Prostatic Acid Phosphatase as Measured with Two Radioimmunoassay Kits in the Detection of Prostatic Adenocarcinoma

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Serum prostatic acid phosphatase concentration was measured with two commercially available radioimmunoassay kits. Results were compared with histological evidence of prostatic adenocarcinoma obtained at autopsy in 33 patients. The serum assay did not differentiate significantly ($p > 0.1$) between patients with adenocarcinoma and those without. We conclude that the test, at least as performed by use of these kits, is of little value in the detection of occult disease.

Additional Keyphrases: cancer - "kit" methods

The conventional enzymic determination of serum acid phosphatase (EC 3.1.3.2) is recognized to be of value in the follow-up of advanced prostatic adenocarcinoma, but is of no value in the early diagnosis of treatable lesions (1). A radioimmunoassay specific for prostatic acid phosphatase has become available in recent years that reportedly has improved specificity and sensitivity as compared with the older conventional spectrophotometric enzymic methods (2-4). We hoped that, with this improved assay, lesions at clinical Stage 1 of prostatic adenocarcinoma might be detected; this lesion is curable but cannot be detected by simple rectal examination (1). In previous studies the prostatic acid phosphatase concentration in serum has been correlated with clinical criteria for the staging of prostatic adenocarcinoma (3-7). It is generally agreed that the radioimmunoassay method was superior to the conventional enzymic techniques for detecting occult disease.

Microscopic evidence of Stage 1 prostatic adenocarcinoma in older males can be found about 46% of the time (8). Thus the yield of such evidence among randomly selected autopsies of men over 35 years of age would be expected to be high. In the present study, we collected prostate glands from such autopsies and attempted to correlate the histological findings with data on prostatic acid phosphatase concentration in serum specimens collected before the patients’ deaths.

Materials and Methods

Subjects. The material for study was collected in the following manner: (a) prostate glands were routinely obtained from all autopsies of men over age 35 years (excluding Medical Examiner cases) from August 13, 1979 to November 29, 1979 and January 3, 1980 to March 14, 1980 at the University of Virginia Hospital (58 patients); (b) glands from only those patients from whom serum (stored at $-20^\circ$ C) was available were studied histologically (43 patients); (c) at histological examination, 10 glands were excluded because of postmortem autolysis (one patient) or insufficient tissue (nine patients).

After collection, each prostate gland was immediately fixed in formaldehyde/water (10/90 by vol) for at least 72 h, block-sectioned completely, and examined histologically for the presence of prostatic adenocarcinoma. The mean number of blocks per case was 15 (range 8 to 33). Histological sections were examined by two histopathologists (J.P.B., B.C.S.) who had no knowledge of the serum prostatic acid phosphatase concentration.

The age distribution by year, the number with adenocarcinoma of the 33 patients who were included in the study was: 35-39, 3 (0); 40-49, 1 (0); 50-59, 7 (2); 60-69, 11 (7); 70-79, 6 (5); 80-89, 5 (3). The primary disease (cause of death) in these patients was: atherosclerosis or cardiac disease, or both, 13; carcinoma or melanoma, nine; haematological malignancy, three; cirrhosis of the liver, three; acute or chronic infections, four; and chronic renal failure, one.

Prostatic acid phosphatase assays. Sera were retrieved from the Clinical Biochemistry Laboratory of the University of Virginia Medical Center upon notification that the patient had died and was to be autopsied. The serum samples had been stored at $-20^\circ$ C for 10 days or less.

We used two commercially available radioimmunoassay kits. (a) The Rianen kit (New England Nuclear, Billerica, MA 01821) is a double-antibody system with $^{125}$I-labeled antigen. Overnight incubation of standard or sample, tracer, and primary antibody was recommended and used for optimal performance. The standards contained 0, 2.5, 5, 10, 25, 50, and 100 µg of human prostatic acid phosphatase per liter. Day-to-day precision of the assay (CV) ranged from 7 to 11%. Normal reference ranges proposed by the vendor were 0.6 to 3.3 µg/L. (b) The GammaDab kit (Clinical Asays, Division of Travenol Laboratories, Inc, Cambridge, MA 02139) is very similar in principle to the Rianen procedure. Again, we used the overnight incubation recommended by the supplier. Standards contained 0, 1, 3, 10, and 30 µg of human prostatic acid phosphatase per liter. Day-to-day precision (CV) ranged from 6 to 9%. The reference interval proposed by the manufacturer is 0 to 2.0 µg/L.

