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Studies on proactivator from the paranasal mucous membrane in chronic sinusitis

T. Kosugi, I. Takagi, K. Kinjo, M. Ura, K. Okamoto, K. Sueno and H. Mihara, Okinawa, Japan

SUMMARY

We succeeded in differentiating the proactivator (PA) in tissue extract of paranasal mucous membrane with chronic sinusitis by the gel filtration technique. Furthermore, the results demonstrated that PA in the tissue extracts of the paranasal mucous membrane with chronic sinusitis and antrochoanal polyp was unrelated to the antigenicity of plasminogen. In particular, it was clarified that the tissue extract of antrochoanal polyp as a source of PA was not related to the antigenicity of plasminogen.

INTRODUCTION

Fibrinolytic enzymes have been reported to exist in various organs of the oto-rhinolaryngological system. Large amounts of tissue plasminogen activator (TA) have been found to occur in the paranasal mucous membrane in cases with chronic sinusitis (Sasaki et al., 1959). On the other hand, only one of the plasminogen activators, SK-reactive proactivator, (proactivator, PA), was found to exist in tissue extracts of antrochoanal polyps (Kosugi et al., 1979) and it was postulated that the PA might play an important role in the growth of such polyps. If this is correct, then since antrochoanal polyps are a by-product of chronic inflammation of the paranasal cavity and originate from the paranasal mucous membrane, the PA, which is distinct from TA, may exist in tissue extracts of the paranasal mucous membrane in cases with chronic sinusitis.

The present study was undertaken to determine whether PA is in fact present in tissue extracts of the paranasal mucous membrane in chronic sinusitis. Furthermore, an attempt was made to determine whether any PA in the tissue extract was a plasminogen related substance originating from the circulating blood.

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MATERIALS AND METHODS

1. *Acetone powder preparation from paranasal mucous membrane and antrochoanal polyp*

Paranasal mucous membrane was surgically removed from 30 patients with chronic sinusitis, and antrochoanal polyps were surgically removed from 6 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 from the acetone powder*

Fifty ml of 2 M KCl was added to 3 g of the acetone powder of the paranasal mucous membrane, 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 diluted 10 times with phosphate buffer (M/15, pH 6.8) for use as starting material in the subsequent investigations. On the other hand, 10 ml of phosphate buffer (M/15, pH 6.8) was added to 1.0 g of the acetone powder of antrochoanal polyp, 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 for the experiment.

3. *Reagents*

Fibrinogen: Cohn's fraction I (bovine) (Miles Laboratory) was dissolved in borate slaine 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 effluent from gel filtration was applied to st. plates and to free plates to determine the TA activity. To estimate the PA activity, a 0.4 ml portion of each effluent from gel filtration 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 and to free plates. After incubation for 18 to 20 h at 37 °C, the lysis areas were measured.

5. *Fractionation of the TA and PA preparation with ammonium sulfate*
The extract (starting material) was fractionated at 4 °C by adding solid ammonium sulfate to 33% saturation. The solution was then centrifuged at 3.000 r.p.m. for 15 min at 4 °C and the resultant precipitate was dissolved in phosphate buffer (M/15, pH 6.8). The supernatant was fractionated at 4 °C by the addition of solid ammonium sulfate to 50% saturation. Finally, the supernatant was fractionated at 4 °C in ammonium sulfate at 75% saturation according to Dixton's method (1953). The 75% ammonium sulfate precipitate was used in subsequent experiments.

6. *Gel filtration of the above fraction*
Gel filtration for desalting the ammonium sulfate from the precipitate and for analyzing the molecular size of the TA and PA, was performed according to Flodin and Killander's procedure (1963). A 2.6 × 40 cm Sephadex G-50 column was equilibrated with phosphate buffer (M/15, pH 6.8) at 4 °C. The eluate was collected using an automatic fraction collector (LKB). A 5.2 ml portion was collected in a test tube after the Sephadex G-50 gel filtration. The protein concentration of the eluate was measured with a spectrophotometer (Hitachi) at 280 nm.

7. *Antigen-antibody reaction*
Double-immunodiffusion analysis was performed by the method of Ouchterlony (1967) at room temperature for 24 h. Agarose gel (Behringwerke) was prepared at a concentration of 1.0% in veronal buffer (I = 0.07, pH 8.6) containing 0.05% sodium azide.

RESULTS

1. *Differentiation of PA from TA of low molecular weight in the paranasal mucous membrane*
Measurement of the TA activities of the fractions on st. plates revealed that the 75% ammonium sulfate precipitate from the extract had two peaks of fibrinolytic activity on the Sephadex G-50 gel (Figure 1). Furthermore the 75% ammonium sulfate precipitate showed two peaks of PA activity on the same gel. However, when the TA and PA activities of the fractions were measured on free plates, no fibrinolytic activities were observed. Eluates containing compounds of high molecular weight (tubes no. 11–15) exhibited the properties of TA and the PA. Addition of SK to these eluates increased the activity of the eluates alone. However, eluates containing compounds of low molecular weight (tubes no. 24–28) exhibited the properties of TA only (Kosugi et al., 1982) and addition of SK to these eluates did not increase the activity of TA alone.

