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Plasma concentration of protein Z and protein Z-dependent protease inhibitor in patients with haemophilia A

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ABSTRACT

The potential role of alterations in protein Z (PZ) concentrations in the pathogenesis of coagulation has been investigated in several studies which, however, yielded conflicting results. Protein Z deficiency may induce bleeding as well as prothrombotic tendencies and it might occur as an inherited disorder. The principal aim of the present study was to explore the concentration of protein Z and protein Z-dependent protease inhibitor (ZPI) in patients with haemophilia A.

In haemophilia A patients mean plasma concentrations of PZ and ZPI were significantly higher than in healthy individuals: PZ (1.87 ± 0.68 μg/mL vs 1.49 ± 0.54 μg/mL) and ZPI (5.02 ± 1.11 μg/mL vs 4.22 ± 0.55 μg/mL), with $p = 0.02$ and $p = 0.03$, respectively. In the subgroup with severe haemophilia A, an in-depth analysis revealed a tendency to modulating effect of the PZ ($r = -0.53$; $p = 0.072$) and a statistically significant one in the case of ZPI (rho $= -0.79$, $p = 0.002$) on the bleeding rate. It simultaneously disclosed a statistically significant correlation between the number of bleeds to the joints (20.18 ± 14.1), PZ ($r = -0.72$; $p = 0.04$) and ZPI (rho $= -0.88$, $p = 0.001$). With reference to this particular group of patients, the study also showed some other statistically meaningful correspondences: between PZ and ZPI (rho $= 0.65$, $p = 0.02$), PZ and FIX ($r = -0.61$, $p = 0.04$), as well as ZPI and FVIII (rho $= 0.78$, $p = 0.002$).

In conclusion, despite the fact that FVIII deficiency is undoubtedly the main mechanism of bleeding in haemophilia A patients, the activity of PZ/ZPI complex may play some modulating role in the matter.

Introduction

Despite its characterization in human plasma in 1984, the physiological function of protein Z (PZ) still remains unclear. Being structured similarly to other vitamin K-dependent factors (factors VII, IX, X, protein C), it could play a role as a cofactor of another protein which down-regulates coagulation [1], likewise protein S. This hypothesis was confirmed by the isolation of protein Z-dependent protease inhibitor (ZPI) which inhibits activated factor X (FXa) in a process that requires presence of protein Z, calcium ions and phospholipids. Consecutive studies revealed a new inhibitory function of this molecule that is also relevant to haemostatic system. ZPI also efficiently and rapidly inhibited FXIa in the mechanism that does not require PZ, calcium or phospholipids [2]. PZ and ZPI form a complex and in pooled normal plasma, which contains excess ZPI, all the PZ appear to be bound to ZPI [3]. Chronic warfarin treatment dramatically reduces PZ plasma level, which is associated with 45% decrease of ZPI [3], and it was speculated that PZ concentration might affect ZPI level by acting either on ZPI secretion or clearance. Consistently with this hypothesis, other clinical studies showed a significant correlation between PZ and ZPI levels in patients with vascular complications [4,5].

The potential role of alterations in protein Z and/or ZPI levels in the pathogenesis of thrombotic and/or haemorrhagic diseases has been investigated in several studies which, however, yielded inconsistent results. Some of these analyses reported low protein Z levels connected with the occurrence and progression of several types of ischemic vascular diseases [6] whereas others neither observed that correlation nor mentioned the association between low levels of protein Z and haemorrhagic diseases [7]. There is no published data evaluating PZ/ZPI complex in patients with inherent deficiency of anti-haemophilic factor.

The purpose of the present study was to explore the concentration of protein Z and protein Z-dependent protease inhibitor in patients with haemophilia A. Additionally; it examined the correlation between PZ/ZPI plasma concentration and bleeding rate per year.