Results

Figure 1 shows the serum prostatic acid phosphatase concentrations for the 33 patients. Sera from all 33 patients were analyzed by the Rianen kit but sera from only 27 were available for analysis by the GammaDab kit. Of the 33 patients studied, 17 had histological evidence of prostatic adenocarcinoma, and two had metastases.

Fisher’s exact test was used to analyze the radioimmunoassay results for their usefulness in distinguishing patients with adenocarcinoma from those without. Neither assay technique distinguished the patients with adenocarcinoma from those without ($p > 0.1$).

The characteristics of the two assays in distinguishing histological evidence of adenocarcinoma from non-carcinoma are defined in Table 1 in terms of the test specificity, sensitivity, and predictive value (9). Consistent with the results for Fisher’s exact test, the data in Table 1 also demonstrate the inadequacies of the two assays in providing a test for the detection of occult adenocarcinoma.

The histological material was also examined to assess the possibility that prostatic hyperplasia was causing a high

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percentage of false-positive results. Of 16 patients without prostatic adenocarcinoma, five had nodular hyperplasia; with the Rianen assay three of these 16 had increased serum prostatic acid phosphatase concentrations. Five of 14 non-carcinoma patients whose sera were analyzed with the GammaDab method had prostatic nodular hyperplasia; in only one of these was the serum prostatic acid phosphatase concentration increased.

Results obtained by the two radioimmunoassay methods were compared for the 27 serum specimens where both analyses were performed. The resulting equation was: \( y = 0.443x + 0.23 (r = 0.961) \), where \( y = \text{GammaDab} \) and \( x = \text{Rianen} \). Thus, results of the two assays correlated reasonably well. The slope of 0.443 apparently resulted from standardization differences, and this also affected the respective normal reference ranges of the two assays.

Discussion

Our objective was to determine the value of radioimmunoassay of prostatic acid phosphatase in serum for detecting prostatic adenocarcinoma. To determine the true sensitivity and specificity of this serum test, we compared the findings with information obtained by histological examination of material collected at autopsy. All of the patients studied were at least 35 years old, but prostatic adenocarcinoma was found only in those over 50 years of age, with an overall incidence for the series of 17 (52%), a percentage similar to that found for a previously reported autopsy series (8).

With neither of the radioimmunoassay kits we evaluated could we significantly differentiate patients with prostatic adenocarcinoma from those without. The predictive values of both methods were also disappointing: the Rianen predicted positive and negative results in only 64% and 58%, respectively, the GammaDab in 50% and 52%. These predictive values are unsatisfactory for a test that may be used to screen large numbers of patients. Serum prostatic acid phosphatase was above normal by the Rianen assay in seven of the 15 patients with occult prostatic adenocarcinoma, but the number of false-positive results precludes its use as a screening test. With the GammaDab assay we detected only one patient with occult adenocarcinoma; the other two patients with above-normal values for prostatic acid phosphatase concentrations in serum had metastatic adenocarcinoma. These latter findings represent no improvement over those obtained with an enzymic assay. Although occult prostatic adenocarcinoma may be difficult to detect by any serum assay, three of the eight false-negative results were for patients in whom more than three of their histological slides contained prostatic adenocarcinoma. Although we included in our study two patients with metastatic disease and four patients who were under the age of 50 years, and thus unlikely to be included in a screening program involving this assay, the exclusion of these patients from the evaluation did not significantly affect the statistical evaluation of our findings.

We emphasize that the results reported here were derived by use of two commercially available radioimmunoassay kits. One of these (Rianen) has been shown (7) to provide results for Stages 1–4 adenocarcinoma of the prostate that compare well with those by an assay developed and evaluated in a research laboratory setting (5), which indicates that this particular commercial kit represents a valid radioimmunoassay for serum prostatic acid phosphatase. However, of the several commercially available kits for this assay, some other conceivably might give more encouraging results.

