Urol. Res. 9, 21-24 (1981)

Urological Research

Enzyme Immunoassay for the Prostate-Specific Acid Phosphatase (E. C. 3.1.3.2.)*

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Accepted: August 20, 1980

Summary. The clinical application of enzyme immunoassay for the determination of the prostate-specific acid phosphatase is reported. 277 sera were investigated in the diagnosis as well as in tumour monitoring and a good correlation with the clinical stage was found. In prostatic carcinomas 5 of 13 with stage T1, 11 of 12 patients with stage T2, 16 of 16 patients with T3 and 19 of 19 patients with stage T4 disease had values above 1 ng/ml. In prostatic adenomas (n = 69) in prostatitis (n = 40) and in other carcinomas of the urogenital tract (n = 28), (renal carcinomas, carcinomas of the bladder and the penis) the values of the prostate-specific acid phosphatase measured by the enzyme immunoassay 131 of 137 were under 1 ng/ml. A comparison of random samples with the radioimmunoassay for this enzyme showed good correlation.

Key words: Prostatic cancer, Enzyme immunoassay, Prostate-specific acid phosphatase, Serological tumour monitoring.

INTRODUCTION

Since 1938, when Gutmann (7) reported increased values of acid phosphatase (E. C. 3.1.3.2.) in metastasizing prostatic carcinoma, this is the most frequently determined laboratory parameter to control the efficiency of therapy in prostatic cancer. Because of the different isoenzymes of acid phosphatase the determination of the total acid phosphatases was, however, non-specific. In 1953 Fishman and Lerner (4) succeeded in increasing the specifity by differentiating the tartrate-labile portion from the total acid phosphatase. However, only half the tartrate-inhibitable portion is derived from the isoenzyme of prostatic origin.

The difficulty in determining the prostate-specific acid phosphatase is that there is no specific substrate for the isoenzyme produced in the prostate and also the serum enzyme activity is stable only for a few hours.

The immunochemical methods by which the enzyme is determined with the aid of its structural characteristics are considered to be an alternative to the functional identification techniques. In 1975 Foti et al. (5) developed a radioimmunoassay; in 1979 Grenner (8) established an enzyme immunoassay. While the management of a radioimmunoassay requires expensive laboratory equipment, only a photometer is needed for the performance of an enzyme immunoassay.

Here we report the clinical findings of enzyme immunoassay for the prostate-specific acid phosphatase (Enzygnost® PSAP) in the diagnosis and follow-up of patients with prostatic carcinoma.

METHODS

The enzyme immunoassay for prostata-specific acid phosphatase is based on the sandwich test principle. The antigen of the acid phosphatase was isolated from prostatic secretion by conventional methods. The purified enzyme had a specific activity of 450 U/mg, when paranitrophenyl phosphate was used as a substrate at a temperature of 37°C. In absorption studies with other human tissues the specificity of the antigen and of the antibody was proved. The human serum used for the preparation of standards was previ-

*E. C. 3.1.3.2 = Enzyme Code according to the International Union of Biochemistry

0300-5623/81/0009/0021/$ 01.00

ously freed from acid phosphatase by means of immunoabsorption. For establishing the enzyme immunoassay synthetic tubes were coated with antiprostatic phosphatase. Peroxidase (E. C. 1.11.1 7.) served as the labelling enzyme (Grenner 1979 (8)).

For the test 0.1 ml of standard or test serum together with 0.1 ml of incubation buffer were incubated in the antibodycoated tubes for two hours at room temperature. After repeated elution the peroxidase-labelled antibodies were added. A renewed two-hour incubation was followed by elution of the free portion of the peroxidase-conjugated antibody. The addition of the substrates phenylendiamine and hydrogen peroxide took place for 30 min. The reaction was then interrupted by the addition of diluted sulphuric acid. The extension of the staining solution was determined at 492 nm. A typical reference curve is represented in Fig. 1.

With the aid of the assay, concentrations from 0.5 ng/ml upwards could be ascertained. To different sera defined amounts of prostatic phosphatase were added in the form of the pure antigen and the recovery amounted to 96% ± 6%. The coefficient of intraassay variability lay between 3 and 5%, the coefficient of interassay variability between 5 and 8%.

While the enzyme activity of the prostatic phosphatase in the serum at 25°C decreased after several hours and was no longer detectable after 2 to 3 days, a perceptible decrease of the immunological activity at 25°C occurred only after 5 to 8 days.

PATIENTS

Two hundred and seventy-seven serum samples from patients were investigated by the method described[1]. Sixty-nine patients had clinically benign hypertrophy and in 140 cases histological and clinical evidence of prostatic cancer was evaluated by means of standard physical findings, skeletal survey, bone scan and assay for serum prostatic acid phosphatase by the p-nitrophenyl phosphate method. In 60 of these patients the examinations were carried out in the course of diagnosis. Eighty patients with prostatic carcinoma were examined for tumour monitoring. In the patients in whom the inquiry was carried out for diagnostic reasons the values determined by the enzyme immunoassay were related to the tumour stage as far as prostatic carcinoma was concerned.

