Radioimmunoassay for Human Pancreatic Ribonuclease and Measurement of Serum Immunoreactive Pancreatic Ribonuclease in Patients with Malignant Tumors

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ABSTRACT

A method for radioimmunoassay of human pancreatic RNase was developed. The method is sensitive, reproducible, and specific. Almost no cross-reactivity exists between human pancreatic and liver RNases. A good correlation was observed between the serum concentration of pancreatic RNase as measured by radioimmunoassay and its enzymatic activity using polycytidylic acid as substrate. The concentration of serum pancreatic RNase correlates well with age, blood urea nitrogen, and albumin contents but does not correlate with serum amylase activity. Using the data of 52 patients with malignant tumors except pancreatic cancer, serum RNAse level could be expressed by a multiple regression equation:

\[
\text{Serum RNAse (ng/ml) = 6.21 \times \text{age (years)} + 16.85 
}\times \text{blood urea nitrogen (mg/dl) } - 169.81 \times \text{albumin (g/dl) } + 722.38
\]

Immunoreactive RNAse content in pancreatic cancer was elevated in patients with complications from renal failure. Serum pancreatic RNAse contents in patients with pancreatic cancer measured by radioimmunoassay agreed well with the values calculated using the equation derived from the data of patients with other malignant tumors.

INTRODUCTION

Serum RNAse has been reported to elevate in patients with malignant tumors (3, 10, 24). In human serum, several types of RNAse, such as pancreatic type, liver-spleen type, and leukocytic type (1, 2, 11, 15, 16), are known to exist. Pancreatic type RNAse has been considered to increase in the serum of patients with pancreatic cancer (20, 23). Several conflicting results (4, 17, 21) have been reported, however, about the specificity of pancreatic-type RNAse as a diagnostic marker of pancreatic cancer. Recently, we have reported the purification of human pancreatic RNAse and the development of a RIA3 of the enzyme (11). In the present paper, the serum content of immunoreactive pancreatic RNAse was determined in patients with various malignant diseases. Several factors influencing the elevation of serum immunoreactive pancreatic RNAse were also investigated, and the significance of the measurement of serum pancreatic type RNAse in patients with pancreatic cancer was discussed.

MATERIALS AND METHODS

Special Materials. Na125I was purchased from New England Nuclear, Boston, MA. Human pancreatic RNase (11), elastase II (7), amylase (13), and liver RNase (16) were purified by the methods reported from our laboratory. Antiserum against human pancreatic RNase was produced in guinea pigs as reported previously. DNase I, RNase T1, (Aspergillus oryzae), and bovine trypsin were purchased from Sigma Chemical Co., St. Louis, MO. The sources of the other reagents are: poly(C), Yamasa Shoyu Co., Japan; Sephadex G-25, Pharmacia Fine Chemicals AB, Uppsala, Sweden; goat anti-guinea pig IgG serum and normal guinea pig serum, Eiken Chemicals Co., Japan.

Serum Samples. Serum samples were obtained from healthy volunteers and hospitalized patients. These patients (except 2 with pancreatic cancer) had normal renal function. Sera were stored at −20° until used. The diagnosis of malignant tumor had been confirmed by X-ray, endoscopy, angiography, and computed tomography findings and was also confirmed in the majority of instances by operation or biopsy.

Radioiodination. The purified RNase (10 μg) was radioiodinated with 1 mCi 125I by the chloramine-T procedure as described previously (9).

RIA Procedures. RIA for pancreatic RNase was a modification of the double antibody technique developed by Morgan and Lazarow (14). Details were described previously (11).

Enzyme Assay. RNase activity was assayed by the method of Reddi (19).

Protein Determination. Protein concentration was determined by the method of Lowry et al. (12).

Serum Albumin Determination. Serum albumin content was measured by the method of Doumas et al. (5).

BUN Determination. The analysis for urea nitrogen is based on hydrolysis of urea to ammonia and carbon dioxide, catalyzed by urease (EC 3.5.1.5).

