

Rapid and Short Communication

Protein Z and protein Z-dependent protease inhibitor

Determinants of levels and risk of venous thrombosis

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Summary

To assess the potential roles of protein Z (PZ) and protein Z-dependent protease inhibitor (ZPI) in venous thrombosis, their plasma levels were measured in 426 individuals with venous thrombosis and 471 control individuals participating in the Leiden Thrombophilia Study. A relationship between the level of

PZ or ZPI and venous thrombosis was not detected in the overall case-control study. PZ and ZPI circulate as a complex and their plasma levels are interdependent. Both PZ and ZPI are increased with oral contraceptive use and reduced with oral anti-coagulant therapy.

Keywords

Coagulation inhibitors, clinical / epidemiological studies, thrombophilia

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Introduction

Protein Z (PZ) is a vitamin K-dependent plasma glycoprotein whose structure is similar to that of factors VII, IX, X, and protein C (1, 2). In contrast to these serine protease zymogens, however, PZ lacks the histidine and serine residues of the canonical catalytic site and does not serve a proteolytic function. Instead, PZ functions as a cofactor to enhance the inhibition of factor Xa by a serpin termed PZ-dependent protease inhibitor (ZPI) (3–5). In addition to inhibiting factor Xa in a PZ dependent fashion, ZPI also inhibits factor XIa in the absence of PZ (5).

Plasma PZ levels in 450 Red Cross blood donors spanned a broad range (0.6–5.7 µg/mL) with a mean concentration of 2.9 ± 1.0 µg/mL in EDTA anticoagulated plasma (~ 2.6 µg/mL, in citrated plasma) (6). Oral anticoagulant treatment with vitamin K antagonists, such as warfarin, reduces both the PZ antigen (1–16%) and its degree of γ-carboxylation (<1%) much more than other vitamin K-dependent factors (6). PZ and ZPI circulate as a complex in plasma and in the normal case of excess ZPI all the PZ in plasma is bound to ZPI (7).

In mice, PZ deficiency is associated with a prothrombotic phenotype and dramatically increases mortality in animals with

the factor V Leiden mutation suggesting that PZ deficiency may be a risk factor for thrombotic disease in humans (8). Studies exploring the relationship between PZ levels and ischemic stroke have produced conflicting results (9–14). Two reported an association of stroke with low PZ levels (9, 11), another found that a PZ genetic polymorphism (intron F g79a), which is reportedly associated with reduced PZ plasma levels, protected from stroke (12, 15), and a third found an association of high PZ levels with stroke (10). In two additional studies there was no relationship between stroke and PZ levels (13, 14). Recent studies have also reported a relationship between PZ deficiency and the acute coronary syndrome and early fetal wastage (16, 17).

Low levels of PZ are common in individuals with antiphospholipid antibodies and are associated with thrombotic complications in the antiphospholipid syndrome (18–20). In regard to venous thrombosis, one study did not find a relationship with low levels of PZ in a small cohort of patients (9) and another study, in which PZ levels were not determined, failed to detect an association with polymorphisms within the PZ gene (21).

As yet, the relationship between ZPI levels and thrombotic disease has not been explored. The aim of the current study was to assess determinants of plasma levels of PZ and ZPI, as well as

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Table 1: PZ and ZPI levels in healthy individuals.

	n	Protein Z Mean (Range) %	ZPI Mean (Range) %
All	471	100 (15-233)	100 (33-191)
Sex			
Men	199	103 (23-210)	98 (49-187)
Women	272	98 (15-233)	102 (33-191)
Use of oral contraceptives*			
Yes	54	121 (30-233)	109 (44-168)
No	99	83 (15-168)	92 (33-149)

*Oral contraceptive use at the time of the venopuncture of premenopausal women between 15-49 years, who were not pregnant, not within 30 days postpartum, did not have a recent miscarriage and had not used only depot contraceptives.

the potential association of plasma levels of these proteins with the risk of venous thrombosis in a large population-based case-control study.

