Reduced Serum Selenoprotein P Concentrations in German Prostate Cancer Patients

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Abstract

Selenium (Se) is essentially needed for the biosynthesis of selenoproteins. Low Se intake causes reduced selenoprotein biosynthesis and constitutes a risk factor for tumorigenesis. Accordingly, some Se supplementation trials have proven effective to reduce prostate cancer risk, especially in poorly supplied individuals. Because Se metabolism is controlled by selenoprotein P (SEPP), we have tested whether circulating SEPP concentrations correlate to prostate cancer stage and grade. A total of 190 men with prostate cancer (n = 90) and “no evidence of malignancy” (NEM; n = 100) histologically confirmed by prostate biopsy were retrospectively analyzed for established tumor markers and for their Se and SEPP status. Prostate specific antigen (PSA), free PSA, total Se, and SEPP concentrations were determined from serum samples and compared with clinicopathologic parameters. The diagnostic performance was analyzed with receiver operating characteristic curves. Median Se and SEPP concentrations differed significantly (P < 0.001) between the groups. Median serum Se concentrations in the 25th to 75th percentile were 95.9 μg/L (82-117.9) in NEM patients and 81.4 μg/L (67.9-98.4) in prostate cancer patients. Corresponding serum SEPP concentrations were 3.4 mg/L (1.9-5.6) in NEM and 2.9 mg/L (1.1-5.5) in prostate cancer patients. The area under the curve (AUC) of a marker combination with PSA, total PSA, and percent free PSA (%fPSA) (6, 7) in combination with the SEPP concentration, yielded the highest diagnostic value (AUC 0.80) compared with the marker combination without SEPP (AUC 0.77) or %fPSA (AUC 0.76). We conclude that decreased SEPP concentration in serum might represent an additional valuable marker for prostate cancer diagnostics. (Cancer Epidemiol Biomarkers Prev 2009;18(9):2386–90)

Introduction

Prostate cancer represents one of the leading causes of cancer mortality in men. Only few biomarkers are available to detect prostate cancer or monitor disease progression. The circulating level of prostate specific antigen (PSA) is frequently used for early prostate cancer detection. However, PSA screening often detects small tumors that are clinically insignificant and not likely to become life-threatening (1). Clearly, more specific biomarkers or meaningful combinations of several informative readouts are needed. Alongside the efforts that aim to rectify detection and prognosis, there are also strong research activities in the field of prevention and treatment of prostate cancer by dietary compounds and pharmacologic means, both in basic and applied sciences. In this respect, the micronutrient selenium (Se) represents a promising candidate. Accordingly, a very large prostate cancer prevention trial (SELECT) was launched in 2001 in order to assess the chemopreventive potency of daily Se and vitamin E supplementation (2). Unfortunately, the trial has recently been brought to a premature end due to a lack of the expected strong chemopreventive effects of the Se supplement in the Se arm and arising concerns about an increased cancer risk by the high vitamin E dosage in the vitamin E arms (3).

Earlier, the promising potential of Se became evident in the Nutritional Prevention of Cancer trial first published in 1996, in which the participants received 200 μg Se-enriched yeast or placebo daily. Prostate cancer risk was reduced by an impressive 65% in the Se arm after an average supplementation time of 4.5 years (4). Follow-up studies indicated that the protective effects were mainly elicited in those participants who entered into the study with relatively low baseline Se levels (5). This finding is in line with most case-control and cohort studies, which indicate a protective association of circulating Se concentrations and prostate cancer risk as well as advanced prostate cancer. The underlying mechanism of Se action during tumor prevention is largely unknown, but both the evident success in supplemented participants with a low Se baseline and the analyses of the importance of functional single nucleotide polymorphisms in selenoprotein genes argue for a critical role of selenoproteins in cancer prevention (6, 7).

