Immunological Assay of Pancreatic Ribonuclease in Serum as an Indicator of Pancreatic Cancer

Joachim L. Weickmann, Erik M. Olson, and Dohn G. Glitz

Department of Biological Chemistry, UCLA School of Medicine, University of California, Los Angeles, California 90024

ABSTRACT

Serum levels of RNase activity, presumed to originate in the pancreas, have been suggested to be of use in the diagnosis of pancreatic cancer. We have used a radioimmunological assay of human pancreatic-like RNase to quantitate this protein in serum from normal blood donors and patients with a variety of diseases. Serum pancreatic-like RNase rises gradually with age, and its level is usually higher in males than females. Although many patients with pancreatic cancer show elevated serum levels of immunologically cross-reactive enzyme, others are apparently normal. In several other types of cancer, a similar pattern of elevated RNase is apparent. However, in kidney or bladder carcinoma and in patients with severe kidney disease, RNase levels are almost always greater than normal. Regardless of the nature of the disease, an elevated level of pancreatic-like enzyme is usually accompanied by above-normal levels of serum urea nitrogen. Hence, elevated circulating levels of pancreatic-like RNase are best related to kidney function and do not serve as a specific marker for cancers of the pancreas or other organs.

INTRODUCTION

In 1976, Reddi and Holland (37) suggested that elevated levels of serum ribonuclease were specific for the detection of pancreatic carcinoma. This has been a controversial proposal; several laboratories have reported results that support the hypothesis (20, 32, 38, 45), while other workers used similar methods and found little (9, 11, 18) or no correlation (22, 24, 33). Moreover, altered serum RNase has been related to such diverse diseases as ovarian cancer (39, 40), leukemia (3, 12), prostatic carcinoma (8, 17), multiple myeloma (19), pancreatic fibrosis and degeneration (4, 44), kidney problems (23, 34, 41), myocardial infarction (42), malnutrition (41), and cancer in general (13, 31).

Several factors contribute to this confusion. Normal serum contains a variety of distinct RNases; Akagi et al. (2) have partially purified 5 serum RNases, and many more molecular species may be present. Polyacrylamide gel electrophoresis allows separation of multiple enzymes (7, 43), and up to approximately 20 activities have been seen (28). Identification or quantitation of any single enzyme of this mixture (presumed usually to originate from and be characteristic of a single tissue) must be very difficult. One of the most common assay procedures uses a polycytidylate substrate (30, 37); we believe it is not technically suited for use in a routine clinical laboratory setting, and its precision and linearity have been questioned (9). Many different assay procedures and substrates have been used by others. It is thus difficult to relate different studies to each other or to know which enzymes were being accurately measured in each case.

Pancreatic cancer continues to be a major form of cancer with a "dismal prognosis" (26); a means for early diagnosis could provide some hope for development of more successful means of treatment. An immunochemical assay of RNase, in which one structural form of RNase was quantitated as a protein entity, could provide a reliable means of measuring that specific protein if it was overproduced or released into the bloodstream from a cancerous pancreas. We have therefore studied the potential use of serum RNase as a tumor marker by a combination of biochemical and immunological means. The major enzyme has been purified from autopsy pancreas (46) and characterized to the level of sequence analysis (5). Antibodies to the enzyme have been used to develop a radioimmunological assay and to study tissue distribution of pancreatic-like RNases (47). In this paper, we report results of the immunological and enzymatic assay of pancreatic-like RNase in serum from normal individuals and patients with a variety of diseases.