Study design

Study population

The Leiden Thrombophilia Study (LETS) includes 474 consecutive, unselected patients aged 18-70 years with a first objectively diagnosed episode of deep venous thrombosis and 474 age- and sex- matched control subjects. Control subjects were friends/partners of patients (22). Since PZ is a vitamin K-dependent plasma protein, individuals using oral anticoagulation were excluded from this analysis (n=49). Citrate anticoagulated plasma samples, obtained >6 months after the thrombotic event, from 426 patients and 471 control subjects were tested for PZ and ZPI. The 100% levels for PZ and ZPI were defined as their mean values in the control group of LETS (n=471).

Citrated plasma samples from an additional group of 20 individuals with venous thrombosis obtained during and four weeks after anticoagulant treatment were also evaluated (23).

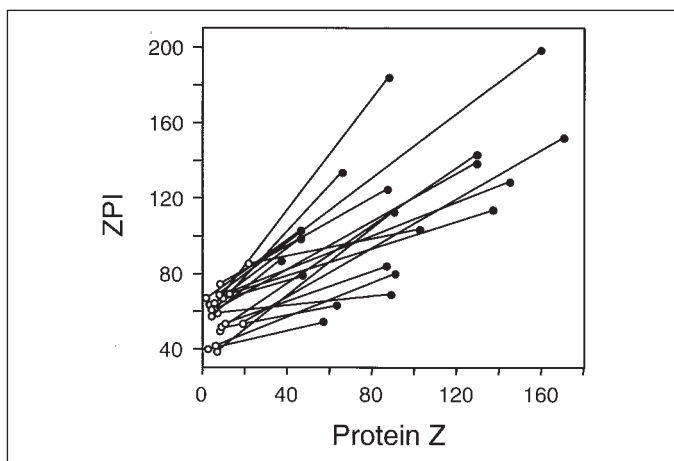


Figure 1: PZ and ZPI levels during and after warfarin therapy. INR during oral anticoagulant therapy 3.1 ± 0.6 ; after oral anticoagulant therapy 1.2 ± 0.1 .

Immunoassays

The monoclonal/monoclonal sandwich immunoassay for PZ (CV=6.5%) and polyclonal/monoclonal sandwich immunoassay for ZPI (CV=7.2%), were performed as previously described (7). Samples were assayed at a 100-fold dilution against a standard curve constructed with pooled normal plasma (George King Biomedical, Overland Park, KS).

INR

Prothrombin times and INRs were determined using Innovin (Dade Behring Inc., Newark, DE) following the manufacturers instructions.

Statistical analysis

Determinants of PZ and ZPI levels were assessed by t-tests and linear regression analysis. To calculate the risk of venous thrombosis, PZ and ZPI levels were dichotomized at the 10th and 90th percentiles measured in the control subjects. Odds ratios (OR) were calculated as estimates of the relative risk, with 95% confidence intervals (95% CI).

Results and discussion

Determinants of PZ and ZPI levels

As noted previously in another group of normal subjects (6), the range of PZ levels in the plasma samples of 471 control subjects was broad (15%-233%). Though less marked than the results for PZ, ZPI also spanned a wide range (33%-191%).

PZ and ZPI plasma levels were similar in men and women (mean difference PZ: 4%; 95% CI: -2%-11%; ZPI: -4%; 95% CI: -9%-1%, respectively) (Table 1). A plot of ZPI versus PZ levels in the control subjects suggests that the levels of these proteins are related (linear regression analysis with ZPI as the dependent variable: slope=0.32, 95% CI: 0.26-0.38; Y-intercept=67.5%, 95%CI 61.0%-74.1%). Women using oral contraceptives had significantly ($p < 0.001$) higher levels of both PZ and ZPI compared with women who were not using oral contraceptives (mean difference PZ: 38.2%, 95% CI: 26.1%-50.2%; ZPI: 16.9%, 95% CI: 8.3%-25.5%) (Table 1).