Selenoproteins are characterized by the 21st proteinogenic amino acid selenocysteine, which becomes incorporated at a specific location during translation. Among the 25 human selenoprotein genes are the families of glutathione peroxidases (GPx) and thioredoxin reductases, which are implicated in the antioxidative defense. Additionally, a number of unique selenoproteins have potential for tumor-relevant functions, as they participate in...
protein folding and quality control in the endoplasmic reticulum or catalyze the reversible reduction of oxidized proteins (8).

The expression of selenoproteins vitally depends on the nutritional supply that controls the Se status of the individual. The concentration of the ubiquitously expressed GPx1 can be measured in circulating blood cells and is one potential biomarker of Se status. Alternatively, the actively secreted GPx3 or selenoprotein P (SEPP) is measured in serum or plasma (9). SEPP seems to represent the better biomarker because its circulating amounts correlate to Se intake over a wider range than does GPx1 or GPx3 (10). Moreover, it carries up to 10 selenocysteine residues per protein, thereby accounting for the majority of circulating Se in humans or rodents (11). Animal models indicate that SEPP not only passively reflects the Se status but also actively determines the Se concentration in SEPP-dependent tissues (12, 13). Thus, SEPP can qualify as a functional biomarker that indicates Se status and actively mediates Se transport and storage.

These functions seem to have a critical impact on tumor growth because single nucleotide polymorphisms in human SEPP1 correlate to advanced rectal adenoma in the colon (14). Moreover, SEPP expression has been shown to be strongly dysregulated in gastrointestinal tumors (15) and human or rodent prostate cancer (16). The biological importance of selenoproteins for prostate tumorigenesis was recently clearly shown in a double transgenic mouse model (17). An experimental analysis on the potential interrelation between SEPP expression and prostate tumors in humans is missing. For these reasons, we examined whether the concentration of circulating SEPP correlates with prostate cancer diagnosis, stage, and grade by using a newly developed assay (18).

Materials and Methods

Patients. A total of 190 patients from the Department of Urology, University Hospital Charité, were included in this study, which had been approved by the local ethics committee. The selection criterion for the inclusion of patients into our retrospective analysis was the availability of suitable sample material, i.e., three unthawed vials of at least 0.5 mL serum each per patient. These sera had been collected between 2001 and 2004. The patients were classified into two groups: 90 men who had a histologically confirmed diagnosis of prostate cancer (median age, 63.5 y; range, 43-77 y) and 100 men who showed “no evidence of malignancy” (NEM group; median age, 65 y; range, 43-81 y) according to a prostate biopsy (8-12 cores). Of all prostate cancer patients, 54 had undergone a radical prostatectomy, whereas the other 36 patients used alternative therapies. The pathologic stages of the prostatectomy specimens were: pT2, n = 39; pT3, n = 15. The pathologic Gleason sum (GS) was GS <7, n = 25; GS ≥7, n = 17, and two cases of missing data. Tumor stages were determined according to the Union Internationale Contre le Cancer (19) and tumor grading according to Gleason (20).

Blood Sample Analyses. Blood samples were taken before any diagnostic or therapeutic procedure involving the prostate, and at least 4 wk after an earlier prostate manipulation. No patient received antiandrogen treatment prior to blood sampling. After sample collection, the sera were stored in aliquots at −80°C and analyzed retrospec-


tively. The measurements of total PSA (tPSA) and free PSA (fPSA) were made as described earlier (21) according to the manufacturer’s instructions on the Elecsys 2010 analyzer (Roche). The percent free PSA (%fPSA) value was calculated as the percentage ratio of fPSA over tPSA. SEPP concentrations were determined from serum samples by the immunoluminometric sandwich assay as described recently (18). Assay characteristics, including detection limit, coefficient of variation, interassay and intra-assay variations, and standardization by a serum pool of healthy donors, are provided elsewhere (18). SE measurements were done by a fluorimetric assay involving piazselenol formation and quantification as described before (13, 22). Two independent reference materials, i.e., a commercially available pooled human serum standard (Serostandard AS) and a Se atomic absorption standard (1,000 mg/mL; Sigma), were used to validate the method. SEPP and Se analyses were conducted in an independent lab from the PSA analyses and in a blinded fashion with respect to patient characteristics.

