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REVIEW

Protein Z: "Light and Shade" of a New Thrombotic Factor

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SUMMARY

Protein Z is a vitamin K-dependent plasma protein described in its human form in 1984. The amino acid sequence of protein Z shows wide homology with many coagulation factors, such as VII, IX, X, and protein C. However, in contrast to other vitamin K-dependent coagulation factors, protein Z is not a serine protease because of the lack of the active centre in its amino acid sequence. The physiological function of protein Z has been uncertain for many years. In vitro and in vivo studies recently suggested that protein Z plays an important role in inhibiting coagulation, as it serves as cofactor for the inactivation of activated factor X by forming a complex with the plasma protein Z-dependent protease inhibitor. The role of alterations of the protein Z levels has been evaluated in different disease states, with conflicting findings. Most of these studies were performed on ischemic vascular diseases. Recently, the possible role of protein Z deficiency in the occurrence of cardiovascular diseases has been evaluated. (Clin. Lab. 2004;50:XXX-XXX)

KEY WORDS

Protein Z, protein Z-dependent protease inhibitor, vascular diseases, coagulation factors, thrombosis

INTRODUCTION

Protein Z (PZ) is a vitamin K-dependent plasma protein synthesized by the liver. Bovine PZ was described for the first time by Prowse & Esnouf in 1977 [1] and purification of human PZ was successfully achieved in 1984 by Broze Jr. & Miletich [2]. It is a single-chain glycoprotein with a molecular weight of 62 kd [3]. The complete amino acid sequence of human PZ shows N-terminal homology with many serine proteases, such as coagulation factors VII, IX, X, and protein C [4]. However, in contrast to other vitamin K-dependent coagulation factors, PZ is not a serine protease because of the lack of the active center in its amino acid sequence [3].

The first report on a large population of healthy subjects showed an estimated half-life of 2-3 days and a wide variability of plasma levels (0.6-5.7 µg/ml), with a profound decrease in patients on stable warfarin therapy [5]. In 1998, the gene encoding PZ was localized on chromosome 13 at band q34 where the genes of other vitamin K-dependent proteins have been clustered, and consists of 9 exons including one alternative exon. Gene organization is essentially similar to that of the other vitamin K-dependent proteins [6].

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Function

Very little has been known about the physiological function of PZ until 1991, when Hogg & Stenflo first suggested a role for PZ as an enhancer of the coagulation cascade [7]. Actually, they demonstrated that PZ was able to increase the association of thrombin with phospholipid vesicles in a calcium-dependent manner. Human PZ, however, was found to bind thrombin poorly (K_d=8.9 µM) with respect to the bovine form and to have a minimal impact on thrombin association with phospholipids [8]. In fact, it has been shown that a 36 amino acid C-terminal extension present in bovine but absent in human PZ is responsible for the enhanced binding of thrombin to bovine PZ [8].

A sharp shift occurred in 1998, when Han et al. demonstrated that PZ circulates in a complex with another plasma protein, the so-called protein Z-dependent pro-