Patients

Hemophilia A is an inherited deficiency in clotting factor VIII, which causes increased bleeding and usually affects males. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1–5% active factor have moderate haemophilia, and those with mild haemophilia have between 5–40% of normal levels of active

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clotting factor. The study was performed on fifty male patients: 25 with severe, 15 with moderate and 10 with a mild form of haemophilia A. Median age at the time of samples taking was 29 (range 19–55 years). None of these patients had undergone prophylaxis of bleeding with FVIII (factor VIII) before the study due to the National Health System regulation which does not subsidize prophylaxis in adult patients. All studied subjects received FVIII on demand. Doses depended on the kind of bleeding event. Neither the patients who underwent analysis nor healthy subjects suffered thrombotic episodes or clinical or biochemical signs of liver dysfunction (Table 1). None one of the study population had co - morbidities like diabetes or obesity and chronic or acute inflammation, that might affect coagulation. All studied individuals did not receive any anticoagulants prior to or at the time of the study. Haemophilia A patients and healthy individuals with confirmed hepatitis B and C or obscure the increase in liver enzymes or haemophilia A with inhibitor of factor VIII had been excluded from the study as well. The control group consisted of 90 healthy male blood donors at median age of 30 (range 19–52). The yearly bleeding rate and joint bleeding rate were calculated on the basis of data from medical records and home-treatment reports from 2005 to 2011, in which patients had recorded the dates and types of bleeding (joint pain and swelling in the knees and elbows, large bruises swelling under the skin and between muscles, internal bleeding, blood in the urine and nose bleeds). Studied patients did not receive any recombinant factor VIII or any plasma derived factor VIII at least seven days before sample collection to avoid potential stimulation of PZ/ZPI, which estimated plasma half-life is about 2.5 days. None of the haemophilia A patients were given blood or platelet transfusion if they had suffered severe bleeding episodes during the period of six months preceding blood collection. Informed written consent was obtained from all participants, and the Local Ethics Committee of Medical University of Bialystok approved the study.

Methods

Venous blood samples were drawn with a butterfly cannula with a minimum of stasis, after at least 4 hours of fat fasting and before 10 a.m. [8]. Tests were performed immediately after blood collection. Quantitative assessment of protein Z and ZPI in the plasma were performed using commercial test (Asserachrom Protein Z Elisa Kits, Diagnostica Stago, France) with expected mean value (±SD, 1.56 ± 0.61 μg/mL) and Enzyme-linked Immunosorbent Assay Kit for Protein Z Dependent Protease Inhibitor (ZPI) (USCN Life Science Inc., China). Quantitative determination of FIX in the plasma was done using Stago® Deficient IX (Stago, France), with expected normal range of factor IX between 60–150%. Quantitative determination of FVIII activity in the plasma was done using Stago® STA FACTOR VIII (Stago Diagnostica, France), with expected normal range of factor VIII between 60–150%.

Statistical analyses

The results are presented as mean ± SD. Distributions of samples were tested by Shapiro-Wilk test. PZ, ZPI, FVIII and FIX assay methods were compared using t-Student test for independent samples with normal distribution and Mann-Whitney test for nonparametric samples. Pearson (r) and Spearman (rho) tests were used to correlate the parameters for normal and nonparametric distribution, respectively. Program Statistica 9.0 PL (StatSoft, Poland) was used to perform statistical analyses. Values ≤ 0.05 were considered as statistically significant.

Results

In haemophilia A patients mean plasma concentrations of PZ and ZPI were statistically significantly higher than in healthy individuals: PZ (1.87 ± 0.68 μg/mL vs 1.49 ± 0.54 μg/mL) and ZPI (5.02 ± 1.11 μg/mL vs 4.22 ± 0.55 μg/mL), with $p = 0.02$ and $p = 0.03$, respectively (Table 1). No differences in concentration of either PZ or ZPI according to degrees of haemophilia A severity were observed (Table 1). However, the results did demonstrate some significant changes in bleeding rate between the subgroup of severe haemophilia A patients and the subgroup of moderate and mild haemophilia A patients, standing at $p = 0.01$ and $p = 0.0004$, respectively (Table 1). Median age of haemophilia A patients and healthy donors did not show important differences, and neither did median age of severe, moderate and mild haemophilia patients (Table 1). We did not find any correlations between age and PZ ($r = -0.08$, $p = 0.68$), ZPI ($rho = -0.07$, $p = 0.67$) or bleeding rate ($r = 0.13$, $p = 0.51$).

