Effectiveness of Radioimmunoassay of Human Prostate-specific Acid Phosphatase in the Diagnosis and Follow-up of Therapy in Prostatic Carcinoma

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ABSTRACT

The radioimmunoassay of human prostate-specific acid phosphatase and the measurement of the catalytic activity of acid phosphatase using p-nitrophenyl phosphate as substrate were compared in the diagnosis and follow-up of therapy of prostatic cancer patients. We monitored 17 patients without metastases and eight patients with metastases for 12 months. We detected elevation of the catalytic activity of acid phosphatase [the upper limit for the reference range was mean + 2 (S.D.)] in 24% of the sera of all these patients (n = 25), and the concentration of prostate-specific acid phosphatase measured by radioimmunoassay [the upper limit for the reference range was mean + 3 (S.D.)] was elevated in 80% of these samples before therapy. The radioimmunological measurement of prostate-specific acid phosphatase was therefore more efficient in detecting prostatic cancer than was measurement of the catalytic activity. Favorable effects of the various forms of endocrine treatment were detected more clearly by the measurement of immunoassayable prostatic acid phosphatase than by the measurement of catalytic activity. Activation of the disease during various forms of endocrine treatment of prostatic carcinoma is possibly more efficiently signaled by radioimmunoassay than by measurement of catalytic activity.

INTRODUCTION

Since the early finding of Gutman et al. (4) and of Huggins and Hodges (6) that serum acid phosphatase (EC 3.1.3.2) activity is markedly increased in patients with prostatic carcinoma, determination of this enzyme activity has been widely used to detect prostatic carcinoma and to monitor therapy. When the assay of serum acid phosphatase is based on the measurement of its catalytic activity, serum phosphatases of nonprostatic origin contribute to the results (12). Another factor complicating these measurements is the great instability of the catalytic activity of the enzyme (8). However, acid phosphatase was the first "tumor marker" to be measured in the blood, and it can still be considered to be one of the most useful tumor markers. We have previously described a specific and sensitive radioimmunoassay for human prostatic acid phosphatase in serum (11). The antigen we used was purified to homogeneity from human prostatic tissue (9), and antiserum against it was raised as a monoclonal antibody. A specific and sensitive radioimmunoassay and measurement of the catalytic activity of the enzyme. Disease classification of tumors, nodes, and metastases was made according to the International Union against Cancer (5). We monitored 17 patients with T2- to T4M0 carcinoma and 8 patients with T2- to T4M1 carcinoma.

The patients were treated with polyestradiol phosphate (E tradurin; Ab Leo, Helsingborg, Sweden; 80 mg i.m. once/month) or by castration, or they underwent both treatment. During the follow-up period, the patients were seen at 0.5, 3, 6, 9, and 12 months after initiation of treatment. The control examination always included clinical examination by the urologist (O. L. or M. K.) and X-ray bone studies. Isotope bone scans were performed at 3, 6, 9, and 12 months of the follow-up period. The response to therapy was classified by the urologist according to the criteria of the National Prostatic Cancer Project (1). Serum samples for radioimmunoassay as for the measurement of the catalytic activity were taken every control examination before rectal palpation of the prostate.

About 10 ml of blood were drawn from an antecubital vein. The serum was harvested by centrifugation at 1500 \( \times \) g for 10 min, and the samples were stored at \(-20^\circ\)C until analyzed.

Preparation of the Antigen and Antibody. Human prostatic acid phosphatase was purified to homogeneity from human prostatic tissue (9), and antiserum against it was raised described previously in rabbits (11). The 2 most important step in the purification of human prostatic acid phosphatase are affinity chromatography using L(+)-tartrate AH-Sepharo