Oral anticoagulant treatment dramatically reduces the level of antigenic PZ in plasma (6) and the effect of this reduction in PZ levels on the plasma concentration of ZPI was examined by evaluating plasma samples from 20 individuals during and after treatment for venous thrombosis. In each case, the rise in plasma PZ (mean 8% vs. 94%; 95% CI of the difference: 68%-103%) that followed discontinuation of anticoagulant therapy was associated with an increase in the level of ZPI (mean 59% vs. 112%; 95% CI of the difference: 36%-70%) (Fig. 1). As a direct effect of warfarin therapy on ZPI plasma levels seems unlikely, we conclude that the rise in ZPI levels following discontinuation of warfarin is related to a yet to be established effect of PZ on ZPI metabolism. A portion of ZPI circulates as a complex with PZ and it is conceivable that the rate of clearance of the PZ-ZPI complex differs from that of ZPI (or PZ) alone (7). Alternatively, the synthesis, secretion or extra-plasma localization of one of the proteins may be affected by the presence of the other.

PZ and ZPI levels and venous thrombosis

No relationship between venous thrombosis and high (>90% percentile, not shown) or low (<10th percentile) levels of PZ or ZPI could be demonstrated in the overall case-control study (Table 2). Subgroup analysis suggested an association of low levels of PZ with venous thrombosis in men (OR: 2.4; 95% CI: 1.2–4.9) and in individuals older than 55 years (OR: 3.3; 95% CI: 1.2–8.7) (Table 2).

There was no relationship between the PZ or ZPI level and thrombosis in those positive/negative for the factor V Leiden mutation and, in contrast to the report of Kemkes-Matthes et al (24), no effect of the PZ (or ZPI) level on age of onset of thrombosis in patients with the factor V Leiden mutation (n=82) was detected (data not shown). It perhaps should be noted in this regard that any effect of PZ on the factor V Leiden phenotype in humans is likely to be much more subtle than that reported in mice (8). In contrast to the situation in humans, in the mice studied (C57Bl/6 x 129 mixed genetic background) the homozygous factor V Leiden genotype leads to a severe phenotype with ~50% perinatal mortality (25). Moreover, although the effect of PZ deficiency was dramatic in murine factor V Leiden homozygotes, its effect in murine factor V Leiden heterozygotes was much more modest (8).

Overall our results suggest that severe deficiencies of PZ and ZPI are unusual and that modestly reduced levels of these proteins are not a major risk factor for venous thrombosis. Due to increased statistical instability, subgroup analysis suggesting that low PZ levels may increase the risk of venous thrombosis in men and older individuals must be viewed with caution. Nevertheless, it is of interest that this same pattern of increased thrombotic risk in men and older subjects was noted by Heeb and colleagues in their studies of the association of PZ with stroke (11).

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Table 2: The risk of venous thrombosis associated with <10th percentile levels of PZ (54%) and ZPI (67%).

		n Pt/Ct*	Protein Z OR (95% CI)**	ZPI OR (95% CI)**
All		426/471	1.3 (0.9–2.0)	0.8 (0.5–1.2)
Men		172/199	2.4 (1.2–4.9)	0.6 (0.3–1.3)
Women		254/272	0.9 (0.5–1.6)	0.9 (0.5–1.6)
Age <55	All	315/348	1.1 (0.7–1.7)	0.7 (0.4–1.2)
	Men	111/132	2.1 (1.0–4.7)	0.5 (0.2–1.2)
	Women	204/216	0.7 (0.4–1.3)	0.8 (0.5–1.5)
Age ≥55	All	111/123	3.3 (1.2–8.7)	1.1 (0.3–4.0)
	Men	61/67	4.2 (0.8–21.1)	1.1 (0.2–5.7)
	Women	50/56	2.9 (0.8–9.9)	1.1 (0.2–8.3)

* Patients/Control subjects
** The risk of venous thrombosis associated with low levels of protein Z or ZPI was calculated for individuals with levels < 10th percentile compared with individuals with levels ≥ 10th percentile

Ongoing investigations should better define the potential roles of both PZ and ZPI in arterial as well as venous thromboembolic disease. The interdependence of the plasma levels of PZ and ZPI and the effect of oral contraceptive use and anti-coagulant therapy on their levels will need to be considered when analyzing the results of these studies.

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