive and healing reaction to injury at locus. Inflammatory responses are caused by both cellular component and mediators inducing immuno-inflammatory cascade. In inflammation, plasmin, one of the chemical mediators, plays an important role. As a result of the proteolytic activity of plasmin, other chemical mediators involved in various inflammatory responses can be produced. The precursor of plasmin, plasminogen is converted to plasmin by the plasminogen activator (PA) which exist in various tissues and body fluids. However, during the inflammatory process the PA derived from the infiltrated leukocytes and macrophages may be closely related to activation of plasminogen. We have analyzed the relationship between the fibrinolytic activity and the inflammatory responses in the field of otorhinolaryngology. Fibrinolytic enzymes have been reported to exist in various body fluids and organs relating to the upper airway, that is, saliva[1], tracheobronchial secretion[2], nasal and paranasal mucous membrane (PMM)[3,4].

In this paper, we summarized the literature concerning physicochemical properties of PA in tissue extract of various tissues, fibrinolytic activity in circulating blood in patients with inflammation in the upper airway.

PLASMINOGEN ACTIVATOR IN TISSUE EXTRACTS OF HUMAN PARANASAL MUCOUS MEMBRANE WITH CHRONIC SINUSITIS

Large amounts of fibrinolytic enzyme are contained in tissue extracts of the PMM in patients with chronic sinusitis. Physicochemical property of PA contained in the tissue extracts of human PMM was studied by the biochemical techniques. The 75% ammonium sulphate precipitate from the tissue extract of PMM revealed two peaks of fibrinolytic activity on Sephadex G-200 gel[5]. The precipitates prepared with 75% ammonium sulphate contained two kinds of PA with different (high and low) molecular weights[6]. The molecular weight of this PA with low molecular weight (LMW) was less than cytochrome c (12,000 daltons), i.e. some 10,000 to 5,000 daltons, based on determinations by gel filtration on Sephadex G-200[7]. It was suggested that PA with low

Table 1 Observations on the proliferation of cells from implanted tissue evidencing chronic sinusitis[13]

days after culture	clot	epithelial cell	fibroblast
1	+	−	−
2	+	−	−
3	+	−	−
4	+	−	−
5	+	+	+
6	+	+	+
7	+	+	+
8	−	+	+
9	−	+	+
10	−	+	+

clot (+:remained, −:not remained); epithelial cell (+:proliferated, −:not proliferated); fibroblast (+: proliferated, −:not proliferated).

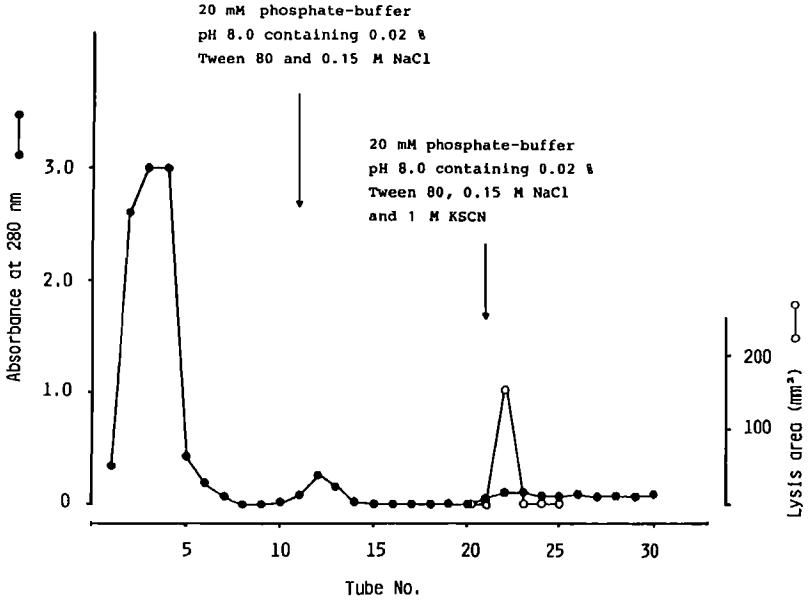

Fig. 1 Isolation of plasminogen activator from conditioned medium on the fifth day after culture of PMM[20]. Affinity chromatography using lysine sepharose was the procedure used to isolate the plasminogen activator.