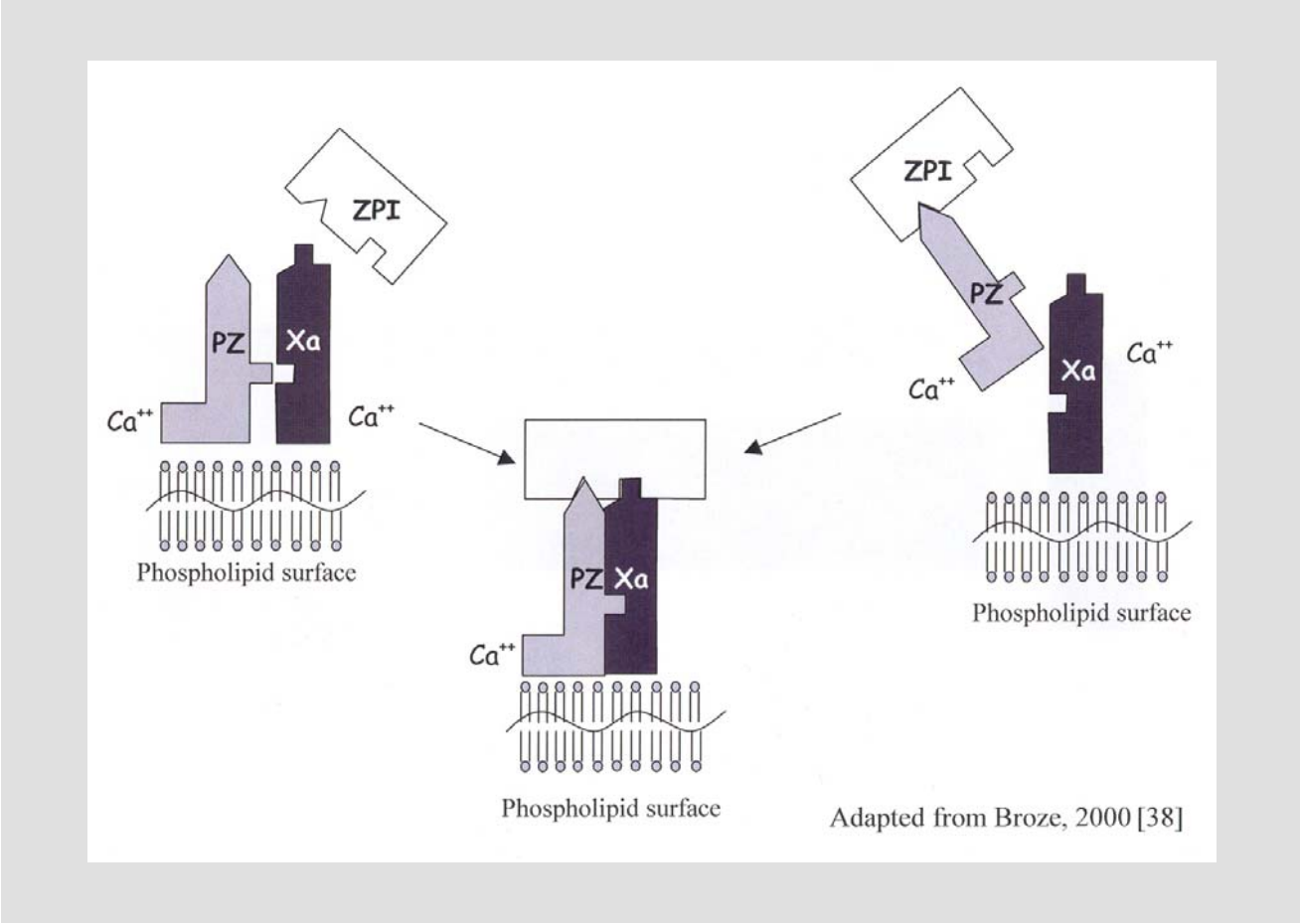

Figure 1: Two potential pathways of forming the Protein Z – Protein Z-dependent protease inhibitor complex
Footnote: PZ = Protein Z; ZPI = Protein Z-dependent protease inhibitor; Xa = Activated factor X

tease inhibitor (ZPI), and limits the coagulation response acting as a co-factor for the inhibition of activated factor X (Xa) [9]. In the presence of PZ, procoagulant phospholipids and calcium it produces a rapid inhibition of factor Xa in a process that appears to involve the formation of a calcium-dependent tertiary complex containing factor Xa, PZ and ZPI at the phospholipid surface. Two potential pathways of forming this tertiary complex have been hypothesized (Figure 1). Whether PZ and ZPI circulate together or form the complex just on the phospholipid surface is still unknown, although the first hypothesis seems to predominate.

ZPI is a 62-kd single chain protein, member of the serpin superfamily of proteinase inhibitors. Besides factor Xa, ZPI also produces a significant inhibition of activated factor XI in a reaction that does not require the presence of PZ, phospholipids or calcium [10].

To investigate the role of PZ alterations *in vivo*, Yin et al., performed an experimental study by disrupting the PZ gene in mice. PZ knock-out mice were then crossbred with mice homozygous for the factor V Leiden mutation, and the potential prothrombotic risk associated with PZ deficiency was observed [11]. As a result, the PZ (-/-) genotype was shown to increase the mortality of Factor V Leiden homozygous mice, indicating a possible role as prothrombotic factor of PZ deficiency.

Genetic control of PZ

Since PZ has a very broad range in the normal population it has been suggested that it may be an acute-phase protein. In 1999, a paper by Undar et al. demonstrated an inverse correlation with interleukin-6 plasma levels in patients with hematological malignancies, whereas similar results were shown with interleukin-1ß or tumor necrosis factor-α [12]. However, an "in vitro" study failed to demonstrate the influence of some inflammatory cytokines on PZ biosynthesis [13]. The authors suggested that the regulation of PZ biosynthesis might be dependent on a genetic control.