MATERIALS AND METHODS

Assay Procedures. The enzymatic assay of RNase activity was based on the formation of acid-soluble nucleotides from a wheat germ RNA substrate (21) in a modification of a published method (15, 46). The assay mixture contained (in 1.00 ml) 0.15 mmol of NaCl, 0.05 mmol of Tris-HCl (pH 8.2), 1.0 mg of RNase-free bovine serum albumin (Miles, Sigma, or Calbiochem), 1 mg of RNA, and enzyme. The reaction components were incubated for approximately 10 min at 37°C, and the reaction was started by addition of substrate. After 15 min at 37°C, the reaction was terminated by the addition of 3.0 ml of ice-cold 10% (w/v) perchloric acid. After approximately 15 min on ice, the samples were centrifuged 20 min at 6000 rpm in a precooled Sorvall SS 24 rotor at 0°C, and the absorbance of the supernatants was measured at 260 nm. One unit of RNase activity is defined as the amount that gives an absorbance of 1.0 greater than that of an enzyme-free blank treated in the same manner. Pure human and bovine pancreatic RNases have specific activities of 3.9 x 10^4 and 3.7 x 10^4 units/mg of protein, respectively, in this assay. Within the range of 0.2 to 1.0 unit, the assay is linear and usually reproducible within 0.02 unit.

RNase activity was also measured using a poly (C)2 substrate (purchased from Calbiochem or Sigma) in the assay above and in the procedure described by Reddi and Dreiling (35). In the latter assay, the specific activity of several preparations of our purified human RNase was at least 13,000 units/mg of protein.

The radioimmunoassay procedure has been described (47). Pancreatic RNase for iodination or for use as a standard was purified from autopsy pancreas (46); in the process of this work, 3 enzyme preparations were used with identical results. The preparations of iodinated RNase usually retained over 90% of their catalytic activity, and in most of the preparations, over 75% of the radioactivity could be precipitated by antibody. For the assays reported in this paper, serum from a single rabbit (No. 153) was used at a final dilution of 1:20,000; in the absence of added inhibitor, about half of the precipitable 125I was bound under these conditions.
Serum RNase in Cancer

Background precipitation with non-immune antiserum was about 2 to 4% of the total 125I.

Serum Samples. Freshly drawn human venous blood samples were allowed to clot for 1 to 2 hr and then centrifuged at room temperature at full speed in a Sorvall GLC-1 table top centrifuge (approximately 1000 × g). The serum fraction was decanted, stored at −20° in small portions, and thawed as needed.

Other Methods. The protein concentration of human pancreatic RNase samples was measured by a variation of the method of Lowry et al. (29) with crystalline bovine pancreatic RNase A (Calbiochem) as a standard. Serum protein was measured using the Bio-Rad dye binding protein assay with crystalline bovine serum albumin (Sigma) as the standard. Serum urea nitrogen was quantitated colorimetrically (10) using an assay kit from Sigma. Any unspecified chemicals were of reagent grade or the highest quality commercially available.

RESULTS

Validity of Assays. A competitive binding RIA procedure has been developed and shown to quantitate pancreatic-like RNases even in the presence of other RNases (47). The reproducibility and sensitivity of the assay are shown in Chart 1; 3 different purified human RNase preparations, assayed at different times, gave indistinguishable results. In addition, 6 preparations of iodinated RNase were used as radioligand; again, the standard curves obtained were indistinguishable. The point of 50% inhibition of 125I-RNase binding corresponds to about 3 units of enzyme activity per tube, equivalent to approximately 8 ng of cross-reactive RNase or the quantity of RNase found in 10 to 15 µl of normal human serum.

Serum samples were usually frozen shortly after collection and stored at −10° until assayed, normally within 1 week. However, enzymatic activity and immunological cross-reactivity were unchanged during at least 1 year of storage at −10°. In addition, several cycles of freezing and thawing did not affect the assay results. In contrast, some of our serum samples had been treated at 56° for 30 min; this resulted in a variable loss of 30 to 70% of the initial enzymatic and immunological RNase activity. Therefore, assays of heat-treated serum samples were never included in our data.

The relationship of enzymatically measured RNase activity and immunologically cross-reactive RNase in normal serum samples is shown in Chart 2. The 2 measurements are not identical; on the average, 82% of the total serum RNase is immunologically cross-reactive, but significant and reproducible variations from the usual ratio occur.