molecular weight was produced by a mechanism involving proteases, i.e. trypsin-like enzymes, at the locus of the chronic inflammation in the PMM. Such proteases are thought to react with the PA of high molecular weight resulting in the production of PA with low molecular weight[8]. On the other hand, purified PA by affinity chromatography using Zn-imminodiacetate Sepharose and lysine Sepharose, formed one peak on a Sephacryl S-200 column. The molecular weight of this purified PA from the PMM was estimated to be about 65-70 kDa using gel filtration with Sephacryl S-200[9,10]. Furthermore, using fibrin autography technique, we characterized and identified a PA derived from tissue extracts of PMM with chronic sinusitis. The result of fibrin autography indicated that the tissue extracts of PMM revealed a single lytic zone at 65 kDa on fibrin-agarose plates. This lytic zone was inhibited by goat immunoglobulin G (IgG) fraction of anti human uterine tissue-type plasminogen activator (t-PA), but not by anti-human LMW urokinase goat IgG. From this result the lytic zone at 65 kDa was identified as t-PA[11]. The binding to fibrin of this purified PA from PMM was stronger than that of urokinase (UK). Furthermore, the binding to human fibrin of t-PA and UK was stronger than that to bovine fibrin. From this result, t-PA from PMM has a high affinity to human fibrin[12]. However, the origin of the t-PA in the tissue extracts of PMM could not be clarified in the above-mentioned study. Subsequently, using a tissue culture of PMM, it was observed that two species of cells, epithelial cells and fibrocytes, proliferated in the implanted tissue (Table 1). PA was isolated from the conditioned medium on the fifth day after culture (Fig. 1). From these results, it appears that the PA may be released from epithelial cells and/or fibroblasts[13].

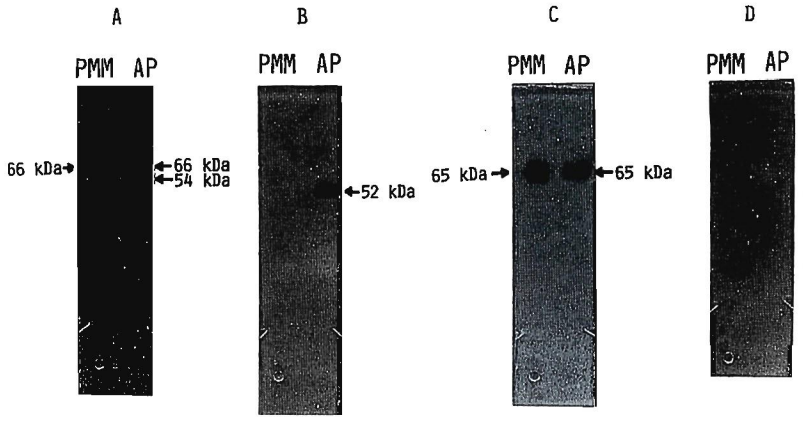

Fig. 2 Characterization of PAs derived from tissue extracts of PMM and AP[11]. A: Lytic pattern on fibrin zymography without goat immunoglobulin G (IgG). B: Lytic pattern on fibrin zymography with antihuman uterine tissue-type PA (t-PA) goat IgG. C: Lytic pattern on fibrin zymography with antihuman LMW urokinase goat IgG. D: Nonlytic pattern on fibrin zymography without plasminogen.