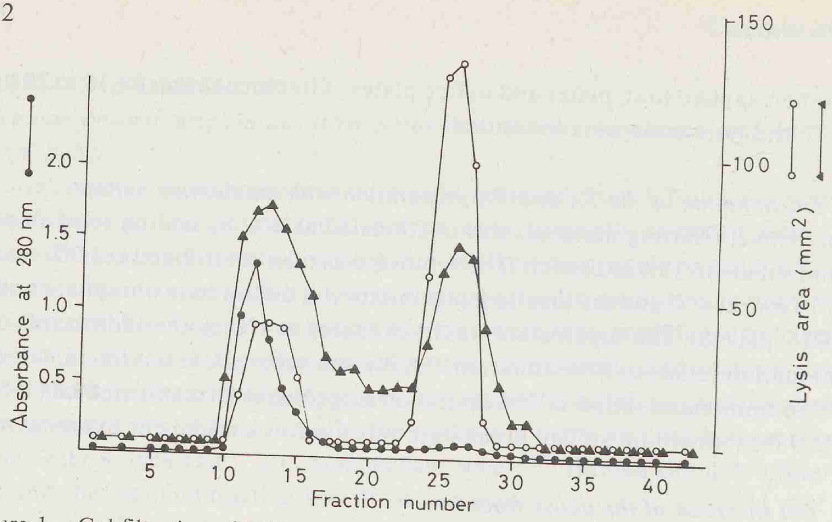

Figure 1. Gel filtration of 75% ammonium sulfate precipitate on Sephadex G-50.
A 2.6 × 40 cm Sephadex G-50 column was equilibrated with phosphate buffer (M/15, pH 6.8) at 4 °C.
The eluate was collected using an automatic fraction collector (LKB). 5.2 ml was collected in a test tube.
●—● absorbance at 280 nm.
○—○ lysis area of TA (eluates alone).
▲—▲ lysis area of proactivator (addition of SK to eluates).

2. *Immunological analysis of tissue extracts and normal human serum with anti-plasminogen sera*

Although plasminogen antiserum revealed an immunoprecipitin line with normal human serum, none of the antisera yielded an immunoprecipitin line with tissue extracts of paranasal mucous membrane with chronic sinusitis or antrochoanal polyp (Figure 2).

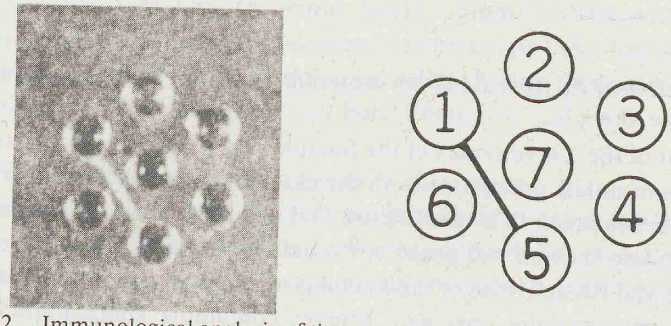

Figure 2. Immunological analysis of tissue extracts and normal human serum with anti-human plasminogen.
1 extracts of antrochoanal polyp.
2, 4 extracts of paranasal mucous membrane with chronic sinusitis (strong).
3, 5 extracts of paranasal mucous membrane with chronic sinusitis (slight).
6 normal human serum.
7 plasminogen antiserum.

DISCUSSION

The presence of PA in tissue extracts has been described in a few papers (Kosugi et al., 1979; Sasaki, 1968). However, differentiation of PA from TA in tissue extracts of the paranasal mucous membrane in patients with chronic sinusitis has not so far been successfully carried out. In the present study, we succeeded in differentiating PA from low molecular weight TA by the gel filtration technique. From the result of gel filtration using the Sephadex G-50, the void volume (the eluated volume of blue dextran) was 62.4 ml, the eluated volume of PA was 67.6 ml. Detailed studies on the characteristics of the proactivator in the tissue extracts are now in progress. On the other hand, tissue extracts of paranasal mucous membrane with chronic sinusitis and antrochoanal polyp did not yield an immunoprecipitin line with plasminogen antibody. On the basis of the results of this immunological analysis, it was concluded that the PA in tissue extracts of paranasal mucous membrane with chronic sinusitis and antrochoanal polyp was unrelated to the antigenicity of plasminogen. In particular, the tissue extract of antrochoanal polyp as a source of PA was not related to the antigenicity of plasminogen.

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T. Kosugi, M.D.
Dept. of Physiology
School of Medicine, University of Ryukyus
207 Uehara Miayazato, Nishihara-cho
Okinawa-ken, 903-01 Japan