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Determination of serum prostate specific antigen in patients with tumours of prostate and tumour cell lines

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Abstract

Tumour markers are biochemical indicators of malignant tumour proliferation. They serve not only for diagnostics of malignant tumour diseases, but also for monitoring of effect of therapy and recurrence of disease. Serum prostate specific antigen (PSA) belongs to group of the best tumor markers, what are available to medicine at the present. In our study the Immune Enzymatic Automated Analyzer AIA 600 II was employed. Calibration curves for PSA and fPSA demonstrates very good linearity by values of R^2 = 0.999 in both cases. We assumed to determine levels of PSA in real samples of patients and compare to cell lines. In comparism with non-tumour cell lines we observed the enormous growth of PSA 129-times and fPSA 170-times by RVL-22 and negligible growth for PC-3 cell lines about 2 times. Correlation of obtained data (method PSA2 and method PA) was very close with R^2 =0.990.

Keywords: PSA, fPSA, oncological markers, prostate carcinoma.

Introduction

Serum prostate specific antigen (PSA) has been first time used as a screening test for prostate cancer in 1991 by Catalona et al. PSA is glycoprotein composed of 237 amino-acids, which originates in prostate gland and is necessary to normal physiological function of sperm. At the physiological state, most of PSA is secernated into sperm, because it causes sperm fluidification. Only small amount of PSA is transported into blood, due to this fact, PSA is detectable in blood serum. In the case of disorganization of inner architecture of prostate gland, majority of PSA is transported into blood, which results in increased PSA level in blood serum. It was determined that increased PSA level is associated with tumour diseases of prostate gland. Determination of PSA level is at the present the best prostate tumour marker, which is presently known and used.

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Materials and methods

Samples were analyzed by the use of apparatus Immune Enzymatic Automated Analyzer AIA 600 II, which serves for measurement of immunochemical parameters in biological liquids; it uses set of reagents AIA–PACK PSA and fPSA. Blood serums of patients suffering from prostate adenocarcinoma were obtained from St. Anne´s University Hospital Brno, all with permission of ethic commission. Cell lines were derived from normal prostate tissue (PNT1A) and tumour tissue - tumour cell lines PC-3 and RV-1. Cells were maintained at 37°C in a humidified incubator with 5% CO_2. The passages of all cell lines were within the range 10-35. Once the cells grew up to 50-60% confluence of the culture and grow medium was replaced by fresh medium for 24 h to synchronize cell growth. In our experiment, we used fully automated immunochemical detection of serum prostate specific antigen. Reaction itself is initiated by pipetting of sample (10 µl) consisting of blood serum or cell lysate with diluents in testing pot AIA-PACK. Samples were incubated at 37°C, antibodies were bonded on surface of paramagnetic particles. Separation of bonded and unbounded antibodies is attained by washing by rinsing solution - unbounded antibodies were washed out. After washing step, substrate - 4-methylumbelliferyl phosphate was added into testing pot and enzymatic activity on paramagnetic particles was fluorescence measured.

Results and discussion

In presented study we compared two methods for determination of total PSA and free fPSA. Calibration curve for PSA was designed

(R^2 = 0.9993, determination error 0.2 %) and fPSA (R^2 = 0.9990, determination error 0.5 %). Firstly we analyzed 26 samples of patients suffering from prostate adenocarcinoma and 3 controls (healthy young men). Levels of total PSA were determined using two procedures PSA2 and PA. Correlation of obtained data (method PSA2 and method PA) was very close with r =0.990. Newly introduced method PSA2 decreased value of determined PSA (method PA) for 1.5 ng/ml at average. In addition, in higher PSA values determined by PA method, PSA level was for about 25-30 % lower in comparison with PSA2 method. At low PSA levels, higher sensitivity of PSA determined by PSA2 method was observed (enhancement of PSA for about 5-7 % in comparison with PA method).

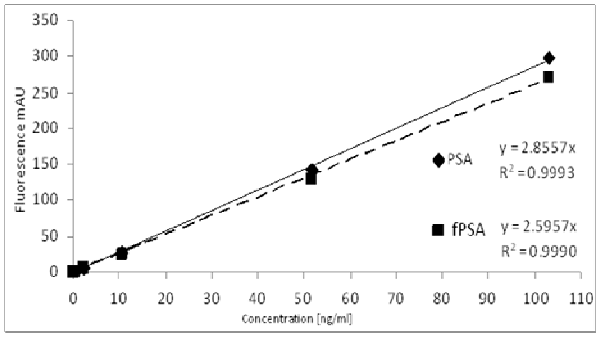

Figure 1: Calibration curve for determination of serum prostate specific antigen of total (PSA) and free (fPSA) PSA.

Proposed methodology for determination of PSA and fPSA was subsequently tested on cell lysates. We determined that proposed methodological procedure is applicable for determination of PSA and fPSA to one million of cells. In non-tumour cell lines, PSA levels were about 0.02 ng/ml and for fPSA 0.02 ng/ml. In the case of tumour cell lines - for tumour cell line PC-3, PSA level was 0.05 ng/ml and level of fPSA was 0.02 ng/ml; tumour cell line RV-1 22 demonstrated significant enhancement of PSA levels to 2.58 ng/ml and fPSA to 3.40 ng/ml.

Conclusion

Determination of serum prostate specific antigen in blood serum of patients is common diagnostic method. We demonstrated in our work that fully automated technique of PSA immunodetection is possible also in cell lysates.

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References

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