1 Blood was sampled from each patient before or 48 h after rectal examination, and stored at -20°C

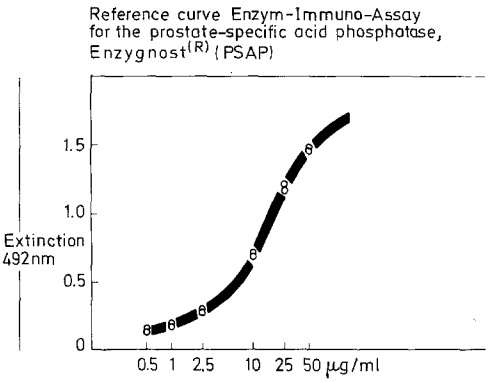

Fig. 1. Reference curve of the enzyme immunoassay for the prostate-spezific acid phosphatase, Enzygnost® PSAP. Each point represents the average of 20 independent determinations

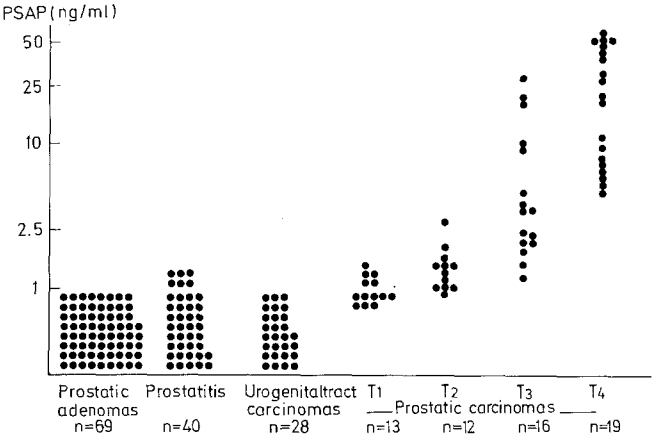

Fig. 2. Comparison between clinical staging of prostatic cancer and results of enzyme immunoassay for the prostate specific acid phosphatase, Enzygnost® PSAP

RESULTS

Figure 2 shows that in 69 patients with benign prostatic adenoma the values of the prostate-specific acid phosphatase measured by the enzyme immunoassay were all under 1 ng/ml. The same was true for 34/40 patients with prostatitis and for 28 patients with other carcinomas of the urogenital tract. In 9 of the 60 patients with prostatic carcinoma in which this investigation was carried out for diagnostic reasons the enzyme immunoassay values were under 1 ng/ml. When 1 ng/ml was taken as the upper standard, 5 of 13 patients with stage T1, 11 of 12 patients with stage T2, 16 of 16 patients with stage T3 and 19 of 19 patients with stage T4 prostatic carcinoma had increased prostatic acid phosphatase values.

As far as the question of follow-up of prostatic carcinoma is concerned, prostatic acid phosphatase concentrations were determined by en-

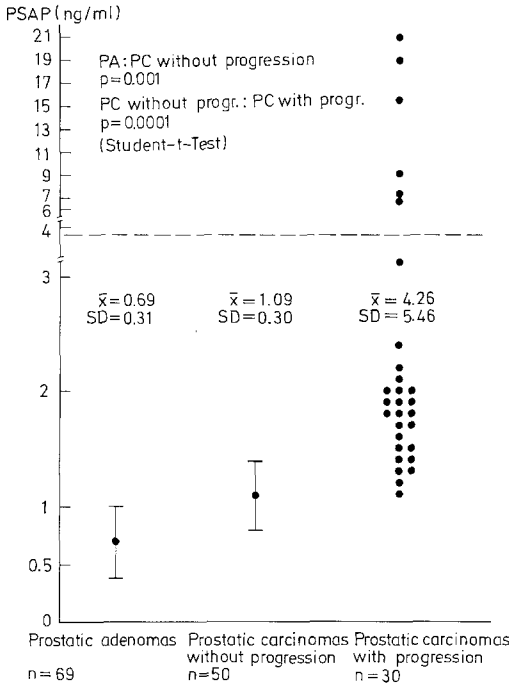

Fig. 3. Alterations in the level of the prostate-specific acid phosphatase and clinical response to therapy in patient with prostatic cancer

zyme immunoassay and compared in 50 prostatic carcinoma patients without progression and in 30 prostatic carcinoma patients with progression (Fig. 3).

As a result a significant discrimination of prostatic carcinoma without progression compared to prostatic carcinomas with progression was found. The progression and the activity of the disease were evaluated with the aid of skeletal scintigraphy and cytosmear after fine needle biopsy of the prostate, respectively.

In 3 patients who had undergone total prostatectomy the prostate-specific acid phosphatase was determined by means of the enzyme immunoassay; the values were always below the detectable limit of this method.

DISCUSSION

The level of serum prostatic acid phosphatase is an important clinical test for cancer of the prostate. Until recently, enzymatic assays have been used exclusively for this diagnostic test. However increased temperature inactivates the enzyme at the pH of normal serum; therefore, results obtained by these methods might not reflect the true activity of this enzyme in serum if scrupulous precautions have not been followed in preparing the serum. Such considerations, as well as questions of substrate specifity, have led to an immunological quantitation of prostatic acid phosphatase. The immunological reactivity of prostatic acid phosphatase is apparently more consistent than enzymatic activity. Therefore, serum samples do not require special treatment and handling.

Normal serum contains several acid phosphatases of different origin. Some of these acid phosphatases are organospecific, some cytospecific and possibly some even specific for subcellular organelles. The serum acid phosphatases can be derived from erythrocytes, leucocytes, thrombocytes or from cells of the reticulo-endothelial system, from osteoblasts or just from the prostatic cells. Consequently, a specific assay that detects enzymes of prostatic origin is required. When Shulman et al. (1964) (10) and Albin et al. (1970) (1) described the possibility of the immunological differentiaion of the prostate-specific acid phosphatase, the door was opened to the so-called methods of immunochemical identification.

The clinical value of the radioimmunoassay has been discussed since 1975 (5, 11, 3). This study was designed to investigate the value of enzyme immunoassay. The shorter incubation time and the less expensive equipment required for this method are important factors in favour of immunochemical determination of the prostate-specific acid phosphatase. The technical laboratory performance comparison with radioimmunoassay and the first clinical results described in this paper confirm the value of this method (2).

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