Trace Element Contents in Adenocarcinoma of Human Prostate Investigated by Energy Dispersive X-Ray Fluorescent Analysis

Abstract

Background: Adenocarcinoma of prostate is an internationally important health problem of the man, particularly in developed countries. The aim of this exploratory study was to evaluate whether significant changes in the prostatic tissue levels of Zn, as an androgen dependent trace element and Br, Fe, Rb, and Sr, as androgen independent trace elements, exist in the malignantly transformed prostate.

Methods and findings: Prostatic tissue levels of Br, Fe, Rb, Sr, and Zn as well as ratio of Zn content to other trace element contents were prospectively evaluated in 35 patients with adenocarcinoma and 37 healthy male inhabitants. Measurements were performed using 109Cd radionuclide-induced energy dispersive X-ray fluorescent analysis. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. Mean values ± standard error of means (M ± SEM) for mass fraction (mg/kg on dry mass basis) of trace element in the normal tissue was: Br 38.8 ± 5.6, Fe 118 ± 8, Rb 16.3 ± 1.1, Sr 2.5 ± 0.4, and Zn 1154 ± 119, respectively. M ± SEM for ratio of mass fractions were: Zn/Br 38.7 ± 6.4, Zn/Fe 11.2 ± 1.3, Zn/Rb 71.7 ± 9.0 and Zn/Sr 534 ± 83, respectively. The contents of Rb and Zn were significantly lower (approximately 2 and 8 times, respectively) and those of Br and Sr was significantly higher (2.7 and 2.2 times, respectively) in cancerous tissues than in normal tissues.

Conclusions: There are great changes in trace element contents and their relationships in the malignantly transformed tissue of prostate.

Keywords: Prostatic adenocarcinoma; Intact prostate tissue; Trace elements; Energy-dispersive X-ray fluorescent analysis

Introduction

Globally, prostate cancer (PCa) is the sixth most common cancer, and the first most common cancer in males in many industrialized countries of Europe [1-5]. In North America, it is also the most common cancer in males and, except for lung cancer, is the leading cause of death from cancer [6]. Moreover, PCa is the leading cancer in terms of incidence and mortality in men from Africa and the Caribbean [7]. PCa in China has also become a major public health concern [8]. Although the etiology of PCa is unknown, several risk factors including such micronutrients as Ca and Zn have been well identified [9-11]. It is also reported that the risk of having PCa drastically increase with age, being three orders of magnitude higher for the age group 40-79 years than for those younger than 39 years [12].

Trace elements have essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death or malignant transformation [13-15].

In our previous study a significant positive correlation between
age and some trace element mass fractions in the prostate was observed [16-26]. For example, a strongly pronounced tendency of age-related exponential increase in Zn mass fraction as well an increase in Zn/Fe, Zn/Rb, and Zn/Sr ratios in prostate was demonstrated by us using $^{109}$Cd radionuclide-induced energy dispersive X-ray fluorescent (EDXRF) analysis [27]. In addition, a significant positive correlation was seen between the prostatic zinc and other trace element contents [27]. It was concluded that high intraprostatic zinc concentrations are probably one of the main factors acting in both initiation and promotion stages of prostate carcinogenesis [28,29]. Moreover, it seems fair to suppose that besides Zn, such trace elements as Br, Fe, Rb, and Sr also play a role in the pathophysiology of the prostate.

This work had two aims. The first was to assess the Br, Fe, and Rb, Sr, and Zn contents in prostatic adenocarcinoma tissue using EDXRF analysis. The second aim was to compare the levels of trace elements in the malignant prostate with those in intact gland of healthy men aged over 40 years.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Material and Methods

All patients suffering from adenocarcinoma of prostate (n=35, mean age M ± SD was 64 ± 11 years, range 40-79) were hospitalized in the Urological Department of the Medical Radiological Research Centre. Transrectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Normal prostates for the control group samples were removed at necropsy from 37 men (mean age 55 ± 11 years, range 41-87), who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer. All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The pounded sample weighing about 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing.

To determine the contents of the trace elements by comparison with known data for standard, aliquots of commercial, chemically pure compounds and synthetic reference materials were used [30]. The microliter standards were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) weighing about 8 mg were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized prostate tissue.

Details of the relevant facility for EDXRF, source with $^{109}$Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF analysis of human prostate tissue [27,31].