PLASMINOGEN ACTIVATOR IN TISSUE EXTRACT OF ANTROCHOANAL POLYP

Nasal polyp (NP) and antrochoanal polyp (AP) are by-products of chronic inflammation of the paranasal cavity and are associated with PMM. In other words, they are polyps in which the PMM proliferate pathologically and invade into the nasal and retropharyngeal cavity. Tissue extract of AP showed a faint lysis area on standard fibrin plates, but streptokinase (SK)-added tissue extract showed a large lysis area. No fibrinolytic activity was observed on plasminogen-free fibrin plates. Therefore, the fibrinolytic activity observed after SK addition is an indication of the existence of SK-responsive protein activator. The addition of UK to the tissue extract slightly enlarged the lysis area compared with UK alone. Dilution led to a proportional decrease in the activity of the SK-added tissue extract. On the other hand, the protease activity of UK-added tissue extract did not vary in this way, but showed an almost constant value on dilution[14]. Furthermore, the result of fibrin autography indicated that the tissue extracts of AP revealed two kinds of lytic zones at 54 kDa and 65 kDa on fibrin-agarose plates. This lytic zone at 65 kDa was identified as t-PA, and the lytic zone at 54 kDa was identified as urokinase-type plasminogen activator (u-PA) by immunological analysis with specific antibody to t-PA and u-PA. Tissue extracts of AP contained both t-PA and u-PA, whereas tissue extracts of PMM with chronic sinusitis contained t-PA only (Fig. 2). Based on the results concerning the presence of u-PA in the tissue extracts of AP, it is considered that u-PA may play an important role in inflammatory enlargement and proliferation[11].

INHIBITORY ACTIVITY OF THE TONSILLAR TISSUE AGAINST UROKINASE

To determine the inhibitory activity of the tissue extract of tonsils and adenoids against UK, the tissue extracts were prepared from the tonsillar tissue resected by tonsillectomy from tonsils having tonsillitis and hypertrophy and the adenoid tissue resected by adenoidectomy from adenoid vegetation. Fibrin plate method was used to determine the inhibitory activity against UK. Tonsillar tissue with hypertrophy and focal tonsillitis demonstrated a complete inhibitory activity against 10 Ploug u/ml of UK, but adenoid tissue demonstrated a complete inhibitory activity against 2.5 Ploug u/ml of UK. By determining the inhibitory activity of human ton-sillar tissue extract with physiological saline against UK, UK inhibitor was observed to exist in the organ. The inhibitory activity of the tonsillar tissue against UK was found to be greater than that of the adenoid tissue[4].

FIBRINOLYTIC ACTIVITY IN TISSUE EXTRACTS WITH VARIOUS LESION OF INFLAMMATION

The distribution and role of PA and proactivator in various diseases of the nasal and paranasal cavity were investigated. We attempted to clarify the role of the fi-

Table 2 Fibrinolytic activity of tissue extract of PMM with chronic sinusitis[16]

	tissue extract		SK+tissue extract			
	slight (n=5)	strong (n=6)	slight (n=5)	strong (n=6)	SK+buffer	
st. plates	73.6±50	54.8±10	91.9±36	68.1±20	0	0
free plates	0	0	0	0	0	0

Paranasal mucous membranes with chronic sinusitis were divided macroscopically into two groups according to the strength of inflammation (strong and slight). The activities of the extract alone and a mixture of SK and the extract were estimated on standard fibrin plates (st. plates) and plasminogen-free fibrin plates (free plates). Data is mean ± SD of the lysis area (in mm²).

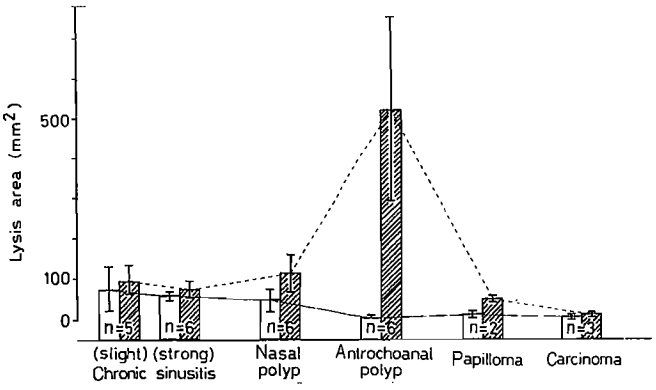

Fig. 3 Fibrinolytic activity of tissue extract in various lesions involving the nasal and paranasal cavity[16]. Open columns indicate the lysis area of the extract alone, and shaded columns indicate the lysis area of a mixture of SK and the extract. The columns and bars show the mean ± standard deviation.

