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The role of fibrinolytic enzymes in inflammation and paranasal mucous membrane

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SUMMARY

The distribution and role of tissue plasminogen activator (TA) and proactivator (PA) in various diseases of the nasal and paranasal cavity were investigated. The stronger the inflammatory and proliferative response of the paranasal mucous membrane, the weaker was the fibrinolytic activity of TA. The fibrinolytic activity of PA tended to be stronger than TA activity. It is considered that PA may play an important role in inflammatory enlargement and proliferation of the paranasal mucous membrane, but does not play an important role in carcinogenic enlargement and proliferation of the nasal and paranasal mucous membrane.

INTRODUCTION

Our previous results have indicated that two plasminogen activators of different molecular weights are present in tissue extracts of paranasal mucous membrane with chronic sinusitis (Kosugi et al., 1982; Kosugi et al., 1981; Kosugi et al., 1981). Furthermore, it was apparent that the tissue plasminogen activator of high molecular weight in such tissue extracts was PA and different from TA with a low molecular weight (Kosugi et al., 1982; Kosugi et al., 1983). The present study examined TA from the paranasal mucous membrane of patients with various diseases originating in the paranasal and nasal cavity, and attempted to clarify the role of the fibrinolytic enzymes, i.e. TA or PA, which may play an important role in the proliferation of the paranasal mucous membrane with chronic inflammation.

MATERIALS AND METHODS

1. Acetone powder preparation

Paranasal mucous membrane was surgically removed from 11 patients with chronic sinusitis and the excised mucous membrane with chronic sinusitis was classified into two groups (strong and slight) at the basis of macroscopic finding;

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the mucous membrane was edematous and thick in strong group, and it was thin in slight group. Nasal polyps and antrochoanal polyps were surgically removed from six patients; papillomas of the nasal cavity were surgically removed from two patients; and carcinomas of the maxillary sinus were surgically removed from three patients. These materials were dissected into small pieces in a cold room (at 4 °C), and washed many times with cold physiological saline to exclude contaminant blood and pus. The fragmented pieces of tissue were then centrifuged at 3.000 r.p.m. for 15 min at 4 °C. After discarding the supernatant, cold acetone was added to the precipitate in the proportion of 10 ml per 1.0 g of tissue, and the mixture was homogenized for 15 min in the cold room using an ultra-homomixer (Nihon Seiki Kaisha Ltd.). The homogenate was then filtered through a filter paper and the retained fraction was dried in vacuo.

2. Extraction of TA and PA from the acetone powder preparation

Ten ml of phosphate buffer (M/15, pH 6.8) was added to 1.0 g of the acetone powder, and the suspended solution was stirred for 6 h at 4 °C. After centrifugation at 3.000 r.p.m. for 15 min, the resultant supernatant was used to determine the activity of TA and PA.

3. Reagents

Fibrinogen: Cohn's Fraction I (bovine) (Miles Laboratory) was dissolved in borate saline buffer (pH 7.8). Thrombin: bovine thrombin (Mochida Pharm, Co. Ltd.) was dissolved in physiological saline. Streptokinase (SK): Kabikinase (Kabi Laboratory) was dissolved in physiological saline. Lysine-Sepharose: Sepharose 4B substituted with L-lysine (Daiichi Pure Chem. Co. Ltd.) was used.

4. Determination of TA and PA activities in the extract

To estimate the TA and PA activities, standard fibrin plates (st. plates) and plasminogen-free fibrin plates (free plates) were employed. The st. plates were prepared from plasminogen-rich fibrinogen and thrombin as described by Astrup and Müllertz (1952).

Assay of TA and PA.

A 0.03 ml portion of each extract was applied to st. plates and free plates to determine the TA activity. To estimate the PA activity, a 0.4 ml portion of each extract and 0.1 ml of SK (1000 U/ml) were mixed and incubated at 37 °C for 10 min, and a 0.03 ml portion of each mixture was then applied to st. plates. After incubation for 18 to 20 h at 37 °C, the lysis areas were measured.