All prostate samples for EDXRF were prepared in duplicate and mean values of trace element contents were used in final calculation. Using the Microsoft Office Excel programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for trace element contents in normal and cancerous prostate tissue. The reliability of difference in the results between the two groups of prostate tissue samples was evaluated by Student’s t-test. For the estimation of the Pearson correlation coefficient between different pairs of the trace element mass fractions in the normal and cancerous prostate tissue the Microsoft Office Excel program was also used.

Results

Table 1 depicts our data for 5 trace elements in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this material.

Table 2 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Br, Fe, Rb, Sr, Zn mass fraction and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in normal and cancerous prostate tissue.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn mass fraction in normal and cancerous prostate tissue [32-44] is shown in Table 3.

The ratios of means and the reliability of difference between mean values of Br, Fe, Rb, Sr, Zn mass fractions and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in normal and cancerous prostate tissue are presented in Table 4.

| Table 1 | EDXRF data | Br, Fe, Rb, Sr, and Zn mass fractions in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis). |
|---------|------------|---------------------------------------------------------------------------------------------------------------------------------|
| Element | Certified values | This work results |
| Mean    | 95% confidence interval | Type | Mean ± SD |
| Br      | 4.1 | 3.5-4.7 | C | 5.0 ± 1.2 |
| Fe      | 49 | 47-51 | C | 48 ± 9 |
| Rb      | 18 | 17-20 | C | 22 ± 4 |
| Sr      | 0.1 | - | N | <1 |
| Zn      | 86 | 83-90 | C | 90 ± 5 |

Mean: Arithmetical mean; SD: Standard deviation; C: Certified values; N: Non-certified values.
Table 2 Some statistical parameters of Br, Fe, Rb, Sr, and Zn mass fractions (mg/kg, dry mass basis) and Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratios in intact and cancerous prostate.

| Tissue         | Parameter | Mean | SD  | SEM | Min | Max | Med | Per.0.025 | Per.0.975 |
|----------------|-----------|------|-----|-----|-----|-----|-----|-----------|-----------|
| Normal, n=37   | Br        | 38.8 | 29.1| 5.4 | 5.0 | 143 | 31.5| 5.63      | 109       |
|                | Fe        | 118  | 41.3| 7.5 | 44  | 244 | 112 | 57.1      | 203       |
|                | Rb        | 16.3 | 6.51| 1.1 | 6.3 | 31.0| 15.6| 7.46      | 31.0      |
|                | Sr        | 2.5  | 2.1 | 0.4 | 0.95| 9.7 | 1.5 | 0.98      | 7.3       |
|                | Zn        | 1154 | 723 | 119 | 229 | 3513| 961 | 233       | 2637      |
| Normal, n=12   | Zn/Br     | 38.7 | 33.2| 6.4 | 2.44| 133 | 27.9| 5.59      | 122       |
|                | Zn/Fe     | 11.2 | 7.4 | 1.3 | 1.70| 28.3| 9.58| 1.95      | 26.0      |
|                | Zn/Rb     | 71.7 | 49.8| 9.0 | 14.3| 196 | 62.9| 16.6      | 188       |
|                | Zn/Sr     | 534  | 382 | 83  | 23.6| 1463| 509 | 48.2      | 1326      |
| Adeno-         | Br        | 105  | 55  | 14  | 11.3| 211 | 115 | 12.9      | 196       |
| carcinomane    | Fe        | 150  | 96  | 17  | 6.90| 389 | 140 | 13.0      | 376       |
| n=35           | Rb        | 8.7  | 6.2 | 1.1 | 1.70| 33.0| 7.80| 2.08      | 20.0      |
|                | Sr        | 5.47 | 2.40| 0.91| 1.50| 9.20| 5.50| 1.91      | 8.90      |
|                | Zn        | 144  | 79  | 13  | 20.0| 328 | 134 | 20.0      | 313       |
| Normal         | Zn/Br     | 1.41 | 0.93| 0.26| 0.095|3.51| 1.04| 0.279     | 3.35      |
| Normal         | Zn/Fe     | 1.39 | 1.28| 0.23| 0.054|5.60| 0.894| 0.163     | 4.34      |
| Normal         | Zn/Rb     | 21.7 | 16.5| 3.0 | 1.30| 65.9| 20.5| 1.36      | 60.4      |
| Normal         | Zn/Sr     | 47   | 51  | 19  | 23.5| 162 | 26.2| 23.5      | 144       |

M: Arithmetic mean; SD: Standard deviation; SEM: Standard error of mean; Min: Minimum value; Max: Maximum value; Med: Median; Per.0.025: Percentile with 0.025 level; Per.0.975: Percentile with 0.975 level.