Radioimmunoassay Procedure. The radioimmunoassay w
a modification of our method published previously (10, 11). In short, it consists of the following steps: (a) incubation of 0.2 ml of serum with 0.2 ml of diluted antiserum for 1 hr; (b) addition of 0.2 ml of an iodinated tracer and incubation of the mixture for 3 hr; (c) separation of bound and free radioactivity by precipitation with anti-rabbit γ-globulin-polyethylene glycol solution for 15 min; (d) counting of radioactivity in the bound fraction. The concentrations of acid phosphatase in sera were calculated with the aid of standards of 0, 0.5, 1.5, 5, 10, and 30 μg of the purified enzyme per liter. The lowest measurable acid phosphatase concentration detected by this method was 0.2 μg/liter. Dilution of aliquots of the samples with acid phosphatase-free sheep serum allowed quantification of concentrations manyfold higher than the standard upper limit (30 μg/liter) of the assay. Within- and between-assay coefficients of variation were between 3 and 14% and between 5 and 11%, respectively, and nonspecific binding was 5%. Concentrations of immunoreactive prostatic acid phosphatase in serum samples from healthy men (n = 394) ranged from 0.3 to 3.6 μg/liter [1.94 ± 0.66 (S.D.)], and, in patients suffering from benign prostatic hyperplasia (n = 56), verified by histology, the concentrations ranged from 0.5 to 3.6 μg/liter [1.71 ± 0.76 (S.D.)]. We used a value of 4 μg/liter [mean + 3 (S.D.)] as the upper normal limit (10).

Enzymatic Assay. Assay of the catalytic activity of acid phosphatase was made by the modified method described by Bessey et al. (2) using p-nitrophenyl phosphate as substrate (Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany) and L(+)-tartrate as inhibitor (E. Merck, Darmstadt, Federal Republic of Germany). The upper limit of the reference range for the total activity of acid phosphatase was 13 IU/liter [mean + 2 (S.D.)], and of tartrate-labile acid phosphatase, it was 2.5 IU/liter [mean + 2 (S.D.)].

Statistical Methods. The t test for paired observations was used in the analysis of the results.

RESULTS

In the present group of T2- to T3M0 patients, serum radioimmunoassayable prostatic acid phosphatase concentrations exceeded the upper limit of our reference range of 4 μg/liter [mean + 3 (S.D.); see "Radioimmunoassay Procedure"] in 12 of 17 patients (T2M0, 3 of 6; T3M0, 5 of 7; T3M0, 4 of 4) prior to the institution of any form of therapy. In comparison, the measurement of catalytic activity, both total and tartrate labile, gave results within the normal reference range in 16 of 17 of these patients. The elevated value was in a patient with T3M0 disease. In Stage T2- to T3M1 patients, the catalytic activity was elevated in 5 of 8 (both total and tartrate labile), whereas immunoassayable prostatic acid phosphatase was elevated in all patients of this group (Chart 1). After the initiation of therapy, elevated radioimmunoassayable serum prostatic acid phosphatase was normalized within 3 months in 6 of 12 Stage T2- to T3M0 patients and in 6 of 8 Stage T2- to T3M1 patients. In the former patient group, the catalytic activity was elevated in only one patient before treatment, and it reached a normal level within 3 months of treatment. In the latter patient group, catalytic activity elevation was found initially in 5 patients, but this became normalized in 3 patients during the same period of therapy (Chart 2). In the T2- to T3M1 group, the mean percentage of decrease in serum concentration of immunoassayable prostatic acid phosphatase was statistically significant (p < 0.001) at 1 month (−79 ± 6% (S.E.)), at 3 months (−73 ± 8%), and at 6 months (−67 ± 11%) after initiation of therapy as compared with the concentrations before any form of therapy. The changes in the catalytic activity of serum acid phosphatase in this group during treatment were not statistically significant. Clinically, a partial response (1) to the therapy was seen in 3 patients of the T2- to T3M1 group within 3 months. Two patients had a stable response, and 3 patients showed progression of the disease at this time. At the end of the follow-up period, there were 2 patients in the T2 to T3M0 group who showed a complete response to the therapy, and 5 of 8 were alive. Six patients of the T2 to T3M0 group showed a partial response, 3 showed a stable response, and 4 showed progression of the disease within 3 months. A partial response to therapy was reflected more clearly by the serum concentrations of immunoassayable prostatic acid phosphatase than by its catalytic activity (Chart 3). In one patient of the T2 to T3M0 group, the immunoassayable prostatic acid phosphatase concentration was elevated at the control examination at 3 months, whereas the bone scan was negative. The bone scan first turned positive after 6 months of follow-up, and X-ray bone studies were not positive prior to 9 months of follow-up (Chart 4).
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Chart 3. Effect of endocrine therapy on immunoassayable prostatic acid phosphatase (PAP) concentrations and on the catalytic activity of this enzyme in the sera of patients with prostatic carcinoma. Staging of the patients: I, T1M0 disease; II, T2M0 disease; III, T3M0 disease; and IV, T4M0 disease. O, prostatic acid phosphatase measured by radioimmunoassay; ●, catalytic activity of acid phosphatase.

