The concentration of 12-lipoxygenase in platelet rich plasma as an indication of cancer of the prostate

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Aim of the study: The aim of this study was to determine whether measuring concentrations of 12-LOX in platelet-rich plasma patients can:
1. Differentiate between the group of patients with prostate cancer and healthy men.
2. Correlate the degree of severity of the disease and the concentration of the enzyme.

Material and methods: The study group comprised 88 men (40–88 years), including 54 patients diagnosed with prostate cancer. The population was divided into 4 groups:
- group 1 (22 men, aged 55–84 years) – with a negative biopsy,
- group 2 (36 men, aged 54–88 years) – with a positive biopsy result,
- group 3 (18 participants aged 58–83) – patients with cancer metastatic disease,
- group 4 of healthy men (12 people aged 40–66 years) – biopsy was not performed.

Routine PSA, morphology and CRP analysis were performed and platelet rich plasma was used for 12(S)LOX determination using an ELISA kit.

Results: 1. There was a weak (r = 0.0487) positive correlation between the number of blood platelets and plasma 12(S)LOX. 2. An inverse relationship between 12(S)LOX and Gleason grade was found. 3. Heterogeneity of 12(S)LOX in the group with prostate cancer metastatic disease may suggest differences in the response to the treatment carried out. 4. There were no statistically significant differences in concentrations of 12(S)LOX in different groups of patients.

Conclusions: Our results suggest that 12(S)LOX is relevant in prostate cancer; however, further study should include a larger, more select group of men with prostate cancer.

Key words: 12-lipoxygenase, prostate cancer, platelet-rich plasma.

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Introduction

Lipoxygenases (LOXs) and their reaction products play an important role in physiology and pathology, particularly in the differentiation and maturation of cells as well as inflammation, carcinogenesis, arteriosclerosis, and degenerative changes of nerve cells [1–3].

In renal cell carcinoma there was found strong expression of 5- and 12(S)LOX, and it was demonstrated that LOX inhibitors reduce the viability of cancer cells by inducing apoptosis [4]. Inhibitors added to cell culture produce chromatin condensation, and reduce cell volume and formation of apoptotic bodies. The results of these studies suggest an important role of 5- and 12(S)LOX in the progression of renal cell carcinoma [5, 6].

Impaired expression of LOX and cyclooxygenase (COX) has been detected in patients with breast cancer, wherein the group of patients with the lowest and highest concentrations of 15LOX, concentrations of 5- and 12(S)LOX and COX-2 had the worst prognosis [7]. Expression of COX-2 and 12(S)LOX mRNA in breast cancer patients was associated with the progression of the disease, whereas there was no connection with the presence of hormone receptors [8, 9]. There is evidence that the increase in expression of 12(S)LOX intensifies the metastatic potential of prostate cancer [10, 11].

Therefore we analyzed a group of healthy men and a group with prostate cancer to find if:
1. There is clear differentiation between the levels of the enzyme in the group of patients with prostate cancer and healthy men.
2. There are correlations between the severity of cancer and the concentration of the enzyme.

A positive answer to any of these questions could indicate a potential use of 12(S)LOX as a biomarker useful for routine clinical diagnosis.

Material and methods

Patients

The study involved 88 men, including 54 patients diagnosed with prostate cancer. Patients signed informed consent and volunteered to participate in the study. Recruitment of patients includes the period from January 2010 to January 2011. The study recruited patients with suspicion of prostate cancer.

Prostate biopsy was performed between the 2nd and 30th days after the blood collection for laboratory testing. Biopsies that were performed in the Department of Urology, the Municipal Hospital in Gdynia, took place between days 2 and 30 after the blood collection for laboratory testing. From 10 to 12 biopsy specimens were taken from the prostate, and sent for the pathology evaluation. Following the pathological outcome, patients were divided into two groups with and without a diagnosis of prostate cancer diagnosis:

- group 1 (22 men, aged 55–84 years) – with a negative biopsy,
- group 2 (36 men, aged 54–88 years) – with a positive biopsy result,
- group 3 (18 participants aged 58–83) – with cancer metastatic disease,
- group of healthy men (12 people aged 40–66 years) – biopsy was not performed.