Table 3 Median, minimum and maximum value of means of Br, Fe, Rb, Sr, and Zn contents in intact and cancerous prostate according to data from the literature in comparison with our results (mg/kg, dry mass basis).

| Prostate tissue | Element | Median of means (n)* | Minimum of means M or M ± SD, (n)* | Maximum of means M or M ± SD, (n)* | M ± SD |
|-----------------|---------|----------------------|------------------------------------|-------------------------------------|--------|
| Normal          | Br      | 14.5 (2)             | 12 ± 8 (4) [32]                    | 17 (12) [33]                       | 39 ± 29|
|                 | Fe      | 150 (14)             | 5.7 ± 0.1 (5) [34]                 | 1040 ± 65 (10) [35]               | 118 ± 41|
|                 | Rb      | 34.5 (3)             | 4.7 (9) [36]                       | 58 ± 33 (4) [37]                  | 16 ± 7 |
|                 | Sr      | 0.94 (4)             | 0.75 ± 0.75 (48) [38]              | 1.4 (12) [33]                     | 2.5 ± 2.1|
|                 | Zn      | 1058 (5)             | 160 ± 20 (11) [39]                 | 3218 ± 41 (10) [35]              | 1154 ± 723|
| Adeno-          | Br      | 1.5 (1)              | 1.5 ± 0.0 (7) [40]                 | 1.5 ± 0.6 (27) [40]              | 105 ± 55|
| carcinomane     | Fe      | 195 (14)             | 12.5 ± 5.0 (23) [41]               | 3530 ± 45 (27) [40]              | 150 ± 96|
|                 | Rb      | 8 (1)                | 8 ± 1 (12) [42]                    | 8 ± 1 (12) [42]                   | 8.7 ± 6.2|
|                 | Sr      | -                    | -                                  | -                                  | 5.5 ± 2.4|
|                 | Zn      | 200 (44)             | 16.7 ± 3.5 (3) [43]                | 840 ± 85 (13) [44]               | 144 ± 79|

M: Arithmetic mean; SD: Standard deviation; (n)*: Number of all references; (n): Number of samples

Data comparison for mean values of Br, Fe, Rb, Sr, Zn mass fractions and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in cancerous prostate tissue of patients of different age and cancer stage are shown in Tables 5 and 6 respectively.

Table 7 contains results of inter-element correlation calculations (values of $r$ - coefficient of correlation) including all trace elements identified in this work.

Discussion

As was shown by us [27] the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the Br, Fe, Rb, Sr, and Zn contents analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of trace elements of the prostate presented in Tables 2-7.

The mean values and all selected statistical parameters were calculated for 5 trace elements (Br, Fe, Rb, Sr, and Zn) and for 4 ratios (Zn/Br, Zn/Fe, Zn/ Rb, and Zn/Sr) of trace element mass fractions (Table 2). The mass fraction of Br, Fe, Rb, Sr, and Zn were measured in all, or a major portion of normal and cancerous tissue samples.

The results for all trace element contents in the prostates of the control group (mean age 55 ± 11 years, range 41-87) are in accordance with our earlier findings in prostates of apparently healthy men aged 41-60 [16-18,23-27]. Values obtained for Br, Fe, Rb, Sr, and Zn contents (Table 3) agree well with median of mean values reported by other researches for the human prostate [32-39]. Data cited in Table 3 also includes samples obtained from patients who died from different non-urolological diseases. A number of values for trace element mass fractions were not expressed on a dry weight basis in the cited literature. Therefore,
Table 4 Comparison of mean values (M ± SEM) of Br, Fe, Rb, Sr, and Zn mass fractions (mg/kg, dry mass basis) in intact and cancerous prostate.