Statistical Analysis. The statistical evaluations were made by linear regression analysis, multiple regression analysis, paired-sample t test, Wilcoxon signed-rank test, Student’s t test, and Fischer’s F test.

RESULTS

Standard Curve of RIA and Specificity of Human Pancreatic RNAse. The standard curve of the RIA for human pancreatic RNAse and dilution curves of human pancreatic amylase, DNase I, human elastase II, bovine trypsin, RNase T1 (A. oryzae), and bovine RNAse A are shown in Chart 1. Almost no cross-reactivity was seen between human pancreatic RNAse and human liver RNAse (about 10% at the point of 50% B/B0). No cross-reactivities to other enzymes could be demonstrated. The quantitative determinations of the unknown samples were made after dilution...
with buffer to bring the concentration of RNase down to less than 500 ng/ml.

**Immuno-cross-reactivity of Pancreatic RNase between Mammalian Species.** The immuno-cross-reactivity of pancreatic RNase between human and other mammalian species (cat, rat, mouse, and rabbit) was investigated by comparing the serum dilution curves of these mammalian species to the standard curve of human pancreatic RNase. No immunological identity was seen between human and other mammalian serum pancreatic RNases.

**Reproducibility.** The reproducibility of the assay was studied by estimating interassay and intraassay variance. Six individual measurements and 30 individual measurements were done for interassay and intraassay variances, respectively. On interassay precision, the coefficients of variance were 9.9 and 10.8%, respectively, using 2 pooled sera with a pancreatic RNase concentration of 532 and 355 ng/ml. On intraassay precision, the coefficient of variance was 7.7% for the serum, with a pancreatic RNase concentration of 417 ng/ml.

**Accuracy.** The percentage of exogenous pancreatic RNase in normal serum was measured by RIA. Pancreatic RNase (500 or 1000 ng) was added to each of two 1-ml samples of serum at concentrations of 370 and 460 ng/ml, respectively. Recoveries were 94 and 90.4% when 500 ng of pancreatic RNase were added and 113 and 119 when 1000 ng of the enzyme were added. The results were also supported by the proportionate decrease in measured RNase content when serum was diluted over a wide range.

**Correlation between the Concentration of Serum Pancreatic RNase as Determined by Immunoreactivity and by Enzymatic Activity.** The concentration of serum pancreatic RNase measured by the RIA significantly correlated with its enzymatic activity using poly(C) as substrate (n = 57, r = 0.6645, p < 0.01).

**Serum RNase Level in Patients with Malignant Tumors as Measured by the RIA.** Chart 2 shows the serum pancreatic RNase contents in preoperative patients with various malignant tumors. In the patients with pancreatic cancer, the average serum RNase level (1165 ng/ml) was about 2 times higher than that in patients with other malignant tumors (574 ng/ml). Patients A and B with pancreatic cancer had complications from renal failure (Patient A: creatinine, 4.0 mg/dl; BUN, 107.6 mg/dl. Patient B: creatinine, 1.6 mg/dl; BUN, 13 mg/dl). When Patients A and B were excluded, the mean serum RNase content was 780 ± 87 (S.E.) ng/ml in the patients with pancreatic cancer, and the median concentration was 690 ng/ml. In contrast, the concentration of serum RNase and the median concentration in other groups were 628 ± 58 and 660 ng/ml in gastric cancer,

**Chart 1.** Standard RIA curve for human pancreatic RNase and dilution curves of human pancreatic amylase, human elastase II, bovine trypsin, DNase I, RNase T1 (A. oryzae), and bovine RNase A: •—•, human liver RNase; X—X, bovine trypsin; Δ—Δ, human amylase; A—A, human elastase-II; •—•, RNase T1 (A. oryzae); ——●, DNase I; □—□, bovine RNase; ——○, standard curve for human pancreatic RNase.
Correlation of Serum RNase Content as Determined by RIA with Patient's Age, Serum Amylase Activity, Serum Albumin, and BUN Contents. The serum concentration of the enzyme is elevated in proportion to age ($n = 160$, $r = 0.7677$, $p < 0.01$). No correlation was seen, however, between the concentration of the enzyme and the amylase activity in serum. There was significant reverse correlation between serum RNase and serum albumin contents ($n = 92$, $r = -0.2138$, $p < 0.05$). There appears to be significant correlation between the concentration of the enzyme and the BUN contents ($n = 102$, $r = 0.3569$, $p < 0.01$).