All those participating in the study were interviewed on co-morbidities. In patients with metastatic prostate cancer information was collected on the current course of treatment and the extent of the disease was assessed on the basis of the available documentation and diagnostic imaging: bone scan, abdominal CT scan and chest X-ray.

Blood tests

Routine laboratory tests were performed in the Laboratory of the Marine Hospital in Gdynia, in accordance with the procedure and standards of the laboratory. To determine the amount of platelets the analyzer CELL-DYN Ruby Abbott, and for biochemical, CRP and PSA (prostate specific antigen) analysis the analyzer Roche Cobas 6000 were used. Platelet (PLT) levels in accordance with the references given in units of laboratory × $10^3/\mu l$, CRP mg/l, PSA in ng/ml.

Concentration of 12(S)LOX was determined at the Department of Clinical Nutrition, Medical University of Gdansk using IMUBIND® test 12-Lipoxygenase ELISA kit. Blood samples for 12(S)LOX were collected into tubes containing 3.2% sodium citrate (9 parts blood, 1 part citrate). Fasting blood was collected from cubital vein puncture. Determination of the concentration of 12(S)LOX was done by 12-IMUBIND® ELISA Lipoxygenase ELISA kit from American Diagnostica Inc. Blood samples after collection were centrifuged (100×g for 10 minutes, 4°C) to give the supernatant of a platelet rich plasma. The plasma was stored in a refrigerator at +2°C to +8°C for 4 hours or frozen at –20°C if analysis could not be done in less than 4 hours after blood collection.

Statistical analysis was performed using the computer program Statistica v.8 StatSoft Company (http://www.statsoft.com/company/).

This work is the first pilot study testing of the human 12 (S)-lipoxygenase in platelet rich plasma of patients using a highly specific antibody.

Results

Outcome of blood analysis (CRP, PSA, LOX) and Gleason grade of 88 men are presented in Table 1.

The mean values of platelets were very similar in the study groups, ranging between 210 and 240 × $10^3/\mu l$. All values were within the reference and the observed differences were not statistically significant.

With the exception of group 2 patients with newly diagnosed histologically confirmed prostate cancer, in all other groups a slight increase in the average level of CRP in relation to the reference values was observed. The average value of CRP in group 2 was within reference values. Differences between groups were not statistically significant.

PSA only in healthy volunteers (group 4) was in the range of the reference values; in group 1 and group 2, the average reference values exceed about 2 and 5 times, respectively. A particularly high average PSA value was obtained in the third group – patients with cancer metastatic disease – PSA values were more than 50-fold higher than the upper limit of the reference values. The differences in PSA concentrations between groups were statistically significant ($p < 0.05$) only when compared to group 4 (healthy).

The average results of 12-LOX ranged from 380 to 640 ng/ml – the differences between the groups were significantly smaller than the differences within the groups. The highest value was obtained in group 3 patients (including patients with metastatic prostate cancer). This group of patients had the largest differences in concentration of 12(S)LOX. Based on statistical analysis there was no significant difference between the two groups ($p = 0.4614$).

Table 1. Plasma levels of CRP, PSA, PLT and 12-LOX patients in the study groups

Parameter	Group 1 ($n = 22$)	Group 2 ($n = 36$)	Group 3 ($n = 18$)	Group 4 ($n = 12$)
Gleason score	– –	6 ±1 4–9	7 ±1 5–8	– –
Platelets ($10^3/\mu l$)	237 ±96 109–462	212 ±64 97–355	226 ±65 105–356	262 ±49 196–354
CRP (mg/l)	7.7 ±17 0–73	2.2 ±2.6 0–13.1	11.5 ±29 0.4–103.3	6.9 ±10.7 0.5–34.6
PSA (ng/ml)	7 ±5.6 0.55–27	21.1 ±27.5 3.1–118.9	223 ±728 0.0–3104	0.7 ±0.4 0.2–1.6
LOX (ng/dl)	517 ±753 0–3411	381 ±629 0–2691	515 ±1108 1.56–4815	636 ±502 14–1644

**Results are expressed as X ± SD (the first row) and as a range (the second row)*

The relationship between the 12(S)LOX and platelet levels in each group of patients studied showed no correlation between these parameters, most likely because of the small number of subjects in the groups (12 to 31 patients) as well as the differences of 12(S)LOX. However, in the whole analyzed population (n = 88) there was found a weak correlation between these parameters (Fig. 1).

