Counterimmunoelectrophoresis in Determination of Prostatic Acid Phosphatase in Human Serum

Andras G. Foti,1 J. Fenimore Cooper,2 and Harvey Herschman3

We evaluated counterimmunoelectrophoresis for use in measuring prostatic acid phosphatase in detection of prostatic cancer. After staining for acid phosphatase, we could detect as little as 0.3 ng of purified enzyme standard complexed with antibody by this technique. However, when serum samples were used as antigen, the method was less sensitive (1.5–2.0 ng) because some of the serum proteins migrate with the phosphatase and decrease the intensity of the stain for acid phosphatase. For this reason we could not detect the phosphatase in serum samples of normal persons; only patients with moderately (or greater) increased activity in their serum showed positive results. In contrast, by radioimmunoassay as little as 1.0 ng of the phosphatase can be detected in serum.

Additional Keyphrases: intermethod comparison • enzyme assay • radioimmunoassay • prostatic cancer • diagnostic aids • screening • procedure for small laboratories

Prostatic carcinoma is one of the leading malignancies for men after age 60. A feasible screening test for the detection of prostatic carcinoma could lead to early recognition of this disease when it is still confined to the prostatic capsule and considered operable. Increased prostatic acid phosphatase (EC 3.1.3.2, PAP) activity in the serum is a potent indicator of prostatic malignancy (1–6).

Biochemical techniques (enzyme assay) are now used to measure serum PAP (7–13). However, it can also be measured by the use of serologic assays. We have previously described in detail a radioimmunoassay for serum PAP (14, 15), which has several advantages: (a) the immunological activity of the enzyme is more stable than its enzyme activity (16); (b) the specificity and the sensitivity of this assay are far better than the conventional enzyme assay (16), which consequently (c) results in substantially improved detection of untreated prostatic cancer, in all stages of the disease, as compared with enzyme assay.4 However, the need to radioiodinate the antigen and the need for an expensive gamma counter led us to investigate the use of alternative serologic procedures for detection of PAP.

Counterimmunoelectrophoresis has been extensively used for the detection and quantitation of many antigens (17–20). Antigen is precipitated with the appropriate antibody after migration in an electric field. It is faster than immunodiffusion and does not require use of radioactive materials as does radioimmunoassay. The technique can be very useful for small laboratories where only a few samples are tested routinely. However, it must be borne in mind that the sensitivity of the radioimmunoassay far exceeds that of counterimmunoelectrophoresis (19).

We describe here our findings.

Materials and Methods

Materials: Disodium p-nitrophenyl phosphate, Fast Red Violet LB salt, and naphthol AS-MX phosphoric acid were obtained from Sigma Chemical Co., St. Louis, Mo. 63178; agarose from Kallestad Laboratories Inc., Chaska, Minn. 55318; and barbitral from Mallinckrodt Inc., St. Louis, Mo. 63147. All other chemicals were of analytical grade and were obtained from J. T. Baker Chemical Co., Phillipsburg, N. J. 08865.

Prostatic acid phosphatase was purified from human prostatic fluid and the antiserum raised in rabbits as described previously (15).

Collection of serum samples: The normal subjects were healthy men who were undergoing routine physical examinations. In all prostatic cancer patients, the stage of the disease was confirmed by rectal examination, prostatic needle biopsy, and bone scan. In all patients, the blood was obtained at least 48 h after rectal examination and separated. Serum was stored at −25 °C until use.

Counterimmunoelectrophoresis: Glass slides (8.3 × 10.2 cm, Kodak 1402130) were precoated with agarose solution in barbital buffer (0.05 mol/liter, pH 8.6), 10 g/liter, and dried. Then 9.0 ml of the agarose solution was layered onto the slides. The gel-covered slides were placed in a moist chamber at 4 °C for at least 20 min. Wells 3 mm in diameter were cut 6 mm apart. A second row of wells was cut 10 mm from the first row. One row of wells was filled with 10 μl of purified PAP standard or serum samples. The other row of wells was filled with diluted antiserum. The electrode vessels were filled with barbital buffer (0.05 mol/liter, pH 8.6). The slide was placed in the electrophoretic chamber in such a way that the wells filled with antibody were on the anodic side, the wells with PAP on the cathodic side. The slide was connected to the electrolyte with a filter-paper bridge. A constant current of 10 mA was applied for 1.5 h at 4 °C. After the electrophoresis, the gel was stained by the Burstone technique (21).

Histochemical staining of acid phosphatase: Fast Red Violet LB salt, 50 mg/dl of water, was mixed with 10 ml of sodium acetate buffer (2.5 mol/liter, pH 5.2) containing 25 mg of naphthol AS-MX phosphate. The electrophoresis slide was incubated in this mixture at 37 °C for 5 h.

Determination of prostatic acid phosphatase: This was determined in all serum samples by both radioimmunoassay (15) and enzyme assay (22).

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Received Aug. 12, 1977; accepted Oct. 4, 1977.
**Table 1. PAP Content of Sera of Patients with Prostatic Cancer, as Measured by Various Methods**

<table>
<thead>
<tr>
<th>Patient</th>
<th>RIA ng/0.1 ml</th>
<th>Enzyme assay Sigma units/1.0 ml</th>
<th>CIE 10 µl</th>
<th>Stage</th>
<th>Treatment</th>
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<tr>
<td>EE</td>
<td>3.0</td>
<td>0.07</td>
<td>—</td>
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</tr>
<tr>
<td>AE</td>
<td>3.2</td>
<td>0.06</td>
<td>—</td>
<td>N</td>
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<tr>
<td>AL</td>
<td>3.2</td>
<td>0.06</td>
<td>—</td>
<td>N</td>
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<tr>
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<td>3.3</td>
<td>0.05</td>
<td>—</td>
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<tr>
<td>FR</td>
<td>3.4</td>
<td>0.07</td>
<td>—</td>
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<tr>
<td>SL</td>
<td>7.2</td>
<td>0.06</td>
<td>—</td>
<td>I</td>
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<tr>
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<tr>
<td>SL</td>
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<td>0.18</td>
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<tr>
<td>CG</td>
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<td>—</td>
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<td>—</td>
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<tr>
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<tr>
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<td>****</td>
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* Abbreviations used are same as in Fig. 2.