brinolytic enzymes, i.e. PA or proactivator from the PMM of patients with various diseases originating in the paranasal and nasal cavity, and they may play an important role in the proliferation of the PMM with chronic inflammation. The fibrinolytic activity of the extracts of PMM with chronic sinusitis is shown in Table 2. In the case of slight inflammation, the average PA activity was 73.6 mm² and the proactivator activity was 91.9 mm². Furthermore, in the case of strong inflammation with chronic sinusitis, the average PA activity was 54.8 mm² and the proactivator activity was 68.1 mm². On comparison of the case of slight inflammation with that of strong inflammation with chronic sinusitis, it was suggested that the activities of both PA and proactivator were somewhat enhanced in the former as compared to the latter. The PA activity was relatively strong in the cases of slight inflammation with chronic sinusitis as shown in Fig. 3. On the other hand, the proactivator activity was strongest in tissue extract of AP, followed by tissue extract of NP (Fig. 3)[15].

FIBRINOLYTIC ACTIVITY IN THE CIRCULATING BLOOD IN PATIENTS WITH INFLAMMATION

Inflammation is the localized characteristic responses of tissue to injury. Plasminogen-plasmin system plays an important role in the process of inflammation as a chemical mediator. Therefore, activation of plasminogen-plasmin system at the locus of inflammation influences the fibrinolytic activity in the circulating blood. Three parameters in blood fibrinolysis, viz, the levels of fibrinogen, plasminogen, and fibrinogen and/or fibrin degradation products (FDP), were measured in patients with acute tonsillitis and the results were compared with those of healthy adults. The content of fibrinogen in acute tonsillitis increased significantly ($p<0.001$), that of plasminogen decreased significantly ($p<0.005$), while FDP showed a higher value ($p<0.01$). The abovementioned changes in the three parameters of the fibrinolytic system in acute tosillitis suggest that PA is released into the blood, activates plasminogen and that the plasmin thus produced digests fibrin or fibrinogen, increasing the level of FDP[16]. Chronic inflammation of the tonsil is temporarily changed to acute inflammation in positive cases of the provocation test. In other words, provocation tests lead to acute exacerbation of chronic inflammation. To clarify the relationship between the positiveness of the provocation test in focal infections and the increase of fibrinolytic activity in the circulating blood, we carried out provocation tests by means of ultra-short wave stimulation in patients with focal infection including palmoplantar pustulosis and examined the changes in fibrinolytic activity of the circulating blood. In the positive group with palmoplantar pustulosis although in both pre and post provocation tests there were no significant differences, the t-PA level was slightly increased after provocation, but in the negative group it decreased. The FDP level in the positive group was increased after the provocation test, while in the negative group it increased slightly although in both groups there were no significant differences. The antithrombin Ⅲ (AT-Ⅲ) activity in the positive group with palmoplantar pustulosis was decreased, but in the negative group it increased slightly although in both pre and post groups there were no significant differences. The anti-plasmin (APL) activity in the positive and negative groups was increased. It is known that α_2-plasmin inhibitor (α_2-PI), which displayed the so-called APL activity, represented one of the acute-phase reactants and this inhibitor could easily undergo changes through simple mechanical stimulation of the tonsil. A positive provocation test is accompanied by an increase in fibrinolytic activity in the circulating blood of patients with focal

Table 3 Summary of results obtained by affinity chromatography of cultured lymphocytes and medium in relation to the existence of plasminogen activator[20]

Days cultured	Nontreated	Nontreated + TGF	Nontreated + BGF	Sensitized	Sensitized + antigen	Arthus (12 h)	Arthus (24 h)
0	−	n.d.	n.d.	−	−	−	−
2	−	n.d.	n.d.	−	−	−	−
4	−	n.d.	n.d.	−	−	−	−
6	−	n.d.	n.d.	−	−	−	−
8	−	n.d.	n.d.	−	−	−	+
10	−	+	−	−	−	−	+

n.d., not determined; +, plasminogen activator present; −, plasminogen activator absent. "Non-treated" indicates lymphocytes from the tonsil of non-treated rabbits. TGF, T-cell growth factor; BGF, B-cell growth factor. "Sensitized" indicates lymphocytes from the tonsil of rabbits sensitized with bovine serum albumin. The antigen was bovine serum albumin. "Arthus" indicates lymphocytes from the tonsil of rabbit with Arthus tonsillitis. Tonsillectomy was performed at 12 and 24 h after the onset of Arthus tonsillitis.

