RADIOIMMUNOASSAY OF PIG PANCREATIC ELASTASE

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1. Introduction

Elastase can be considered a proteolytic enzyme capable of specifically cleaving elastin in connective tissue. Since the early concept of Balo and Banga [1], it has been suggested that elastase of pancreatic origin might be responsible, via the systemic circulation route, for the development of atherosclerosis [2]. However, there has been no firm evidence to support such a hypothesis due to the lack of a reliable method for measurement of the serum elastase content. We have been studying a radioimmunoassay for the measurement of pig pancreatic elastase and succeeded in measuring the serum elastase content of pigs. This is a report on this radioimmunoassay and the results of cross immunity between pig, rat and human pancreatic elastase.

2. Materials and methods

Trypsin (Miles), α-chymotrypsin (BDH) and *N*-suc. $(Ala)_3$*p*NA (Tanpakuken, Osaka, Japan) were used.

2.1. *Preparation of Purified Elastases*

Purification of pig pancreatic elastase was carried out in accordance with the method of Loeven [3]. Elastase crystallized three times was used as the antigen. The purification of rat and human elastase was principally based on the method of Feinstein et al. [4] and included lima-bean trypsin inhibitor Sepharose chromatography followed by SP Sephadex chromatography and DEAE–Sephadex chromatography. The method of purifying rat and human elastase and the enzymatic properties of these elastases will be described in a separate paper.

2.2. *Immunization procedure*

Five milligrams of pig elastase in 1 ml phosphate buffered saline was mixed with an equal volume of Freund complete adjuvant. The mixture was injected into the foot-pads or the backs of male rabbits. Sensitization was continued at biweekly intervals until the antibody titer rose. At 7 days after the fifth and final injection, blood samples were taken by heart puncture and centrifuged at 3000 rev/min for 10 min. Sera were collected and treated at 56°C for 30 min. Antibody titer was estimated by radioimmunoassay, and the sample with the highest value in this respect was utilized as the anti-pig elastase antiserum.

2.3. *Radioimmunoassay procedure*

Radioiodine was purchased from Dainabot Radiochemical Co., Tokyo, Japan. Iodination of elastase was carried out by the chloramine-T method [5]. More than 85% of the labelled compound was

recovered, and its radiological specific activity was 214.3 μCi/μg. A phosphate buffer (50 mM, pH 7.4) containing 2% BSA was used as a routine diluent in the radioimmunoassay. Antiserum (0.1 ml), ^{125}I-labelled elastase (0.1 ml, 8000 cpm) and the buffer (0.4 ml) were successively introduced to a 7 X 75 mm glass tube and incubated for 24 h at 4°C. Standard elastase or test sample (0.1 ml) was added after the first incubation and the mixture left standing for 72 h at 4°C. Goat anti-rabbit γ-globulin antiserum (0.1 ml) and normal rabbit serum (0.1 ml, 1:900 in final dilution) were added after the second incubation and the mixtures left to react overnight at 4°C. The tubes were then centrifuged at 3000 rev/min for 30 min at 4°C and the immune precipitates checked with a γ-counter.

3. Results and discussion

3.1. *Purity of pig pancreatic elastase*

SDS–Polyacrylamide gel electrophoresis of pig pancreatic elastase indicated a homogeneous protein band (fig.1).

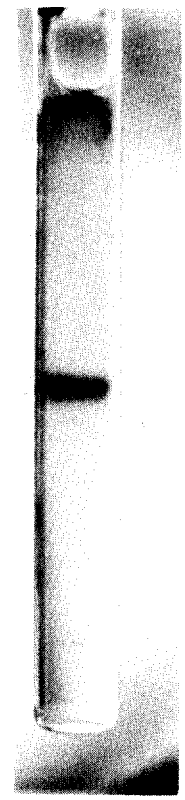

Fig.1. SDS–Polyacrylamide gel electrophoresis of pig pancreatic elastase.

3.2. *Sensitivity and specificity of the radioimmunoassay*

The typical standard curve of the radioimmunoassay is shown in fig.2, and indicates that (1) 8 ng/ml cold elastase can replace 50% of the total ^{125}I-labelled elastase bound to antibodies (50% Bo/To) and (2) the minimum level detectable by this assay, if this level is assumed to be the point of 10% replacement (90% of Bo/To), is approx. 1 ng/ml. Since it has been demonstrated that measurement of enzymatic activity requires more than 50 ng/ml elastase content, our radioimmunoassay appears to be 50 times as sensitive as the enzymatic methods [6,7].