Also we have analyzed the impact of the additional conditions (groups 1, 2, 3) such as coronary artery disease, hypertension and Parkinson's disease and no significant differences were observed in the 12(S)LOX expression.

Our results suggest that 12(S)LOX is relevant in prostate cancer, but further study should include a larger, more select group of men at risk of becoming ill with prostate cancer and patients with prostate cancer metastases.

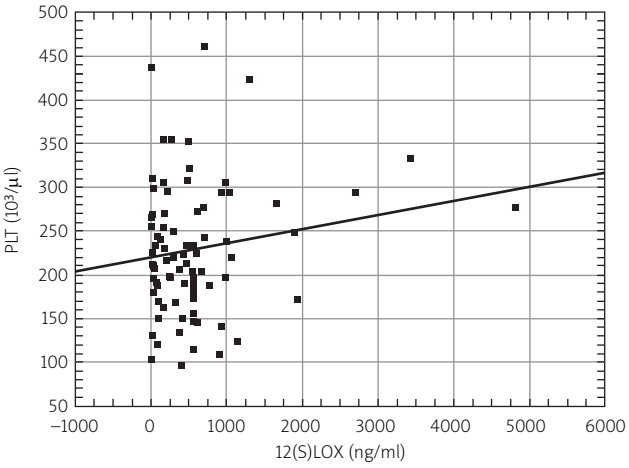

Fig. 1. Correlation between concentrations of 12(S)LOX and PLT in the overall study population (p = 0.039, r = 0.0487)

Discussion

A literature review suggests the existence of a causative link between 12(S)LOX and malignancies depending on stages of carcinogenesis [12, 13]. 12(S)LOX and its reaction product HETE (hydroxyeicosatetraenoic acid) have an effect on cell growth, inhibition of apoptosis, increased angiogenesis, and regulation of cell adhesiveness to tumor stroma and endothelium [14]. Through these mechanisms it can contribute to increasing aggressiveness of the tumor [15]. Also the impact of lipoxygenases on carcinogenesis is complex because it is dependent on the expression and activity of LOX, as well as the availability of substrates in the tissue. Individual expression of LOX isoforms and their products in tumor tissues determines the balance between pro- and anti-cancer enzyme isoforms [16].

Existing literature data almost exclusively analyze mRNA level of 12(S)LOX in the cell lines [17]. Studies by Goa [18] showed that mRNA expression platelet 12(S)LOX was significantly higher in prostate cancer cells than in normal prostate tissue [18]. Additionally, studies of tissue of more than 130 patients showed an average 38% increase in the 12(S)LOX mRNA in tumor tissue as compared with the normal noncancerous prostate tissue. Increased expression of 12(S)LOX in prostate carcinomas with high-grade tumors compared to low-grade and intermediate tumors was statistically significant [18]. In addition, the level of 12-HETE produced by 12(S)LOX in the urine of patients with prostate cancer was higher than in the healthy population. Moreover, there was a statistically significant difference in the concentration of 12-HETE in the urine of patients with prostate cancer compared to patients diagnosed with benign prostatic hyperplasia. Interesting was that in cancer patients after removal of the prostate, eicosanoid (HETE) concentrations were similar to those observed in healthy subjects [19].

Platelet rich plasma is the only readily available biological material containing 12(S)LOX. Until now this material has not been used for diagnosis or monitoring of cancer. Platelet rich plasma is also a difficult material testing require particularly strict observance analytic technology because platelet recovery is dependent on the conditions of centrifugation of blood [20]. That is evidenced by the positive but weak correlation between the number of platelets in the starting material and the concentration of 12(S)LOX (see Fig. 1).