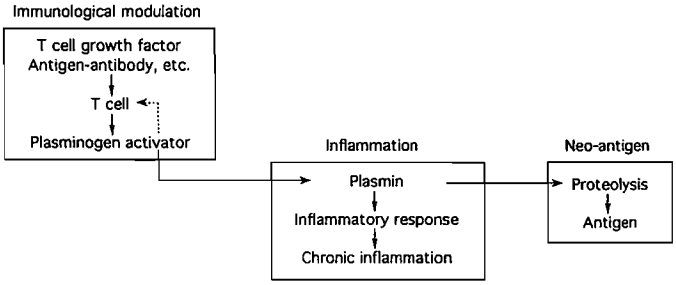

Fig. 4 A proposed mechanism for the development of tonsillogenic focal infection from the view of proteolysis.

infection of the tonsil, and the increase in fibrinolytic activity is closely related to the positiveness of the provocation test[17].

FIBRINOLYTIC ACTIVITY IN ANIMAL MODEL OF INFLAMMATION

It is known that various inhibitors of the coagulation-fibrinolysis system are acute phase reactants, and the levels of these inhibitors in inflammation increase with the strength of the inflammatory response. Furthermore, the changes of fibrinogen in the blood have been found to parallel the changes in acute phase reactants and gamma-globulin, and the fibrinolytic activity in the blood has been found to increase at the initial stage of non-specific inflammation. The possible role of α_2-PI in inflammatory responses was investigated using an animal model of inflammation. After subcutaneous injection of carrageenin into the dorsum of rats, the APL in the plasma was significantly higher than that in the plasma of non-treated rats ($p<0.001$). On the other hand, the APL activity in the exudate of carrageenin pouch was significantly decreased[18].

As an experimental model of human tonsillitis, Arthus tonsillitis of the rabbit was produced[19]. It was found that the changes of fibrinolytic activity occurring in the circulating blood of rabbits with Arthus tonsillitis were similar to those of humans with acute tonsillitis. In comparison to the control group, an increase of fibrinogen content, slight decrease of plasminogen content and remarkable decrease of whole plasmin were observed from the first to the third day at the onset of Arthus tonsillitis. Fibrinolytic activity of blood correlated to the inflammatory process of Arthus tonsillitis, which may modify the clinical process of the tonsillitis. The mechanism of the increased proteolytic activity at the tonsil with Arthus-type tonsillitis was examined using cell culture of lymphocytes originating from the tonsil with Arthus-type inflammation[20]. That is, lymphocytes isolated from tonsil tissue of the rabbit were cultured in a culture medium for the short term. PA did exist in the culture medium of lymphocytes originating from the tonsil of rabbits with Arthus tonsillitis but did not exist in that without Arthus tonsillitis (Table 3). Therefore, PA originating from lymphocytes of the tonsil may play an important role in the increased proteolytic activity observed at the local site of Arthus tonsillitis. Accordingly, it was suggested that the development of the focal infection on tonsil was closely related to the increased activity of PA derived from lymphocytes and proteolytic degradation of constituent protein by plasmin (Fig. 4).

Fibrinolytic activity of mucous membrane of tympanic cavity with carrageenin-induced otitis media of guinea pig was determined[21]. After induced otitis media, the fibrinolytic activity at day 7 increased, compared with the activity at day 3 after injection and that of non-treated mucous membrane. It was suggested that the increased fibrinolytic activity at the late stage of experimental otitis media played an important role in repairing the inflammatory locus.

CONTROL OF INFLAMMATION WITH ANTIPLASMINIC AGENT

Experimental pleuritis in rats induced by turpentine appears to be a useful model for observing the relationship between systemic fibrinolytic enzymes and the process of inflammation at the local site[22]. After the injection of sesquiterpene, marked fibrinolytic activity was observed in the plasma, and slight but transient fibrinolytic activity appeared in the pleural effusion. Fibrin deposition on the pleural walls reached a maximum on day 7, after which adhesion of the pleural walls was observed. Administration of trans-aminomethylcyclohexane carboxilic acid (t-AMCHA), one of the antiplasminic agents, suppressed the fibrinolytic activity of both the plasma and pleural fluid, and fibrin deposition reached a maximum on day 3. Adhesion was also observed to occur early. That is, fibrinolytic enzymes play a significant role in pleurisy and it is clarified that an antiplasminic agent can promote pleural adhesion.