RESULTS

The fibrinolytic activity of the extracts of paranasal mucous membrane with chronic sinusitis is shown in Table 1. In the case of slight inflammation, the aver-

	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

Table 1. Fibrinolytic activity of tissue extract of paranasal mucous membrane with chronic sinusitis.
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 st. plates and free plates. The data in the table show the mean ± standard deviation of the lysis area (in mm^2).

age TA activity was 73.6 mm^2 and the PA activity was 91.9 mm^2. Furthermore, in the case of strong inflammation with chronic sinusitis, the average TA activity was 54.8 mm^2 and the PA activity was 68.1 mm^2. Comparison of the case of slight inflammation with that of strong inflammation with chronic sinusitis, suggested that the activity of both TA and PA was somewhat enhanced in the former as compared to the latter.
The fibrinolytic activity of the tissue extracts in various diseases is summarized in Figure 1. The TA activity was relatively strong in cases of slight inflammation with chronic sinusitis. In cancer of the paranasal cavity, the TA activity tended to be slight. On the other hand, the TA activity was strongest in tissue extract of antrochoanal polyp, followed by tissue extract of nasal polyp.

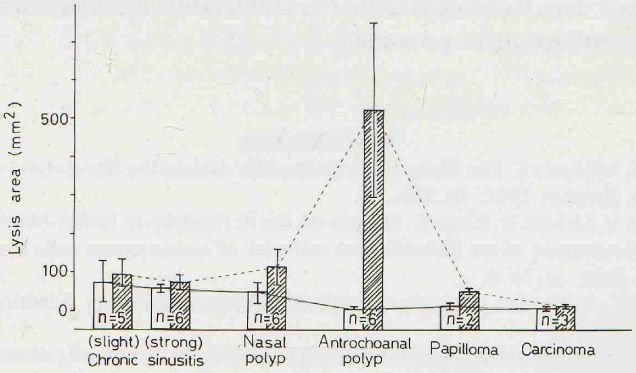

Figure 1. Fibrinolytic activity of tissue extract in various diseases involving the nasal and paranasal cavity. Open columns indicate the lysis area of the extract alone, and shaded columns indicate the lysis area of a mixture of SK and the extract. The columns and bars show the mean ± standard deviation.

DISCUSSION

Participation of protease and antiprotease in the growth and proliferation of cells has been reported in the case of human embryonic kidney cells (Nolan et al., 1977) and transformed cells (Ossowski et al., 1973). The role of fibrinolytic enzymes in the growth and proliferation of tumour cells has been investigated (Funahara et al., 1965; Khato et al., 1975; Kinjo et al., 1963) and protease inhibitor has been applied in an attempt to inhibit the carcinogenicity of some tumour cells (Hozumi, 1976).

In the present study it was shown that the stronger the inflammatory and proliferative response of the paranasal mucous membrane, the weaker was the fibrinolytic activity of TA, while the fibrinolytic activity of proactivator tended to be stronger than the TA activity.

Based on our results for the fibrinolytic activity in tissue extracts of various diseases, it is considered that PA may play an important role in inflammatory enlargement and proliferation of the paranasal mucous membrane, but does not play an important role in carcinogenic enlargement and proliferation of the nasal and paranasal mucous membrane.

RÉSUMÉ

Etude de la distribution et du rôle des plasminogènes-activateurs (TA) et proactivateurs (PA) dans différentes maladies des cavités nasales et paranasales. Plus la réaction inflammatoire et prolifère de la membrane muqueuse paranasale était forte, plus l'activité fibrinolytique des TA était faible. L'activité fibrinolytique des PA avait tendance à être plus forte que celle des TA. On a considéré que les PA pourraient jouer un rôle important dans l'accroissement et la prolifération inflammatoires de la membrane muqueuse paranasale, sans cependant jouer un rôle important dans l'accroissement et la prolifération carcinogènes de la membrane muqueuse nasale et paranasale.

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