Several polymorphisms have been recently reported in the PZ gene. In 2001 Rice et al screened the entire PZ gene identifying 14 novel polymorphisms [14]. The study was set up to genotype four important polymorphisms (intron Fg79a, promoter a-13g, exon 8 Arg255His and intron C insertion/deletion) in relation to venous thrombosis. In 564 patients with deep venous thrombosis and in 492 age- and sex-matched controls no significant differences of genotype frequencies were found. More recently, Lichy et al. investigated the possible association between 2 common single nucleotide mutations in the PZ gene (intron Fg79a and promoter A-13g) and the risk of cerebral ischemia [15]. They analyzed these polymorphisms in 200 young (< 50 years) patients with cerebral ischemia and in 199 control subjects, also investigating the potential role of the PZ gene polymorphisms on PZ levels in 42 control subjects. As a result they found a genetic control of plasma PZ levels and a possible protective role for the A allele of the intron polymorphism on the occurrence of cerebral ischemia.

PZ ALTERATIONS IN CLINICAL STUDIES

Over the last years PZ has been evaluated in different disease states, observing controversial data concerning the alteration of levels.

PZ in bleeding tendency

The first clinical study was performed in 1995 by Kemkes-Matthes & Matthes who suggested a role for PZ as a factor involved in hemorrhagic tendency. The authors reported low PZ plasma levels in 36 patients with bleeding tendency, supposing that PZ deficiency might be the cause of the disease state [16]. By contrast, Gamba et al. and Ravi et al. (1998), failed to detect a relationship between low PZ levels and the bleeding disorder in two additional case-control studies on a total of 63 patients with bleeding tendency [17,18].

PZ in ischemic stroke

According to the identification of ZPI, clinical interest on alterations of the PZ levels moved to the assessment of its possible association with thrombotic diseases. Most of these studies were performed on ischemic cerebrovascular disease, but conflicting findings have been reported. In 2001, Kobelt et al. investigated PZ concentrations in 157 young patients (median age: 40 years) with an episode of ischemic stroke that had occurred during the previous two months, observing a higher frequency of high PZ concentrations associated with an increased relative risk of ischemic stroke [19]. A few months later, Vasse et al. performed a similar case-control study in 169 young patients (<60 years) with an episode of ischemic stroke that had occurred at least three months before the investigation [20]. In sharp contrast to Kobelt et al. they found that low PZ levels were associated with the occurrence of the disease. Furthermore, a group of 59 patients with deep venous thrombosis was analyzed but no significant differences of the PZ plasma levels were observed [20]. The conflicting reports have been a topic of subsequent correspondence without resolution.

Recently, additional studies on the same topic have been conducted. In 2002 Lopaciuk et al. demonstrated no relationship between PZ plasma levels and ischemic stroke in 99 young adults (<50 years) with a previous episode of ischemic stroke (i.e. within the least 6 months) [21]. Moreover, there were no significant differences of PZ concentration according to the etiology of the disease. In the same year, Heeb et al. investigated within 4 days the PZ levels of 154 patients (mean age: 58 years) with a first episode of ischemic stroke [22]. Low PZ levels were found to be significantly associated with ischemic stroke (OR 2.6; 95%CI 1.5-4.3) except in diabetic subjects and females. On this basis, low PZ levels have been hypothesized to be a risk factor for thrombosis.

A further step towards the identification of PZ as a risk factor for thrombosis was determined by McQuillan et al who conducted a case-control study on 173 patients with a first event of ischemic stroke. PZ was measured during the first 7 days and 3 to 6 months after the acute event [23]. Plasma levels of PZ measured within 7 days were significantly higher in cases than in the controls with an odds ratio of 1.75 (95%CI 1.0-3.07). Interestingly, however, this association was no longer detectable 3 months after the acute event. The authors concluded that elevated PZ levels were significantly associated with ischemic stroke, and that elevated levels during the acute phase could be explained by an acute-phase response. More recently, a study conducted on Sneddon's syndrome, a syndrome characterized by ischemic cerebrovascular events with or without antiphospholipid antibodies (aPL), showed a higher prevalence of low PZ values in 26 young patients (<50 years) with an aPL-negative subset of the disease compared to control subjects [24].