RNase in Normal Samples. Serum samples from apparently healthy blood donors were obtained through the UCLA Blood Donor Center and immunologically assayed for RNase. Charts 3 and 4 show the relationship between RNase and age for males and females, respectively. In each case, RNase levels rise with age, but values in males are usually higher than those in females of the same age. In a few cases, serum samples were obtained from the same person 3 to 8 times over an approximate 2-year period. The RNase level in each individual was constant (within experimental error) throughout the time examined.

Serum RNase in Cancer Patients. Levels of pancreatic-like RNase were determined in a large number of samples from patients with various types of cancer. In each instance, duplicate
J. L. Weickmann et al.

assays were performed and then related to patient age and sex. Diagnoses were obtained from hospital records, but (except that the samples were from patients with well-diagnosed and active disease) little or no information was available regarding patient condition, extent of tissue involvement at the time of diagnosis, tumor burden, or metastatic spread and involvement of other organs. Results are shown for pancreatic cancer (Chart 5), lung and tracheal cancer, cancers of the stomach and gastrointestinal system, and cancers of the sexual organs (Chart 6), lymphatic and breast cancers (Chart 7), and bladder and kidney cancers (Chart 8).

For use as control values, serum samples were also obtained from patients with active noncancerous disorders including pancreatitis and Crohn’s disease and recent victims of myocardial infarctions. In Table 1, these data are compared with the results shown in Charts 5 to 8. Because RNase levels in cases of bladder and kidney carcinoma were especially high (Chart 8), serum samples were also obtained from patients with well-identified nonmalignant kidney diseases, including individuals undergoing dialysis. RNase assay results are shown in Chart 9. Chart 10 relates serum RNase levels to values of one common measure of kidney function, serum urea nitrogen. Normal subjects, cancer patients, and individuals with kidney disease are all shown in Chart 10, but only those cancer patients whose RNase or serum urea nitrogen level was significantly elevated are included; other cancer patients were normal in both measurements.

DISCUSSION

Elevated levels of circulating RNase have been related to a bewildering variety of diseases, with several mutually exclusive claims that RNase is a highly specific marker. There are many potential reasons for this confusion. RNase is a stable and easily detected enzyme. But the assay substrates (including our own) are mixtures of complex macromolecules (e.g., wheat germ RNA or yeast RNA) or homopolymers of undefined molecular size [e.g., poly(C)], and the reaction products measured are rarely characterized mixtures of nucleotides and oligonucleotides that are soluble under a given set of conditions. Although serum or plasma RNase is often discussed as if it were a simple protein, it is a complex mixture of enzymes (2, 7, 28, 45). Protein RNase inhibitors exist in several organs (6) and could affect activity measurements if released into the blood. We therefore question the validity of any conclusions based on enzymatic measures of RNase in serum.

The use of an immunologically based procedure for assay of RNase reduces the potential for confusion. Rather than an ill-defined activity, a specific protein structure is quantitated, and measurements from different laboratories should be directly comparable. We have shown (47) that our RIA is specific for pancreatic-type RNase and that there is little or no interference by nonhuman pancreatic RNase or human RNases characteristic of...
other organs such as liver or spleen. But the assay will measure all serum RNases encoded by the pancreatic RNase gene or its close relatives, regardless of the tissue of origin, and probably with only partial recognition of different levels or forms of glycosylation.

Two other laboratories have used immunologically based assays previously to quantitate pancreatic RNase. Reddi and Dreiling (36) used anti-RNase antibodies to inhibit serum RNase activity against poly(C). But their purified RNase preparation shows a specific activity of only 2,840 units/mg of protein3 (in contrast to the enzyme used in these studies with a specific activity of approximately 13,000 units/mg in the same assay). Thus, it is probable that their initial immunogen was no more than 25% RNase and that antibodies to contaminating proteins (perhaps including other nuclease species) could well predomi-