No link was established between tested parameters (PZ/ZPI) and clinical diseases presentation in the whole group of haemophilia A patients. The study did not show statistically correlation between PZ and activity of factors FVIII and FIX, $r = 0.09$, $p = 0.65$ and $r = -0.006$, $p = 0.97$, respectively. Additionally, the study did not revealed statistically significant correlation of ZPI and activity of FVIII and FIX, $rho = 0.23$, $p = 0.24$ and $rho = -0.25$, $p = 0.2$, respectively. In performed study we did not observed modulating effect of PZ and ZPI on bleeding rate, $r = -0.26$, $p = 0.19$ and $rho = -0.23$, $p = 0.25$. There was no significant correlation between PZ and ZPI in all haemophilia A patients ($r = -0.11$, $p = 0.65$) and in mild and moderate haemophilia A, respectively $rho = 0.09$, $p = 0.78$ and $rho = -0.54$, $p = 0.16$.

However, the study observed a tendency towards a modulating effect of PZ concentration on bleeding rate in the subgroup with severe haemophilia A ($r = -0.53$; $p = 0.072$) and a statistically significant correlation for ZPI ($rho = -0.79$, $p = 0.002$). Moreover, it revealed a statistically significant correlation between joint bleeding (20.18 ± 14.1), PZ ($r = -0.72$; $p = 0.04$) and ZPI ($rho = -0.88$, $p = 0.001$).

Table 1
Characteristic of healthy donors and haemophilia A patients.

	Healthy donors n = 90 95%CI (range)	All hemophilia patients n = 50 95% CI (range)	Hemophilia severe n = 25 95% CI (range)	Hemophilia moderate n = 15 95% CI (range)	Hemophilia mild n = 10 95% CI (range)
Age [years]	30 ± 7.67	29 ± 8.99	29.9 ± 6.98	34.5 ± 12.4	29.16 ± 6.8
PZ [μg/mL]	1.49 ± 0.54 (1.29–1.68)	1.87 ± 0.68* (1.59–2.15)	1.89 ± 0.76 (1.50–2.24)	1.88 ± 0.38 (1.61–2.12)	1.73 ± 0.87 (1.4–2.33)
ZPI [μg/mL]	4.22 ± 0.55 (3.85–4.59)	5.02 ± 1.11** (4.57–5.47)	5.35 ± 0.48 (4.73–5.73)	4.78 ± 1.03 (4.22–5.12)	4.65 ± 1.87 (4.19–5.24)
FVIII [%]		1.64 ± 1.54	0.33 ± 0.27	2.38 ± 0.21	5.50 ± 1.41
FIX [%]		95.1 ± 17.7	97.8 ± 14.37	96.6 ± 9.33	87 ± 15.47
Bleeding rate [per year]	0.0	23.96 ± 18.33	25.91 ± 16.3	8.73 ± 7.84	4.0 ± 2.67
Alanine transaminase (IU/L)	21 ± 13.24	22 ± 10.21	21 ± 11.27	23 ± 12.23	21 ± 13.87
Total bilirubin (mg/dl)	0.5 ± 0.23	0.51 ± 0.33	0.49 ± 0.29	0.52 ± 0.41	0.51 ± 0.16
C Reactive Protein (mg/dl)	0.9 ± 0.43	0.8 ± 0.32	0.6 ± 0.26	0.9 ± 0.22	1.0 ± 0.47

PZ - protein Z, ZPI - protein Z-dependent protease inhibitor, FVIII - factor VIII, FIX - factor IX, n = number of patients or healthy individuals, p = power, 95% CI - confidence intervals, *p = 0.02, **p = 0.03.