Chart 4. Activation of prostatic carcinoma during endocrine treatment of 2 patients with prostatic carcinoma. The monitoring of acid phosphatase of Patients I (T1M0 disease) and II (T2M0 disease). ○, prostatic acid phosphatase (PAP) measured by radioimmunoassay; ●, catalytic activity of acid phosphatase.

DIscussion

The radioimmunoassay of prostate-specific acid phosphatase seems to have distinct biochemical and clinical advantages over standard enzymatic techniques using measurements of the catalytic activity of the enzyme. Foti et al. (3) have made a comparison of their radioimmunoassay technique with a standard enzyme assay for prostatic acid phosphatase, using p-nitrophenyl phosphate as substrate, in the diagnosis of prostatic cancer. Using the radioimmunoassay, they diagnosed prostatic cancer in 33, 79, 71, and 92% of patients with Stage I, II, III, and IV disease, respectively, when the limit for their reference upper range was mean + 4 S.D. In contrast, using the enzyme assay, they detected elevations of enzyme activity in the sera of 12, 15, 29, and 60% of the patients, respectively. In this case, the upper limit for their reference range was mean + 2 S.D.

Lee et al. (7) have tested the use of a solid-phase immunoabsorbent assay and a chemical assay using α-naphthyl phosphate as the substrate for acid phosphatase. Using the chemical assay, they detected elevation of enzyme activity in 36% of the sera of prostatic cancer patients (Stages A to D, n = 39), and by immunoabsorbent assay in 79%. The upper limits for their reference ranges were mean + 2 S.D. in both assays. Our results are in agreement with the above studies, showing the better sensitivity of radioimmunoassay over the measurement of catalytic activity of acid phosphatase in detecting prostatic cancer. We found an elevation of catalytic activity of acid phosphatase in 24% of the sera from prostatic cancer patients (n = 25), and our radioimmunoassay detected elevated concentrations of prostatic acid phosphatase in 80% of the sera of these patients. Our radioimmunoassay detected elevation of prostatic acid phosphatase concentration in 71% of prostatic cancers without metastases and in 100% of metastasizing cancers. Using the measurement of catalytic activity, the comparative results were 6 and 63%, respectively. The upper limit for our reference range was mean + 2 S.D. in the catalytic activity assay and mean + 3 S.D. in the radioimmunoassay. Additional studies are needed to clarify whether the use of mean + 2 S.D., as is conventional for the establishment of upper limits of reference ranges for laboratory measurements, would be more suitable. At present, we prefer the use of mean + 3 S.D. in order to ensure the exclusion of false-positive results. Despite this, the radioimmunoassay was clearly more sensitive than the measurement of catalytic activity in detecting prostatic carcinoma, as described above.

We describe here, for the first time, the use of radioimmunoassay of human serum prostate-specific acid phosphatase in the follow-up of therapy of prostatic carcinoma. The percentage of changes of radioimmunoassayable prostatic acid phosphatase was larger than that found by the measurement of catalytic activity. Responses to the various forms of endocrine treatment were monitored more clearly by the measurement of immunoassayable enzyme than by the measurement of its catalytic activity. Progression of the disease during various forms of endocrine treatment of prostatic carcinoma is possibly more efficiently signaled by radioimmunoassay than by measurement of the catalytic activity. However, more patients must be followed to clarify this possibility.

The results suggest that the radioimmunoassay of prostatic acid phosphatase is a reliable and sensitive method for the diagnosis and follow-up of therapy of human prostatic carcinoma and clearly superior to the conventional measurement of the catalytic activity of the enzyme.

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REFERENCES

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