Statistical Analysis. Statistical calculations were done with SPSS, version 17.0 (SPSS Software). Medians and ranges were calculated for all markers. Mann-Whitney U test for unpaired samples was used to evaluate the differences between groups. Spearman correlation coefficient was used to assess the statistical significance of the correlation between different serum parameters. The diagnostic accuracy of each marker was evaluated using receiver operating characteristic (ROC) curve analysis with calculations of the area under receiver operating characteristic curve (AUC) using MedCalc 9.01.0 (MedCalc Software, Mariakerke) and GraphROC 2.1 for Windows (23), which had the ability to compare curves at a certain sensitivity or specificity cutoff. The ROC program version 1 was used to calculate the best marker combinations with inclusion of tPSA, %fPSA, age, total Se, or SEPP. That program implements an approach of combining the ROC curves of several tumor markers or test values by the best linear combination, which maximizes the AUC based on the hypothesis of a multivariate Gaussian distribution (24). The obtained diagnostic values (AUC and diagnostic accuracy at 90% sensitivity or specificity, respectively) were compared. P < 0.05 was considered to indicate statistical significance.

Results

Baseline Characteristics of tPSA, %fPSA, Se, and SEPP. Table 1 gives the median values from prostate cancer as well as NEM patients for tPSA, %fPSA, Se, and SEPP, and significance levels (data for age with P = 0.08 and tPSA with P = 0.01 are not shown). No significant differences between the two groups were observed for tPSA. In contrast, %fPSA, total Se, and SEPP concentrations were significantly (P < 0.001) lower in prostate cancer patients. The total serum Se and circulating SEPP concentrations were found to correlate significantly (r = 0.52; P < 0.0001). No such strong correlations were detected between SEPP concentrations and age (r = 0.15; P = 0.045), tPSA (r = 0.05; P = 0.49), or fPSA (r = 0.18; P = 0.015) levels in the combined group of all participants or in the prostate cancer patients or NEM group separately.

SEPP Concentrations in Relation to Prostate Cancer Stage and Grading. The subgroup of prostate cancer
patients undergoing radical prostatectomy (n = 54) were analyzed in more detail due to the availability of their confirmed pathologic tumor characteristics. No significant difference in SEPP concentrations were found between pT2 (mean SEPP, 2.94 ± 0.72 mg/L) and pT3 (mean SEPP, 2.86 ± 0.90 mg/L) tumors. However, the cases with aggressive prostate cancer (Gleason sum ≥7; mean SEPP, 2.67 ± 0.75 mg/L) showed distinct, but not significantly (P = 0.134) lower SEPP serum concentrations compared with those prostate cancer patients with less aggressive tumors (Gleason sum <7; mean SEPP, 3.12 ± 0.71 mg/L; Fig. 1).

Diagnostic Validity of the Single Markers and Marker Combinations. To analyze the diagnostic performances of the established parameters (age, tPSA, and %fPSA), as well as the two Se markers (total Se and SEPP), ROC curves were generated and the AUC calculated (Table 2). From all available single markers, %fPSA had the highest AUC (0.76); SEPP and total Se reached an AUC of 0.68 each. In addition, multivariate analyses were done using the program mROC to test the diagnostic performance of different marker combinations (age, tPSA, %fPSA with and without SEPP). The resulting ROC curve characteristics are summarized in Table 2. In comparison with the best single marker %fPSA, the combination of %fPSA with age and tPSA without SEPP (AUC = 0.77) showed no further improvement of diagnostic performance (P = 0.69). Inclusion of SEPP to the established common markers yielded an improvement of the AUC from 0.77 to 0.80 (P = 0.06). Notably, the model with inclusion of SEPP reached a significantly higher AUC than %fPSA alone (P = 0.02). Sensitivities and specificities at given cutoffs of 90% sensitivity and specificity were also significantly (P < 0.05) increased by adding SEPP into the calculation (Table 2).