PZ in pathologic pregnancies

Another field of interest of PZ has been the evaluation of its role on the pathogenesis of pathologic pregnancies. In 2002, Gris et al. evaluated PZ concentrations in three groups of women with pathologic pregnancies of unexplained origin compared to a group of healthy mothers [25]. Two-hundred patients had unexplained primary recurrent miscarriage before the 8^{th} week of gestation, 200 had an unexplained primary episode of early fetal death (10^{th}-19^{th} week) and 50 had an unexplained episode of late fetal death (>20^{th} week). This study indicated a higher frequency of PZ deficiency in women with early fetal loss with respect to the other three groups, suggesting that PZ deficiency may induce

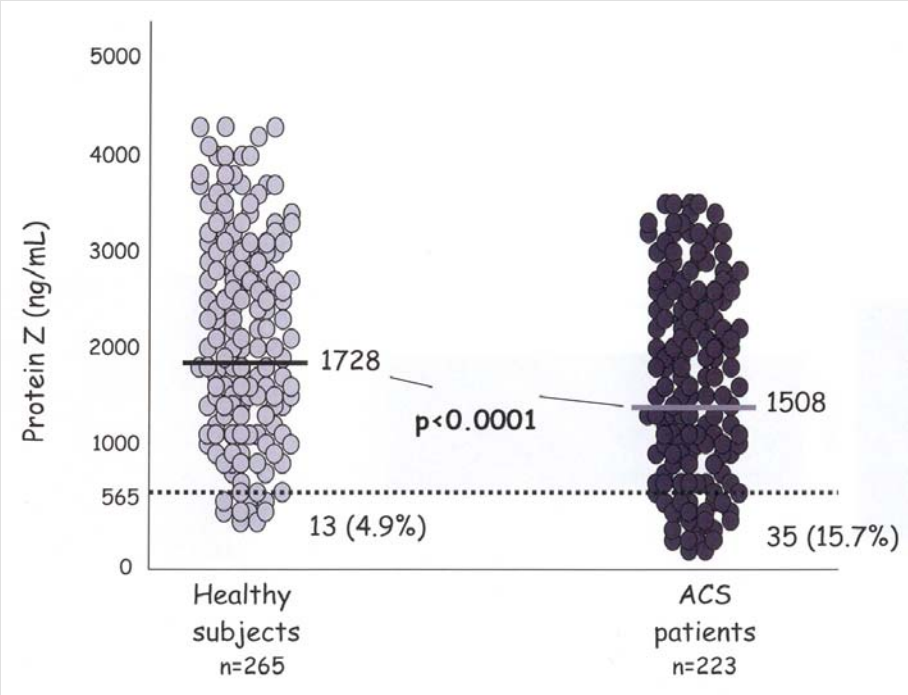

Figure 2: Protein Z levels in patients with acute coronary syndromes
Solid line = mean values of protein Z; broken line = 5th percentile of the PZ values of healthy subjects population; ACS = acute coronary syndromes

an enhanced risk of severe placental insufficiency soon after the connection of the maternal and the fetal circulation.

A novel mechanism by which PZ can induce a thrombotic complication during pregnancy has been hypothesized by the same author in 2003 [26]. Actually, Gris et al. investigated the incidence of anti-protein Z antibodies in 216 women with abnormal pregnancies, showing that high levels of these antibodies were associated with the risk of recurrent embryo losses or fetal deaths. However, anti-protein Z IgG and IgM antibodies were not correlated with plasma PZ concentrations. Furthermore, it has recently been demonstrated that PZ deficiency and positive anti-PZ antibodies were independent risk factors for a poor outcome of treated pregnancies in women with a positive thrombophilic pattern and a previous unexplained fetal loss [27].

PZ in antiphospholipid antibodies syndrome

During the last few years it has also been suggested that PZ might be associated with aPL as PZ possibly acts as potential antigen for aPL. On this basis, Steffano et al. evaluated PZ plasma levels in 53 patients with persistent aPL and in 36 healthy subjects [28]. They found low PZ levels in patients with aPL compared to the controls, especially in those with a lupus anticoagulant activity, whereas no significant difference was found in patients with anticardiolipin antibodies in comparison to control subjects. This observation was confirmed by McColl et al. who showed low PZ plasma levels in women with aPL compared to a group of female control subjects and a group of women with a previous episode of venous thromboembolism without evidence of aPL [29]. The mechanism by which PZ plasma levels could be decreased in patients with aPL is not yet known.