Multiple Regression Analysis of the Concentration of Serum Pancreatic RNase, Age, Serum Albumin, and BUN Contents. Data on the serum immunoreactive pancreatic RNase concentration, age, serum albumin, and BUN contents were obtained from 103 sera from 52 patients with malignant tumors except pancreatic cancer. By multiple regression analysis, the following equation was derived:

$$\text{Serum RNase (ng/ml)} = 6.21 \times \text{age (years)} + 16.85 \times \text{BUN (mg/dl)} - 169.81 \times \text{albumin (g/dl)} + 722.38$$

($r = 0.501336$, $F = 11.0786$, $p < 0.01$).

Comparison of Serum RNase Concentrations Measured by RIA and Those Calculated from the Multiple Regression Equation in Patients with Pancreatic Cancer. Table 1 shows the serum RNase concentrations measured by the RIA and the concentration calculated from the regression equation for patients with pancreatic cancer. The results for Patients A and B (Chart 2) were excluded because they were complicated by renal failure. No significant difference was demonstrated between serum pancreatic RNase measured by the RIA and that calculated by the equation (paired-sample $t$ test and Wilcoxon signed-rank test). There was also significant correlation between serum pancreatic RNase measured by RIA and that calculated by the equation ($n = 12$, $r = 0.714$, $p < 0.01$).

**DISCUSSION**

We have developed a sensitive, specific, and reproducible RIA for human pancreatic RNase. As reported previously, the immunoreactive RNase in human serum and urine could be determined at several different dilutions (11). The pancreatic RNase concentrations in serum measured by RIA correlated well with those measured by enzymatic assay using poly(C) as substrate. In comparison with the enzymatic assay, the RIA showed a much wider range of measurement than the enzymatic assay method, and the procedure such as sample dilution gave a smaller influence on the measured value of RNase.

Recently, Dekker$^4$ and Francesconi et al. (6) revealed that serum RNase levels elevated proportionally with age. Our results are similar. Since Sigulem et al. (22) have reported earlier that serum RNase is elevated in malignancy, we checked the serum albumin and BUN contents of patients with their nutritional state. There was a significant positive correlation between serum RNase and BUN contents, and significant reverse correlation between serum RNase and albumin contents.

A multiple regression equation for serum pancreatic RNase was obtained by statistical analysis of the data from patients with malignant tumors (except pancreatic cancer), and significant correlation was found. Also, serum immunoreactive RNase contents measured by the RIA of patients with pancreatic cancer were compared with those calculated using the equation. Two patients with pancreatic cancer were excluded because of complications from renal failure (8, 18). The pancreatic RNase contents measured by the RIA agreed well with the values calculated using the equation. Therefore, serum RNase level could be estimated by the same equation in the patients with pancreatic cancer as other malignant tumors. It is concluded that serum pancreatic RNase cannot be a diagnostic marker of pancreatic cancer.

In the present study, the average serum immunoreactive RNase content in pancreatic cancer was higher than that in other malignant tumors. An elevation of serum RNase in patients with pancreatic cancer has been reported, but it might be due to pathological status such as renal failure or malnutrition in the advanced stage of the patients’ illness or to the advanced age of patients. However, there was one patient whose serum RNase content measured by the RIA differed markedly from the value calculated from the equation. Thus, there could be a possibility that the equation may not be applicable to the estimation of the serum RNase level in all patients with malignant tumors or that there is a specific pancreatic cancer which supplies a larger amount of RNase to the serum than the amount expected from patient’s status, although the characteristics of such a pancreatic cancer cannot be elucidated presently.

**REFERENCES**


$^4$ C. A. Dekker, University of California at Berkeley, personal communication.


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