Discussion

The interrelation of Se status, cancer risk, and tumor growth is a complex and controversial issue. The vast majority of experimental animal studies and human analyses have indicated that a high Se status provides some protection against mutagenic noxae and reduces the relative cancer risk of different tissues (6, 9, 25). Yet, a recent nested case-control study among men in the European Prospective Investigation into Cancer and Nutrition trial detected no association of plasma Se concentrations with prostate cancer risk (26). In this study, total plasma Se was analyzed and pathologic scoring was done in different clinics by different pathologists. Nevertheless, this study analyzed a large number of cases and controls recruited from the same geographical area, as we have done, and did not find a respective correlation. Currently, we are unable to explain the inconsistent findings, but the choice of serum versus plasma, the number of pathologists involved, and the selenoprotein nature of SEPP as a functional readout might have made a difference.

In the aforementioned Nutritional Prevention of Cancer trial (4), Se proved chemopreventive even in a prospective trial setting, especially as the poorly supplied individuals who were still better supplied than our probands profited most from the nutritional supplement (5). These results led to the perception that taking Se-containing supplements and increasing one's own Se status is in general a safe strategy to reduce personal prostate cancer risk. A recent cross-sectional analysis has indicated that there might be an upper limit (27). In principle, American men need no Se supplementation due to the self-dependent supplementation efforts and the increasing use of Se as an antioxidant supplement in a variety of US foodstuffs. The situation in Europe, however, is fundamentally different. Mean serum or plasma Se concentrations of most Europeans are relatively low (28).

In our cohort of German men we found a strong correlation of total Se and SEPP concentrations in serum samples. Such interrelation is restricted to Se-deplete subjects (10) and is not observed in Se-replete probands whose selenoproteins are maximally expressed. Moreover, it might explain why the Se status correlates to the pathology in
our probands, and why we observed a significant lower serum concentration of total Se and SEPP in tumor patients compared with men with NEM. The average Se status in our study was below the saturation level at which surplus Se is no longer used for selenoprotein biosynthesis (29). This situation is fundamentally different from the participants in the SELECT trial whose serum Se concentrations averaged at 136 μg/L (3) and who therefore seemed unlikely to respond favorably to an increasing Se supply. Therefore, we assume that in contrast to the men in the SELECT trial, both the NEM and the prostate cancer men included in our study were responsive to alterations of their nutritional Se intake or to disease-dependent pathways that impair regular selenoprotein biosynthesis.

Our finding of decreased Se and SEPP status with higher tumor grade, i.e., more aggressive prostate cancer, raises the principal question of whether this effect is a consequence of the disease or rather represents a predisposition. Both notions seem plausible and do not exclude each other. The published SNP correlation of SEPP1 genotype and increased advanced colon adenomas (14) argues for a predisposition, whereas the known decline of serum Se and serum SEPP in inflammatory conditions argues for a consequence (18, 30, 31). Yet, the mechanisms of action of Se in cancer prevention are far from clear. Several meaningful theories have been put forward and are currently being discussed and experimentally tested (9, 25, 32). Independent of the underlying mechanism, Se supplementation efforts clearly need to be considered in males who display a relatively low Se concentration, at which the selenoproteins are not expressed to their full extent. These men might profit from increased Se availability, thus reducing their cancer risk and tumor growth progression.

Furthermore, SEPP concentrations might represent an additional valuable marker for prostate cancer diagnostic in German men. We could show in multivariate analyses using mROC, a computer program that implements an approach of combining the ROC curves of several tumor markers (24), that SEPP had a diagnostic impact. In combination with the established parameters of age, tPSA, and %fPSA, we reached a significantly higher AUC compared with tPSA only or %fPSA alone, and we yielded a significantly higher sensitivity at a given specificity of 90% compared with the combination without SEPP. No significant correlations between SEPP and tPSA or %fPSA were found in our population, indicating that SEPP is an independent serum marker for prostate cancer. Therefore, we suggest measuring of SEPP concentrations in order to improve the possible diagnosis of prostate cancer risk, and at the same time providing a more direct rationale to consider an individually tailored adjuvant Se supplementation effort.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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