However, Forastiero et al. recently suggested that the PZ/ZPI complex is commonly impaired in aPL patients [30]. In this case-control study it was observed that factor Xa inactivation by PZ/ZPI was slightly delayed in the presence but not in the absence of B_2-glycoprotein-I antibodies, and that concomitant PZ deficiency in subjects with aPL could contribute to the development of the clinical features associated with the aPL syndrome.

PZ in different clinical disease states

PZ alterations have been also evaluated in association with several different clinical disorders. Increased levels of PZ were found in patients undergoing hemodialysis

procedures [31] whereas low PZ levels were found in patients with nephrotic syndrome [32]. The prothrombotic tendency associated with low levels of PZ was studied by Kemkes-Matthes et al. in 46 patients with factor V Leiden mutation and a past thromboembolic episode [33]. This study demonstrated that patients with a concomitant PZ deficiency had experienced deep vein thrombosis at an earlier age. PZ levels were also evaluated in atrial fibrillation by Marco et al., but no significant differences between patients and controls and according to aspirin therapy were observed [34]. Moreover, data on the association of low PZ levels with inflammatory chronic diseases, such as Behçet's disease and ischemic colitis have been recently reported [35, 36].

PZ and acute coronary syndromes

Recently, a possible association between PZ levels and arterial thrombosis has been demonstrated [37]. In this study the role of PZ in 223 patients with acute coronary syndromes (ACS) referred to the Coronary Intensive Therapy Unit of the University of Florence for coronary angiography and in 265 healthy subjects was evaluated. None of the patients had liver or renal dysfunction. None was under oral anticoagulant treatment. Prothrombin time was within the normal range (> 80%) in the entire study population, and patients with positivity for antiphospholipid antibodies were excluded from the study. Significantly lower PZ levels in patients (1508 ± 730 ng/mL) than in controls (1728 ± 594 ng/mL) were found (Figure 2). No significant differences of PZ levels were observed when grouping the subjects for the traditional cardiovascular risk factors (smoking habit, dyslipidemia, hypertension, diabetes and BMI).

As PZ levels below the 5th percentile (565 ng/mL) of the normal distribution of values in the control subjects was considered, a higher prevalence (15.7%) of patients with respect to healthy subjects (4.9%) was observed. Moreover, logistic regression analysis performed in order to investigate the possible relationship between PZ deficiency and the disease showed an increased risk of ACS with reduced PZ levels (OR=3.6; 99%CI 1.5-8.6; $p < 0.0001$). From the multivariate analysis, adjusted for all traditional cardiovascular risk factors and for fibrinogen, the increased susceptibility to disease with low levels of PZ was confirmed (OR=3.3; 99%CI 1.1-9.7; $p = 0.004$). Furthermore, the contemporary presence of PZ levels < 5th percentile and smoking habit led to an increased susceptibility of ACS (OR 9.5; 99%CI 2.4-37.2; $p < 0.0001$). Also performed was a stratification of patient groups according to the different manifestations of disease, acute myocardial infarction and unstable angina, and to the number of vessels with stenosis > 75% (monovessel and multivessel disease) but no difference was found between the PZ levels in both groups.

This report was the first study investigating PZ in patients with ACS. It documented that in both manifestations of ACS the PZ plasma values were significantly lower than in comparable controls, and that PZ levels lower than the 5th percentile were associated with ACS, at univariate (OR=3.6) and multivariate (OR=3.3) analysis. The observation of a higher susceptibility to ACS associated to the contemporary presence of smoking habit and low levels of PZ may stem from the wider involvement of inflammatory reactions secondary to smoking damage. Therefore, these results add further weight to the possible role of PZ in the occurrence of arterial thrombosis and stimulate the need for further studies to investigate this protein in relation to the prothrombotic state and in different phases of activity of coronary heart disease.

CONCLUSION

Protein Z is a glycoprotein with structural similarities to some coagulation factors. Many studies have attempted to determine the pathophysiological role of PZ on the occurrence of vascular diseases. Unfortunately, the clinical significance of PZ remains to be determined. Recent researches have shown that low PZ plasma levels are associated with both ischemic stroke and coronary artery disease. The explanation for the decrease of PZ levels in patients suffering from this kind of diseases is uncertain at present. Whether low PZ levels concur with the hypercoagulability state documented in these patients, thus preceding the occurrence of the ischemic event or, on the other hand, whether they are just the expression of a consumption occurring during this pathologic process needs to be demonstrated by additional studies.

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