From our data we conclude that measuring serum prostatic acid phosphatase by radioimmunoassay (at least with these two commercial kits) is not effective in detecting Stage 1 adenocarcinoma of the prostate and therefore would be of no value as a screening test for this disease.

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References

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Insulin Degradation by Human Erythrocyte Lysates

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In vitro hemolysates of isolated human erythrocytes degrade $^{125}$I-labeled insulin. Ten- to 100-fold dilutions of the hemolysate give a proportionally decreased degradation of $^{125}$I-labeled insulin at 37 °C, while dilutions of up to eightfold do not. Like the control, diluted “Buffer G” containing 5 mmol/L Tris and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer alone, more than 500-fold dilutions of the hemolysate or boiled hemolysate (in buffer) caused negligible (<1%) degradation of the labeled insulin. We conclude that accurate insulin-binding data during erythrocyte insulin radioreceptor assay under optimum conditions (Clin. Chem. 23: 1590–1595, 1977) depend on avoiding or minimizing hemolysis.

Additional Keyphrases: insulin receptor binding - erythrocyte membranes

An insulin radioreceptor assay for human erythrocytes was described earlier (1). Recently we found (unpublished observations) that the plasma membranes of human erythrocytes, unlike the membranes of monocytes, show no insulin-degrading activity. Thus the insulin-degrading activity observed with erythrocyte lysate evidently is ascribable to erythrocyte contents. In any event, this degrading activity of human erythrocytes makes it important to avoid or minimize lysis of erythrocytes during the assay if insulin-binding data are to be accurate. Here we present evidence for degradation of $^{125}$I-labeled insulin in vitro by human erythrocyte lysate and show that under the optimum conditions (15 °C, pH 8.0, and 3.5-h incubation) for the assay (2), insulin-binding data may be unreliable unless lysis of erythrocytes is avoided or minimized.

Materials and Methods

Monocomponent insulin (lot no. JM95AF) was purchased from Elanco Laboratories, Indianapolis, IN 46206 for iodination with Na$^{125}$I (by New England Nuclear). “Fentex” bovine albumin was from Miles Laboratories Inc., Elkhart, IN 46515. The other chemicals were of the highest purity available commercially.

From six non-obese, non-diabetic men with no family history of diabetes, ranging in age from 20 to 40 years, 4 to 10 mL of blood was obtained by venipuncture before breakfast, after an overnight fast.

Erythrocytes, isolated by Hypaque–Ficoll gradient centrifugation (1), were used for hemolysis. “Buffer G,” a mixture that includes Tris and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, prepared as described earlier (1), but without bovine albumin, was diluted to 5 mmol/L and used to lyse erythrocytes and in the insulin-degradation studies. A 10 g/L bovine albumin solution was prepared in the diluted buffer. A 200 g/L trichloroacetic acid solution was made in de-ionized glass-distilled water.

The purified erythrocytes were hemolyzed by adding an equal volume of the diluted buffer at 23 °C. After 15 min, hemolysis was observed to be almost complete; no residue was visible after the mixture was centrifuged (400 × g, 10 min, 23 °C).

A mixture of 900 µL of hemolysate with 100 µL of $^{125}$I-labeled insulin (500 pg, spec. acty. 130–175 Ci/g) was incubated at 37 °C. At various time intervals, 50-µL aliquots, in duplicate, were removed into 16 × 100 mm plastic tubes (placed in ice) containing 200 µL of the 10 g/L bovine albumin solution. The proteins in these aliquots were immediately precipitated by adding 200 µL of a 200 g/L solution of trichloroacetic acid. The precipitates and supernatants were separated and their radioactivity measured with a gamma counter. As the controls, 900 µL of the buffer and 900 µL of boiled hemolysates were incubated individually with 100 µL of $^{125}$I-labeled insulin (500 pg) each at 37 °C. Aliquots taken at similar time intervals were treated as described above. The