Trypsin and α-chymotrypsin both failed to inhibit binding of ^{125}I-labelled elastase to the antibody. Also, human and rat elastase showed either no or weak cross immunity to pig elastase. The antigenic difference between pig and human elastase is consistent with previous findings that the amino acid compositions of these elastases differ [8]. Our human elastase appeared to be similar to the elastase I of Feinstein et al. [4] and protease E of Mallory and Travis [8] with respect to its anionic nature and elution behaviour on ion-exchange Sephadex gel chromatography. We are presently conducting amino acid analysis of human and rat elastase. Rat elastase is a cationic protein with a molecular weight of 25 000.

3.3. *Estimation of serum immunoreactive elastase*

Serum dilution resulted in a replacement curve (fig.3) similar to the standard curve and indicating that the serum elastase level in castrated male pigs, which is not detectable by the enzymatic method, is about 8 ng/ml. The presence of serum elastase inhibitors, namely α-1 antitrypsin and α-2 macroglobulin, did not have an effect on the radioimmunoassay of elastase.

The inhibition curve in fig.3 levels off at about 40%, or higher than in fig.2, due to the difference in antibody concentrations in the two experiments. The plateau level of elastase radioimmunoassay rose with

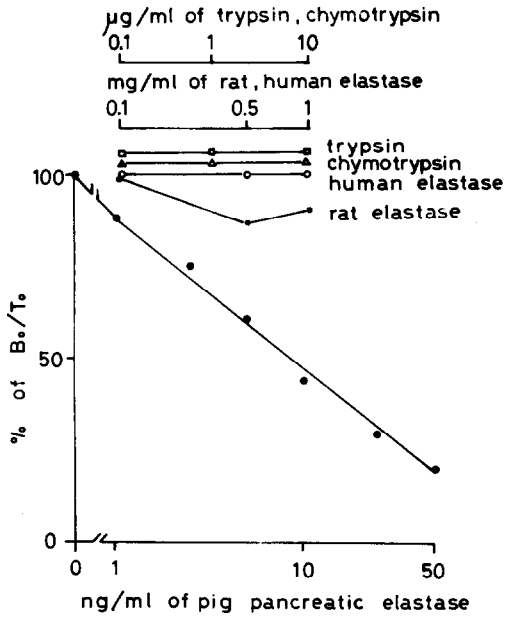

Fig. 2. Radioimmunoassay of pig pancreatic elastase. The effect of elastases or trypsin and chymotrypsin on pig elastase radioimmunoassay was tested. Bo/To represents the ratio of antibody-bound labelled elastase (B) to total labelled elastase (T) in the absence of cold elastase, and % of Bo/To the degree of inhibition. Trypsin, chymotrypsin and human elastase showed no cross immunity to pig elastase, while rat elastase indicated a weak cross immunity. An antibody concentration of 1:800 000 in the final dilution was used in this experiment.

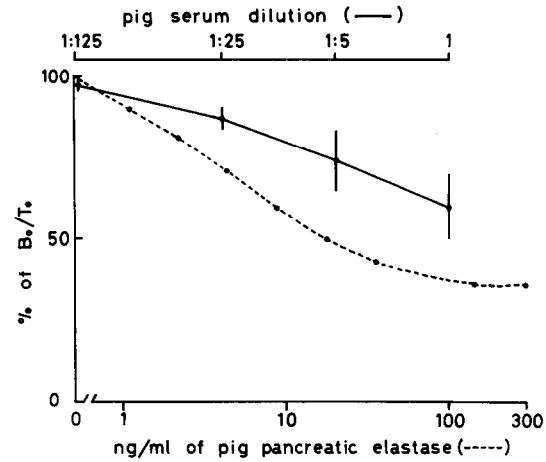

Fig. 3. Serum dilution curve in pig elastase radioimmunoassay. Sera from 9 castrated male pigs were diluted and added to the radioimmunoassay system instead of cold elastase. Each point on the pig serum curve represents the mean % of Bo/To standard deviation. An antibody concentration of 1:400 000 in the final dilution was used in this experiment. The pig serum inhibition curve paralleled the standard curve for purified pig elastase.

the increase in antibody concentration. Utilizing a radioimmunoassay method, Carballo et al. [9] previously reported that canine serum had a rather higher elastase content of 0.1 μg/ml to 1 μg/ml. However, the specificity of their radioimmunoassay is questionable as they did not indicate the criteria for purity of the canine elastase they used as the antigen. Their data on the serum elastase level, therefore, may not exactly reflect the true elastase content as it appears to be a much lower level.

4. Conclusion

A radioimmunoassay for the measurement of pig pancreatic elastase was developed which permits measurement of an elastase content of 1 ng/ml. The measured elastase content of pig serum was about 8 ng/ml, which is not detectable by conventional enzymatic methods. The presence of serum inhibitors did not have an effect on the radioimmunoassay. Isolated rat and human pancreatic elastase indicated either none or a weak cross immunity to pig pancreatic elastase. Trypsin and chymotrypsin also did not demonstrate cross immunity.

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