| Element | Prostatic tissue | Adenocarcinoma | Ratio | Student’s /t-test | P ≤ |
|---------|-----------------|----------------|-------|------------------|-----|
|         | Normal 41-87 year | Adenocarcinoma 40-79 year | Adenocarcinoma to Normal |                  |     |
| Br      | 38.8 ± 5.4 105 ± 14 | 2.71           | 0.00033 |
| Fe      | 118 ± 8 150 ± 17 | 1.27           | 0.106 (NS) |
| Rb      | 16.3 ± 1.1 8.7 ± 1.1 | 0.53           | 0.000013 |
| Sr      | 2.5 ± 0.4 5.5 ± 0.9 | 2.2           | 0.015 |
| Zn      | 1154 ± 119 144 ± 13 | 0.12           | 0.000000039 |
| Zn/Br   | 38.7 ± 6.4 1.41 ± 0.26 | 0.036          | 0.0000037 |
| Zn/Fe   | 11.2 ± 1.3 1.39 ± 0.23 | 0.12           | 0.000000048 |
| Zn/Rb   | 71.7 ± 9.0 21.7 ± 3.0 | 0.30           | 0.0000058 |
| Zn/Sr   | 534 ± 83 47 ± 19 | 0.088          | 0.00001 |

M: Arithmetic mean; SEM: Standard error of mean; NS: Not significant difference.

Table 5 Differences between mean values (M ± SEM) of Br, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) and between mean values of Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratio in cancerous prostate glands of patients of two age groups.

| Parameter | Adenocarcinoma Age group 1 40-65 year n=15 | Adenocarcinoma Age group 2 66-79 year n=20 | Ratio /Group 2 / Group 1 | Student’s t-test |
|-----------|------------------------------------------|------------------------------------------|-------------------------|-----------------|
| Br        | 127 ± 27 91 ± 14 | 0.72 | 0.278 (NS) |
| Fe        | 165 ± 10 140 ± 27 | 0.85 | 0.409 (NS) |
| Rb        | 10.3 ± 2.1 8.9 ± 1.7 | 0.86 | 0.604 (NS) |
| Sr        | 5.5 ± 0.9 5.4 ± 1.6 | 0.98 | 0.979 (NS) |
| Zn        | 159 ± 22 133 ± 14 | 0.84 | 0.328 (NS) |
| Zn/Br     | 0.97 ± 0.28 1.68 ± 0.36 | 1.73 | 0.147 (NS) |
| Zn/Fe     | 1.03 ± 0.19 1.62 ± 0.36 | 1.57 | 0.160 (NS) |
| Zn/Rb     | 18.1 ± 4.2 23.4 ± 4.1 | 1.29 | 0.372 (NS) |
| Zn/Sr     | 31.1 ± 4.5 58.8 ± 34.4 | 1.89 | 0.481 (NS) |

M: Arithmetic mean; SEM: Standard error of mean; NS: Not significant difference.

Table 6 Differences between mean values (M ± SEM) of Br, Fe, Rb, Sr, and Zn mass fraction (mg/kg, dry mass basis) and between mean values of Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratio in adenocarcinoma of two groups patients with cancer stage in range (T1–T2) and (T3–T4).

| Parameter | Adenocarcinoma Stage (T1–T2) n=12 | Adenocarcinoma Stage (T3–T4) n=23 | Ratio (T3–T4)/(T1–T2) | Student’s t-test |
|-----------|---------------------------------|---------------------------------|-----------------------|-----------------|
| Br        | 82 ± 16 125 ± 21 | 1.52 | 0.114 (NS) |
| Fe        | 119 ± 28 169 ± 21 | 1.42 | 0.170 (NS) |
| Rb        | 9.4 ± 1.4 8.3 ± 1.6 | 0.88 | 0.625 (NS) |
| Sr        | 5.4 ± 2.2 5.5 ± 0.6 | 1.02 | 0.979 (NS) |
| Zn        | 174 ± 26 128 ± 14 | 0.74 | 0.131 (NS) |
| Zn/Br     | 1.92 ± 0.44 0.96 ± 0.20 | 0.50 | 0.087 (NS) |
| Zn/Fe     | 1.54 ± 0.31 1.30 ± 0.32 | 0.84 | 0.591 (NS) |
| Zn/Rb     | 22.4 ± 4.4 21.3 ± 4.2 | 0.95 | 0.859 (NS) |
| Zn/Sr     | 70 ± 46 29.9 ± 3.4 | 0.43 | 0.479 (NS) |

M: Arithmetic mean; SEM: Standard error of mean; NS: Not significant difference.

we calculated these values using the medians of published data for water ~83% [45] and ash ~1% on wet mass basis [46] contents in nonhyperplastic prostate of adult men. In cancerous prostate tissues (Table 3) our results were comparable with published data for Fe, Rb, and Zn contents and almost two orders of magnitude higher for Br [40-44]. No published data referring Sr contents of cancerous prostate tissue were found. From Table 4, it is observed that in cancerous tissue the mass fractions of Rb (p<0.00001) and Zn (p<0.0000000004) are lower whereas mass fractions of Br (p<0.0003) and Sr (p<0.015) are significantly higher than in normal tissues of the prostate. In tissue of adenocarcinoma the Zn/Br, Zn/Fe, Zn/ Rb, and Zn/Sr mass fraction ratios are significantly (p<0.000001) lower than in normal prostate tissues.

