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Measurement of a small amount of t-PA antigen in tissue extract derived from nasopharyngeal carcinoma

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ABSTRACT

We developed a method for estimating tissue-type plasminogen activator (t-PA) antigen in tissue extract (TE) derived from nasopharyngeal carcinoma (NPC) by means of rocket immunoelectrophoresis (RIE). Initially, the TE sample without treatment failed to shape the immunoprecipitate line by RIE. However, when the TE sample was treated with final concentration of 0.2% SDS, we observed that the precipitate line was so clear that we could measure the height of the rocket. *Ryukyu Med. J., 16(1)23~25, 1996*

Key words: t-PA, tissue extract, rocket immunoelectrophoresis

INTRODUCTION

Plasminogen activator (PA) can be divided into two types: tissue type (t-PA)[1] and urokinase-type (u-PA)[2,3]. It has been reported that PA plays an important role in inflammatory enlargement and proliferation of the paranasal mucous membrane. Cell proliferative diseases such as nasopharyngeal carcinoma (NPC) and antrochoanal polyp have been found to present u-PA in their tissue extracts and inflammatory diseases such as paranasal mucous membrane with chronic sinusitis to present t-PA in their tissue extracts[4~6]. It appears that the cells could maintain a normal structure of epithelial tissue through their secretion of t-PA.

We are currently attempting to classify NPC based on the level of t-PA antigen in its tissue extracts in relation to the progression of the carcinoma. In the present study, we developed a method for measuring low levels of t-PA antigen in tissue extracts from NPC.

MATERIALS AND METHODS

Reagents

Specimens of NPC were obtained from patients without previous treatment. Biopsy samples were taken at the Department of Otorhinolaryngology, Guangxi Medical College Affiliated Hospital in China. Goat anti-human uterine t-PA antibody was purchased from Biopool (Umea Sweden). Agarose C was purchased from Pharmacia Fine Chemicals Co. (Uppsala, Sweden). TRIS (Tris (hydroxymethyl) aminomethane), barbital, and sodium dodecyl sulfate (SDS) were purchased from Nacalai Tesque (Kyoto, Japan). Human t-PA (Hapase®) was obtained from Kowa Pharmaceutical Co., Ltd. (Nagoya, Japan). Coomassie Brilliant Blue R 250 was purchased from Merck Co. (Darmstadt, Germany). Unless otherwise stated, other reagents used were reagent-grade chemicals.

Preparation of tissue extract[7]

Three milliliters of buffer (0.07 M potassium acetate, 0.3 M NaCl, 10 mM EDTA, 0.1 M L-Arginine, 0.25% Triton X-100, pH 4.2) was added to a 1 mg piece of carcinoma tissue. The tumor tissue was minced with scissors and homogenized in the suspension solution. This procedure was carried out at 4℃ in a cold room. The homogenate was then sonicated for 5 min by means of an ultrasonic bioruptor (Model 80 W; Taiyo, Tokyo, Japan) at 20 kHz for 30s. After sonication, the homogenate was centrifuged at 5,000 rpm for 15 min. The supernatant was dialyzed against Tris-HCl buffer (pH 7.8), and used as the tissue extract of the cancer tissue in subsequent experiments. The protein content of the tissue extract was measured according to Lowry's method[8].

Preparation of the antibody-containing goat anti-human uterine t-PA[9]

To 4 ml of Tris-barbital buffer of pH 8.6 and ionic strength 0.02 (prepared by dissolving 8.9 g of TRIS, 4.5 g of barbital, 0.5 g of calcium lactate, and 0.2 g sodium azide, in distilled water, and adjusting the volume to 1 liter)

was added 0.06 g of Agarose C. The resultant suspension was placed on a boiling waterbath and stirred until all the agarose had completely dissolved. This 1.5% agarose solution was allowed to cool down to 55℃, and 4 μl of goat anti-human uterine t-PA antibody (final protein concentration, 32 μg/ml) was then added. The mixture was poured onto a plastic sheet (size, 4.3×5.0 cm), so that the sheet was covered evenly with the antibody-containing agarose solution. After the latter had been allowed to harden at room temperature, small antigen wells (2 mm in diameter and 2.5 mm apart) were cut in the agarose using a tubular cutter and a plastic template.