The number of healthy volunteers in this study (group 4) was small in comparison to clinical trials – just 12 people could be involved in the study. In the control group, 12(S)LOX was in the range 14–1000 ng/ml. Due to the small size of this population it was not possible to establish reference values for 12(S)LOX.

Also, group 1, with a negative biopsy, cannot be treated as a reference group. A negative biopsy does not rule out the existence of prostate cancer. This group of patients should be particularly interesting to keep track of its future fate. It seems that this group of patients should undergo re-examination or be specifically monitored.

In group 3, patients with prostate cancer metastasis and even ill for 10 years, considerable heterogeneity was observed between the sensitivity limit of the analytical values up to 5000 ng/ml. It appears that effective treatment may reduce the synthesis of 12(S)LOX and activation of apoptosis. However, no therapeutic effect could result in high levels of 12(S)LOX [21–23]. This hypothesis will need to be confirmed in other studies.

Among the factors influencing the measurement result obtained 12(S)LOX, depending on the patient's individual variation, replace the enzyme concentration and activity. Studies *in vivo* have shown that the activity of 12(S)LOX may change in the case of a single nucleotide polymorphism (SNP). A mutation in the active site (N544L) led to complete deactivation of the enzyme, while present in the peripheral part of a single nucleotide polymorphism of the enzyme resulted in a change of the enzyme kinetics. It was found that even in the peripheral SNP may change the mechanism for allosteric enzyme, and the change of signals at the border of the cell membrane [24]. Gly-Ala point mutation can change not only the twisting SR enzyme, but also the place of oxidation. Mutations in other places around the iron atom location may have a similar effect on the catalytic specificity.

Currently, only the concentration of PSA in serum is a marker used in clinical practice. PSA level not exceeding the reference value (up to 4 ng/ml) is associated with more than 25% risk of prostate cancer revealed with abnormal DRE test.

In this study there was no correlation between the amount of 12(S)LOX and PSA in any group; no correlation was also found in the overall study population.

In group 2 patients (newly diagnosed with prostate cancer) there was a statistically significant positive correlation between the amount of 12(S)LOX and CRP ($p = 0.002$). Often, the development of prostate cancer may be accompanied by inflammatory changes in the prostate gland. Another factor that may affect CRP levels and, consequently, 12(S)LOX is diet [25]. There was no statistical difference in the level of 12(S)LOX between the two groups. It must be emphasized that high fat intake and obesity are correlated with elevated CRP and 12(S)LOX (23), which may influence our results. No statistical differences were found in concentrations of 12(S)LOX that would allow patients with metastatic cancer to be separated from a group of newly diagnosed prostate cancer. This can be explained by the small sample size of the population involved in the study, and above all, great diversity in both groups (especially group 3 – with metastatic carcinoma of the prostate).

Literature data suggest a possible association between prostate cancer progression and metastasis formation and increased expression of 12(S)LOX [26]. In the group of patients with prostate cancer we found an inverse correlation with the Gleason score, but there was no correlation with the degree of tumor malignancy. There may be several reasons for this. First, advancement of tumor in Gleason score was assessed at the time of diagnosis of the tumor, that is 2 to 10 years earlier, and the examination was performed by different pathologists. Second, this result may be associated with additional illnesses identified in the study group and therapies, which can lead to both increase and inhibition of expression of 12(S)LOX. Another probable cause of the negative correlation of 12(S)LOX with Gleason score may be genetically determined high heterogeneity of prostate cancer [27, 28]. Here one must also take into account that the study group was small (46 patients, group 2 + 3).

We hope that further studies will clarify the hypothesis and determine the utility of the 12(S)LOX activity test in the diagnosis of prostate cancer. Future studies should take into account not only the malignancy and the degree of its severity but also such factors as inflammation, the coexistence of other diseases, type of diet and the amount and profile of dietary fats, as well as the dynamics of the disease process type of the therapy.

The authors declare no conflict of interest.

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