No age- or cancer stage-related differences in the mass fraction of Br, Fe, Rb, Sr, Zn and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios were found (Tables 5 and 6 respectively).

Inter-element correlations between trace elements are...
leading cause of interobserver variability was insufficient quality control of results in these studies. In many reported papers tissue samples were ashed or dried at high temperature for many hours. In other cases, prostate samples were treated with solvents (distilled water, ethanol, formalin etc). There is evidence that by use of these methods some quantities of certain chemical elements, including Zn, are lost as a result of this treatment [47-49].

Characteristically, elevated or deficient levels of trace elements observed in cancerous tissues are discussed in terms of their potential role in the initiation, promotion, or inhibition of prostate cancer. In our opinion, abnormal levels of many trace elements in adenocarcinoma could be the consequence of malignant transformation. Compared to other soft tissues, the human prostate has higher levels of Zn and some other trace elements. These data suggests that these elements could be involved in functional features of prostate tissue. Malignant transformation is accompanied by a loss of tissue-specific functional features, which leads to a significant reduction in the contents of elements associated with functional characteristics of the human prostate tissue (Zn and, probably, Rb). Therefore, it is plausible that the reason for the emergence and development of adenocarcinoma is associated with abnormally high concentration of some metals in the prostate tissue of older men.

| Table 7 | Interco relations of pairs of the trace element mass fractions or mass fraction ratios in intact and cancerous prostate glands (r – coefficient of correlation). |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tissue | Element | Fe       | Rb      | Sr       | Zn       | Zn/Br    | Zn/Fe    | Zn/Rb    | Zn/Sr |
|---------|---------|----------|---------|----------|----------|----------|----------|----------|-------|
| Normal  | Br      | 0.001    | -0.07   | 0.23     | -0.10    | -0.43    | 0.02     | 0.06     | 0.14  |
| 41-87 years | Fe | zzzzz    | -0.12   | -0.07   | 0.12     | -0.12    | -0.45    | 0.18     | -0.14 |
| n=37    | Rb      | zzzzz    | -0.22   | -0.27   | -0.34    | -0.33    | -0.58    |         |       |
| Sr      | zzzzz   | zzzzz    |         |         |          |          |          |          |       |
| Zn      | zzzzz   | 0.83     | 0.78    | 0.80     |          |          |          |          |       |
| Zn/Br   | zzzzz   | 0.76     | 0.59    |          |          |          |          |          |       |
| Zn/Fe   | zzzzz   | zzzzz    | 0.56    |          |          |          |          |          |       |
| Zn/Rb   | zzzzz   | 0.69     |         |          |          |          |          |          |       |
| Zn/Sr   | zzzzz   |          |         |          |          |          |          |          |       |
| Adenocarcinoma | Br | 0.04     | 0.44    | 0.17     | -0.60    | -0.78    | -0.17    | -0.54    | -0.67 |
| 44-87 years | Fe | zzzzz    | 0.03    | 0.26     | -0.21    | 0.29     | -0.66    | -0.08    | -0.61 |
| n=35    | Rb      | zzzzz    | -0.55   | -0.10   | -0.08    | -0.61    |          |          | 0.47  |
| Sr      | zzzzz   | -0.09    | -0.39   | -0.39    | 0.51     | -0.76    |          |          |       |
| Zn      | zzzzz   | 0.73     | 0.37    | 0.42     |          |          |          |          | 0.65  |
| Zn/Br   | zzzzz   | 0.52     | 0.21    |          |          |          |          |          | 0.88  |
| Zn/Fe   | zzzzz   | 0.29     |          |          |          |          |          |          | 0.87  |
| Zn/Rb   | zzzzz   | 0.07     |          |          |          |          |          |          |       |
| Zn/Sr   |        |          |          |          |          |          |          |          |       |

Statistically significant values with p ≤ 0.01 are in bold.
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