Rocket immunoelectrophoresis[10]

6 μl portions of TE sample were placed in the wells of the agarose plate, and electrophoresis was performed with a flat bed electrophoresis apparatus (FBE 3000, Pharmacia Fine Chemicals Co., Uppsala, Sweden) at a constant 30 volts for 8 hr. After completion of the electrophoresis, the gel was soaked in saline overnight, and washed with distilled water for 2 hr. It was then stained with 0.05% Coomassiee Brilliant Blue R 250 for 30 min and destained with 5% acetic acid. The height of the rocket was measured to estimate the concentration of t-PA antigen.

RESULTS

Selection of detergents

8 μl of TE or t-PA was treated with 2 μl of Tris-barbital buffer (pH 8.6) containing a detergent (2.5% (W/V) SDS, 2.5% (V/V) Tween 20, or 2.5% (V/V) Triton X-100) for 1 hr at room temperature and analyzed using RIE. As shown in Fig. 1, TE treated with SDS shaped the rocket precipitate but TE treated with the other detergents did not. In addition, the height of the rocket for t-PA

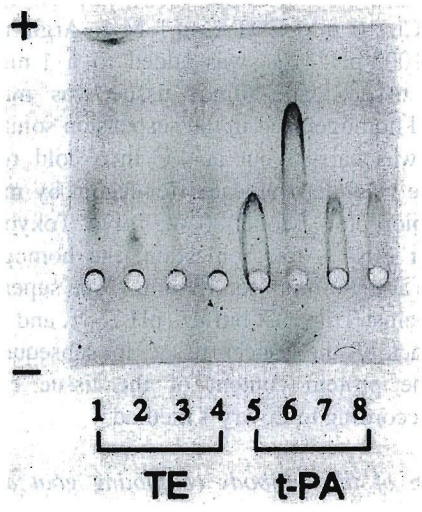

Fig. 1 Effect of detergents on the measurement of TE samples or 1 mg/ml of t-PA. The TE sample (protein concentraton, 1.05 mg/ml) or t-PA underwent no treatment (lanes 1, 5), was treated with SDS (lanes 2, 6), Triton X-100 (lanes 3, 7), or Tween 20 (lanes 4, 8), respectively.

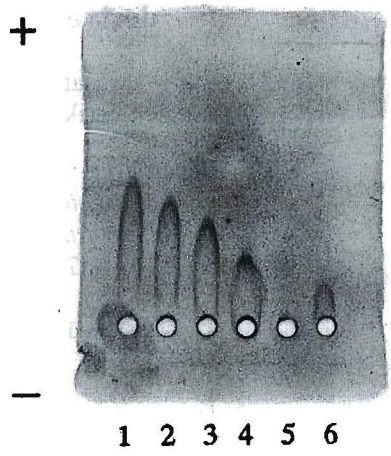

Fig. 2 Determination of the optimal concentration of SDS in RIE. SDS was added at a final concentration of 0.4% (lane 1), 0.2% (lane 2), 0.1% (lane 3), or 0% (lane 4); TE alone (lane 5); t-PA alone (lane 6).