Experimental studies of acute inflammation in the tracheobronchial lumen of rats suggest that protease inhibitor increases in tracheobronchial secretions in order to control inflammation[2]. The polyvalent protease inhibitor, Miraclid®, derived from human urine, is useful for treating DIC and acute pancreatis. Effects of administration of Miraclid® by means of ultrasonic nebulization was investigated on the inflammation of the upper airway in rats. Compared to the administration of physiological saline into the tracheobronchial lumen, administration of Miraclid® by means of ultrasonic nebulization decreased the fibrinolytic activity in tracheobronchial secretions[23].

Fibrinolytic activity was increased in the circulatory blood of rabbits with Arthus tonsillitis, and then t-AMCHA was administered intravenously to rabbits, in order to clarify its role as an antiplasminic agent in the control of Arthus tonsillitis[24]. After the injection of the antiplasminic agent, fibrinolytic activity decreased and whole plasmin was not consumed as a result of the administration of t-AMCHA during the early stage of tonsillitis. The process of Arthus tonsillitis can be controlled by the administration of the antiplasminic agent.

DISCUSSION

It has been known that two different types of PA existed in various cells, tissue extract and body fluid. The two PAs could be differentially classified into t-PA and u-PA based on its immunological characteristics. That is to say, it has been reported that t-PA and u-PA are immunologically distinct molecules encoded by different genes[25,26]. Unlike t-PA, u-PA does not have a specificity for the binding to fibrin. t-PA is found in vascular endothelial cell, liver cell and neuron, and plays an important role in the intravascular fibrinolysis and thrombolysis. On the other hand, u-PA does not play an important role in the intravascular fibrinolysis but plays an important role in extravascular fibrinolysis or proteolysis. Accordingly, it has been known that u-PA is related to tissue remodelling and cell migration under physiological and pathophysiological conditions. In addition, it is known that plasminogen activator inhibitors (PAI) which inhibit the activities of t-PA and u-PA exist in various organ and cell. PAI-1, especially, is often found in association with adhesive glycoprotein, vitronectin or S protein under physiological conditions. This association of PAI-1 with the extracellular matrix stabilizes PAI-1 in its active conformation, but does not interfere with the inhibition of the PAs[27]. PAI-1 inhibits the activity of t-PA and u-PA and accelerates the degradation, internalization or clearance of t-PA and u-PA via the complex formation of t-PA, u-PA and PAI-1. Although PAI-1 activity and antigen in tissue extracts of PMM with chronic sinusitis and AP were not determined in this study, it was inferred that the inhibition of t-PA and u-PA induced by PAI-1 may be weak in tissue extracts of PMM and AP. In addition, the existence of u-PA in tissue extracts of AP was confirmed in our study and it was emphasized that the proliferation, enlargement and remodelling of PMM in paranasal cavity is induced by u-PA. It is known that the depression of t-PA and enhancement of u-PA is observed in synovial fluid with rheumatic arthritis. Furthermore, in joint inflammation, the depressed t-PA mediated plasminogen activation, although more than compensated by the enhanced u-PA mediated plasminogen activation, results in protraction of fibrin removal[28]. The elevated u-PA in local inflammation induces plasmin generation and the formation of fibrin degraded products. It has been reported that FDP, especially FDP-D dimer induces the secretion of interleukin-1 and u-PA, and regulates inflammation[29]. In addition, it is also known that FDP plays an important role in the acceleration of angiogenesis and tissue remodelling of chronic inflammation and wound healing[30]. From the previous results obtained from our experiments and the other literatures, it was clarified that fibrinolytic enzymes in inflammation have an important role in extension, ending and repair at the local inflammatory sites. Furthermore, it was suggested that the suppression of the elevated activity of fibrinolytic enzyme at the initial stage of acute inflammation could control the process of inflammation. On the other hand, the enhancement of fibrinolytic activity at locus of inflammation may accelerate the tissue repair and angiogenesis in chronic inflammation.