**Results**

We altered a variety of conditions for counterimmunoelectrophoresis (the buffer system and its pH, the current, duration of electrophoresis, and the amount of gel used to coat the slides), to maximize the measurement of PAP. Of those buffers we examined, the most suitable was sodium barbital (0.05 mol/liter, pH 8.6), used at 10 mA. Under these conditions, the electrodendosmosis of the antibody is equal to the migration of PAP in the 10 g/liter gel. The PAP migrates towards the anode at pH 8.6. Consequently, the enzyme and its antibody meet between the two wells. The antibody/antigen precipitate is made visible with acid phosphatase stain, because the acid phosphatase/antibody complex retains the ability to hydrolyze phosphate groups from the substrate (23, 24). Figure 1 shows the results of counterimmunoelectrophoresis when purified enzyme standards were used. The limit of detection is between 0.21 and 0.43 ng with a 25-fold diluted antisera. When 1.75 and 3.5 ng of PAP were used against antisera dilutions of 10-, 50-, 100-, 500-, 1000-, 1500-, 2000-, and 3000-fold, a visible precipitate was detected up to 2000-fold dilution. For subsequent studies a 100-fold diluted antisera was used.

In contrast to our results for purified enzyme, we were unable to detect PAP in serum of normal patients, even when more serum (20 µl) was used in the wells. Similarly, we could not detect PAP in most cases in sera from patients with intracapsular prostatic tumors (Stage I or II). Detection was not improved when serum proteins other than immune precipitates were washed out of the gels after electrophoresis, when the volume of the gel was increased to accommodate an increase volume of sample (25-50 µl), or when antibody dilutions of 10- to 25-fold were used.

The mean concentration of PAP in the serum of normal persons is 65 µg/liter by radioimmunoassay and 150-200 Sigma units/liter of serum by enzyme assay. Figure 2 shows our results by counterimmunoelectrophoresis for patients with different stages of prostatic cancer. For sera containing less than 200 µg/liter of PAP, counterimmunoelectrophoresis gave no visible stain for acid phosphatase/antibody complex. Table 1 summarizes serum PAP measurements by radioimmunoassay, by enzyme assay, and by counterimmunoelectrophoresis.

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*Fig. 1. Counterimmunoelectrophoresis of purified PAP with anti-PAP*

PAP was applied into the upper wells and 10 µl of antisera (25-fold dilution) into the lower wells. The upper wells from left to right: 0.21, 0.43, 0.87, 1.75, 3.5, 7.0, 14.0, and 24.0 ng of PAP per 10 µl. After electrophoresis the immunocomplex was stained by the Burstone (2f) method.

*Fig. 2. Counterimmunoelectrophoresis of sera from prostatic carcinoma patients*

Serum, 10 µl, was applied into the upper wells. Left to right: CK, St, III, RAD, DES; TC, St, II, DES; LM, St, IV, DES; JA, St, IV, RAD, DES; BL, St, IV, DES; BM, St, IV, untreated; IG, St, IV, DES; BM, St, III, untreated. Lower wells contained 10 µl of antisera (100-fold dilution). The immunocomplex was stained by the Burstone (2f) method. Abbreviations are (in order given) patients’ initials, stage of the cancer, and treatment (radiation; diethylstilbestrol) being received.
phoresis (the degree of intensity of the stained antibody/antigen precipitate is indicated with one to four asterisks), along with the stage of the disease and the treatment for some patients. Only a very few with Stage II of the disease and none with Stage I gave positive test results. Patients with Stage III or Stage IV disease showed positive counterimmunoelectrophoresis tests. Patients with a PAP concentration of about 2 mg/liter by radioimmunoassay and about 7000 Sigma units/liter by enzyme assay showed the greatest PAP/antibody precipitate after staining. The least amount of complex detected corresponded to 180–300 μg/liter of PAP by radioimmunoassay and 290–580 Sigma units/liter by enzyme assay. Although radioimmunoassay and enzyme assays do not show complete correlation, the trends are clearly seen. Serum samples that gave positive results by counterimmunoelectrophoresis always had an increased concentration of PAP by both of the other assay techniques. In contrast, many samples showing increased concentration of PAP by radioimmunoassay did not show increased activity by the spectrophotometric enzymatic assay or positive results by counterimmunoelectrophoresis.

Discussion

Counterimmunoelectrophoresis is one of the most widely used serologic methods for detecting and measuring antigens. It is fast and theoretically is more specific than the enzyme assay for the detection and quantitation of PAP. However, its sensitivity appears to be less than that for the enzyme assay or the radioimmunoassay. Different buffers were studied for the PAP assay by counterimmunoelectrophoresis.

We evaluated more than 100 serum samples from patients with prostatic cancer by counterimmunoelectrophoresis, with no false-positive results. However, the technique is not sensitive enough to detect PAP from serum of normal males, and so we have no mean value of PAP for normal men by it. No prostatic cancer patients with Stage I disease gave a positive test, by this technique, and only a few patients with Stage II disease did so.

We conclude that the method in its present form is not suitable as a clinical screening test.

This work was supported in part by Southern California Permanente Medical Group and by Contract EY-76-03-0012 between the Energy Research and Development Administration and the Regents of the University of California.

References