3 Reddi and Dreiling (35) have estimated the specific activity of their enzyme if it were to be measured in our RNase assay; their calculated value ($5.7 \times 10^6$ units/mg) can be derived by multiplying the RNase activity in their assay (2840 units/mg) by 13.2 (the molar extinction coefficient of cytidylic acid) and again by 15 (the time of our reaction, in min). Their calculation did not include a dilution factor of 4, which reduces the calculated specific activity of their preparation to $1.4 \times 10^6$ units/mg in our procedure. It is equally difficult to compare their calculated value with the result we obtained when our enzyme was assayed in our usual way, but with a poly(C) substrate ($17.2 \times 10^6$ units/mg) since both pH and acid precipitation conditions were different in the 2 procedures, and the protein assays were based on different standards. These calculations do demonstrate the problems inherent in attempting to relate RNase activities measured in different assay procedures.

The RIA of Kurihara et al. (27) is similar to our procedure, except that it is about one-sixth as sensitive and shows more cross-reactivity with liver RNases. However, their measurements of serum and urine pancreatic-like RNases are compatible with our results, confirming our expectations about interlaboratory comparison of data.

The control experiments of Charts 3 and 4 show levels of serum pancreatic-like RNase to depend on age and sex. The age dependence of activity against a poly(C) substrate has been noted before (e.g., Refs. 9 and 32), and our data thus help relate the enzymatic and immunological results. Many of the studies linking serum RNase to a disease do not consider factors of age or sex, but the data of Charts 3 and 4 show that comparison of a population of healthy 60- to 70-year-old "patients" with a group of 20- to 30-year-old student volunteers, laboratory workers, or blood donor "controls" would suggest a near doubling of RNase concentration.
kidney or bladder carcinoma, most assays are well above normal (Chart 8). Several studies have tied elevated RNase activity in serum to renal malfunction (e.g., Refs. 23, 34, and 41), and our data link elevated pancreatic-like serum RNase to kidney disease (Chart 9) and to kidney function regardless of disease (Chart 10). But the relationship between elevated RNase and abnormal serum urea nitrogen levels is not perfectly consistent, in agreement with the enzymatic measurements of Abramson et al. (1). The kidney is a fairly rich source of pancreatic-like RNase (18, 47), but it is not obvious if the enzyme is synthesized in the kidney, cleared by the kidney from the blood, or both. Our results certainly suggest that a large proportion of the serum pancreatic-like RNase that is elevated in diseased individuals accumulates because of kidney malfunction. Much work (e.g., Refs. 14 and 25) indicates that an abnormal serum or urine level of small proteins, such as β2-microglobulin, is associated with problems in kidney function. It is thus possible that RNase measurements could be of value in assessing renal function or in evaluation of the overall effects of therapy in specific individuals. Both Peterson (33) and Abramson et al. (1) strongly question the pancreatic origin of most serum RNase, since RNase activities in serum were found to rise after pancreatectomy. We find significant levels of pancreatic-like RNase in several organs (47), and in some instances, it appears that the tissue specificity of human RNases is quite unlike that of similar enzymes in other species. For example, most of the RNase in human brain is immunologically and catalytically identical to the pancreatic enzyme (47), while bovine brain RNase is quite unlike the cow pancreatic protein (15, 16). It thus seems that not only was serum RNase misconstrued as a marker for pancreatic cancer, but also that the pancreatic-like enzyme in serum may originate in one or more other organs.

ACKNOWLEDGMENTS

We thank the UCLA Blood Donor Center for samples of serum from healthy volunteers and personnel of the UCLA Bowyer Cancer Clinic for serum samples from cancer patients. We are particularly grateful to Dr. Robert Lechner for his aid and advice in sample acquisition and to Janice Trask of the Bowyer Clinic who was instrumental in expediting this process.

REFERENCES


Immunological Assay of Pancreatic Ribonuclease in Serum as an Indicator of Pancreatic Cancer

Joachim L. Weickmann, Erik M. Olson and Dohn G. Glitz

*Cancer Res* 1984;44:1682-1687.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/4/1682

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.