REFERENCES

1) Albrechtsen, O. K., and Thaysen, J. H.: Fibrinolytic activity in human saliva. Acta Physiol. Scand. 34: 138-145, 1955.
2) Kosugi, T., Morimitsu, T., Matsuo, O., and Mihara,

H.:Fibrinolytic enzyme in tracheobronchial secretion of rats:Existence of plasminogen activator. Laryngoscope XC: 1045-1051, 1980.
3) Sasaki, Y., Okamoto, S., Ohwada, K., and Nishibata, T.: Some observations on a remarkable fibrinolytic activity in the extract of nasal tissues and related tissue. Keio J. Med. 8: 235-246, 1959.
4) Kosugi, T., Hamaya, M., Wakayama, T., Oikawa, S., Nakajima, M., and Nomi, K.: Fibrinolytic activity in tonsillar tissue of man. J. Saitama Med. School 4: 175-180, 1977.
5) Hirano, K., Yotsukura, K., Kosugi, T., Matsuo, O., Mihara, H., and Hamaya, M.: Studies on tissue plasminogen activator-Characteristics of tissue plasminogen activator from human paranasal mucous membrane with chronic sinusitis-Otologia (Fukuoka) 25: 1102-1114, 1979.
6) Kosugi, T., Takagi, I., Ariga, Y., Kinjo, K., Matayoshi, S., and Noda, Y.:Differentiation of proactivator from tissue plasminogen activator with low molecular weight in the paranasal mucous membrane. Auris. Nasus. Larynx (Tokyo) 9: 99-104, 1982.
7) Kosugi, T., Matsuo, O., Mihara, H., Hirano, K., and Hamaya, M.: Characteristics of tissue plasminogen activator from paranasal mucous in chronic sinusitis. Ann. Otol. Rhinol. Laryngol. 91: 101-105, 1982.
8) Kosugi, T., Takagi, I., Matsuo, O., Mihara, H., Morimitsu, T., Hirano, K., and Hamaya, M.:Characteristics of tissue plasminogen activator from paranasal mucous membrane in chronic sinusitis. Tissue plasminogen activator with low molecular weight. Rhinology Suppl. 1: 125-130, 1981.
9) Kosugi, T., Nakamura, M., Tsukayama, T., Haraguchi, S., and Noda, Y.: Some chemical properties of tissue plasminogen activator purified from para-nasal mucous membrane. Rhinology 25: 129-132, 1987.
10) Yamashiro, Y., Nakamura, M., Huang, G.-W., and Kosugi, T.: Fibrin binding, fibrinolytic and fibrinogenolytic activity of plasminogen activator derived from the paranasal mucous membrane of humans. Laryngoscope 103: 197-202, 1993.
11) Yamashiro, Y., Nakamura, M., Huang, G.-W., and Kosugi, T.: Presence of urokinase-type plasminogen activator(u-PA) in tissue extracts of antrochoanal polyp. Laryngoscope 102: 1049-1052, 1992.
12) Kosugi, T., Ura, M., and Noda, Y.: Amidolytic and fibrinolytic activity of tissue plasminogen activator from paranasal mucous membrane in chronic sinusitis : New dimensions in otorhinolaryngology-head and neck surgery (Myers, E., ed), Vol. 2, pp336-338, Elsevier Science Publishers B.V. (Biomedical Division), 1985.
13) Kosugi, T., Nakamura, M., Koja, S., Haraguchi, S., and Noda, Y.: Fibrinolytic activity in medium from tissue culture of paranasal mucous membrane. Rhinology 25: 195-198, 1987.
14) Kosugi, T., Morimitsu, T., Matsuo, O., and Mihara, H.: A protease-antiprotease system in antrochoanal polyp. I. Evidence for the existence of proactivator. Arch. Otorhinolaryngol. 225: 97-102, 1979.
15) Kosugi, T., Kinjo, K., Takagi, I., Sueno, K., Noda, Y., and Mihara, M.:The role of fibrinolytic enzymes in inflammation and paranasal mucous membrane. Rhinology 22: 119-123, 1984.