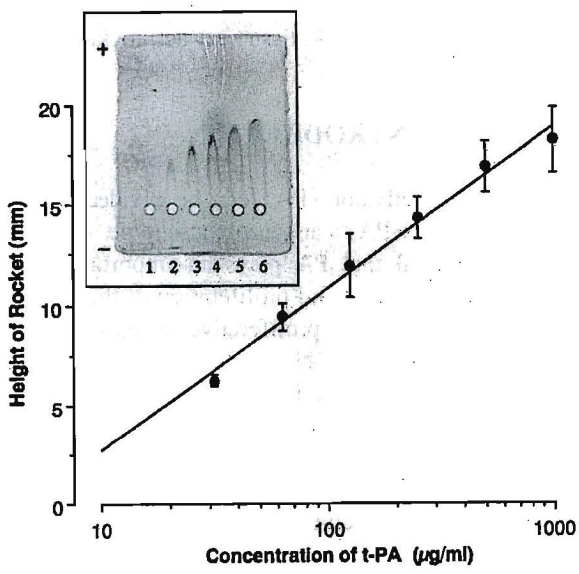

Fig. 3 Standard curve for the estimation of t-PA antigen. The serial dilutions of t-PA were: 31.25 μg/ml (lane 1), 62.5 μg/ml (lane 2), 125 μg/ml (lane 3), 250 μg/ml (lane 4), 500 μg/ml (lane 5), and 1000 μg/ml (lane 6). Plotted values are means ± S.D. (n = 4). The correlation coefficient for the regression line exceeded 0.98.

treated with SDS was higher than that treated with the other detergents.

Optimal concentration of SDS for treatment of the TE sample

t-PA solutions were mixed with TE and SDS added at various final concentrations (0%, 0.1%, 0.2% and 0.3%), and then analyzed using RIE. As shown in Fig. 2, 0.2% SDS as the final concentration shaped the rocket precipitate more clearly than did the other concentrations.

Standard curve for estimating t-PA antigen

To prepare a standard curve for estimating t-PA antigen, 5 mg/ml of t-PA solution was serially diluted with Tris-barbital buffer. To each diluted solution was then added SDS solution at a final concentration of 0.2%. The heights of the rocket increased in a dose-dependent manner. The standard curve is shown in Fig. 3.

DISCUSSION

When TE samples from NPC were treated with 0.2% Triton X-100 or Tween 20 as the final concentration, we were unable to measure the height of the rocket. It was suggested that t-PA might exist as t-PA · plasminogen activator inhibitor (PAI) complex[11] in TE. Because the nonionic detergents such as Triton X-100 or Tween 20 could not dissociate t-PA · PAI complex, goat anti-human uterine t-PA antibody could not react with t-PA in TE. However, following treatment with 0.2% SDS, we succeeded in measuring the height of the rocket (Fig. 1). Since SDS which was an anionic detergent could dissociate t-PA · PAI complex, t-PA in TE could shape the rocket precipitate. Furthermore, the height of the rocket was dependent on the type of detergent and its final concentration. In the case of t-PA (1 mg/ml) with no treatment, with Triton X-100 treatment, and with Tween 20 treatment, the height of the rocket was almost equal, but that with SDS was twice as high as the others (Fig. 1). The height of the rocket increased with increase in the concentration of SDS, and when the SDS concentration exceeded 0.2%, the bottom of the rocket lifted off from the wells. Treatment with 0.2% SDS yielded a standard curve (Fig. 3).

Although it has not been reported that the level of t-PA activity in circulatory blood of patients with NPC was measured, Kosugi *et al.* has reported that the fibrinogen level and FDP content of the circulatory blood were significantly increased in patients with both NPC and other cancers of the head and neck[12,13]. It was suggested that the increase of FDP content was based on the increase of released PA to circulatory blood from the tumor. The precise relationship between activity of t-PA or u-PA in TE of NPC and that in the circulatory blood was not well analyzed until recently. On the other hand, an important role of PA, especially u-PA, has been implicated in the mechanism of tumorigenesis, tumor invasion and metastasis[14]. We now aim to measure the levels of t-PA antigen in TE samples from various stages of NPC, so that we can clarify the relationship between the level of t-PA antigen in TE from NPC and the progression of the carcinoma.

In conclusion, we succeeded in measuring the t-PA antigen in TE from NPC treated with 0.2% SDS as the final concentration.

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