16) Kosugi, T., Hamaya, M., Matsuo, O., and Mihara, H.: Fibrinolytic activity in patients with acute tonsillitis. Ann. Otol. Rhinol. Laryngol. 88: 366-367, 1979.
17) Ura, M., Nakahodo, K., Nakamura, M., and Kosugi, T.: Evaluation of provocation tests in tonsillogenic focal infection from the standpoint of fibrinolytic activity. Auris. Nasus. Larynx (Tokyo) 19: 243-249, 1992.
18) Kosugi, T., Kinjo, K., Matsuo, O., Mihara, H., and Nishikaze, O.: Antiplasmin activity in carrageenin-induced inflammation of rats. Int. J. Tiss. Reac. III: 173-176, 1981.
19) Kosugi, T.: Studies of tonsillitis from the aspect of protease- antiprotease. 3. Arthus tonsillitis of rabbit and fibrinolytic activity of blood. Jap. J. Otol. (Tokyo) 81: 1531-1539, 1978.
20) Nakahodo, K., Nakamura, M., Kosugi, T., Ura, M., and Taira, A.: Plasminogen activator derived from tonsillar lymphocytes of rabbits with Arthus tonsillitis. Auris. Nasus. Larynx (Tokyo) 18: 145-151, 1991.
21) Nakayoshi, H., Noda, Y., Nakamura, M., and Kosugi, T.: The fibrinolytic enzyme in carrageenin-induced otitis media of guinea pig:New dimensions in otorhinolaryngology-head and neck surgery (Myers, E., ed), Vol. 2, pp150-152, Elsevier Science Publishers B.V. (Biomedical Division), 1985.
22) Kosugi, T., Hamaya, M., Tanimoto, H., Matsuo, O., and Mihara, H.: Acceleration of pleural adhesion by the administration of antiplasminic agent. J. Saitama Med. School 7: 189-195, 1980.
23) Kosugi, T., Nakamura, M., Noguchi, S., and Huang, G.-W.: Effect of ultrasonic nebulization of Miraclid® on the proteolytic activity in tracheobronchial secretions of rats. Laryngoscope 99: 1281-1285, 1989.
24) Kosugi, T., Takagi, I., Kinjo, K., Nakagawa, S., Noda, Y., and Mihara, H.: Control of Arthus tonsillitis by the administration of an antiplasminic agent. Int. J. Tiss. Reac. V: 205-215, 1983.
25) Ny, T., Elgh, F., and Lund, B.: The structure of the human tissue-type plasminogen activator gene: Correlation of intron and exon structures to functional and structural domains. Proc. Natl. Acad. Sci. USA 81: 5355-5359, 1984.
26) Riccio, A., Grimaldi, G., Verde, P., Sebastio, G., Boost, S. and Blasi, F.: The human urokinase-plasminogen activator gene and its promoter. Nucleic. Acids Res. 13: 2759-2771, 1985.
27) Seiffert, D., Mimuro, J., Schleef, R.R., and

Loskutoff, D.J.: Interactions between type 1 plasminogen activator inhibitor, extracellular matrix and vitronectin. Cell Diff. Dev. 32: 287- 292, 1990.
28) Brommer, E.J.P., Dooijwaard, G., Dijkmans, B.A.C., and Breedveld, F.C.: Depression of tissue-type plasminogen activator and enhancement of urokinase-type plasminogen activator as an expression of local inflammation. Thromb. Haemost. 68: 180-184, 1992.
29) Hamaguchi, M., Morishita, Y., Takahashi, I., Ogura, M., Takamatsu, T., and Saito, H.: FDP D-dimer induces the secretion of interleukin-1, urokinase-type plasminogen activator, and plasminogen activator inhibitor-2 in a human promonocytic leukemia cell line. Blood 77: 94-100, 1991.
30) Thompson, W.G., Harvey, J.A., Kazmi, M.A., and Stout, A.J.: Fibrinolysis and angiogenesis in wound healing. J. Pathol. 165: 311-318, 1991.