

Pilot Screening Study of the Angiogenesis Factor Pleiotrophin (PTN) in Serum Samples from Patients of Various Disease Groups

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Abstract

Introduction: The secreted growth factor pleiotrophin (PTN) belongs to the midkine family of heparin-binding growth factors and is tightly regulated during embryogenesis. In contrast to its very limited expression in normal adult tissues, PTN protein levels are markedly increased in different tumors, with PTN showing mitogenic, chemotactic, transforming, pro-angiogenic, pro-invasive and pro-metastatic activity. However, little is known about PTN upregulation in diseases other than cancer. The aim of this study was to investigate PTN serum levels in patients with various non-malignant chronic or acute disorders, and in pregnant women, compared to healthy non-pregnant blood donors as negative control group.

Materials and Methods: PTN serum levels were determined by a sandwich ELISA.

Results: PTN serum levels were found to be significantly elevated with a p-value of <0.05 in patients with the acute non-malignant disorders, acute inflammation, acute vascular disease and acute trauma. In patients with chronic leukemia and solid tumors, and pregnancy, increased PTN serum levels were detected as well.

Conclusion: Beyond its described functions in solid tumors and in the central nervous system, these data indicate that PTN is an acute phase protein in the adult organism and that, due to its upregulation under various non-cancer conditions, special caution must be taken when exploring PTN serum levels as potential tumor marker.

Keywords: Pleiotrophin; Acute and chronic disorders; Serum samples; ELISA; Inflammation; Vascular disease

Introduction

The secreted growth factor pleiotrophin (PTN), also called heparin-binding growth-associated molecule (HB-GAM), is a cytokine belonging to the midkine family of heparin-binding growth factors [1]. PTN expression is tightly regulated during embryogenesis, with high expression in the developing nervous system, and shows very limited expression in normal adult tissues [2]. PTN is involved in the development and maintenance of the central nervous system [1-3], with protective effects upon its upregulation in neurodegenerative diseases and drug addiction [4], and in tissue regeneration [5]. In the hippocampus, PTN involvement in learning and memory has been shown [1-3]. Moreover, PTN shows mitogenic, chemotactic, transforming as well as pro-angiogenic, pro-invasive and pro-metastatic activity [6-9].

Concomitantly, a marked PTN upregulation on the mRNA and protein level was found in various human tumors and tumor cell lines [10-13]. For different tumors, increased PTN protein levels have also been demonstrated in patient serum and linked to tumor stage, thus suggesting a possible role as prognostic marker [14-19]. However, little is known about PTN upregulation in disorders other than malignant tumors, despite of its role in neurologic disorders (see above). This,

however, will be important to determine (i) whether PTN may serve as putative target molecule in pathologies other than solid tumors, (ii) if a therapeutic PTN inhibition in cancer patients may lead to possible side effects, and (iii) if PTN (upregulation) may actually serve as biomarker for the early detection of tumors or for tumor progression, without being impaired by other effects that may lead to elevated PTN levels. Concomitantly, this study focused on the analysis of PTN levels in serum samples taken from patients with various non-malignant diseases.

Materials and Methods

In a municipal hospital, serum samples were collected within 2 consecutive days from 135 in or out patients with chronic or acute disorders, and from pregnant women. In addition samples were collected from 4 patients treatment refractory with chronic leukemia (3 patients with chronic lymphatic and 1 with chronic myeloid leukemia) and 10 patients with treatment refractory solid tumors (2 patients with colon carcinoma, 2 with breast cancer, 2 with pancreatic cancer, 1 with ovary cancer, 1 with kidney cancer, 1 with prostate cancer and 1 with lung cancer) were collected. Details are given in Table 1. All blood draws had been done for medical reasons, independently from this study, and no additional blood samples were needed. Healthy non-pregnant blood donors served as normal control group. Appropriate institutional review board approval was obtained

prior to sample collection. The pleiotrophin ELISA was performed as described previously [15]. Briefly, a mouse anti-PTN monoclonal antibody (4B7) [19], diluted to 1 µg/ml in TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl), was used for coating 96-well ELISA plates at 4°C overnight. After washing with TBST (TBS+Tween-20), residual free binding sites were blocked for 1 hour at room temperature with 1% bovine serum albumin in TBST.

Serum samples were 1:1 diluted in 2X TBST and 100 µl/well of this dilution was added and incubated at room temperature for 1 hour. After washing, 100 µl/well biotinylated affinity-purified goat anti-(human PTN) secondary antibody (R&D, Wiesbaden, Germany) was added at a concentration of 500 ng/ml and incubated for 1 hour at room temperature. Following another washing step, the plate was incubated in the dark with 100 µl/well p-nitrophenyl phosphate substrate solution for two hours. Absorbance was measured in an ELISA reader at 405 nm. Recombinant human pleiotrophin (R&D, Wiesbaden, Germany) served as the standard. The lower limit of detection under these assay conditions was determined at 1.5 ng/ml which is comparable to the literature [15]. ELISA results up to 30 ng/ml were in the linear range, requiring appropriate dilution of samples when PTN levels were found to be higher. For statistical analysis, the Wilcoxon Rank-Sum Test and Student's t-Test were used. Differences in pleiotrophin serum levels with two sided p-values of <0.05 were considered as statistically significant, as done previously [19].

Disease		Number	Mean age	Male	Female
Inflammation	Acute inflammation	19	60	9	10
	Chronic inflammation	11	62	8	3
Vascular disease	Acute vascular disease	23	69.5	13	10
	Chronic vascular disease	54	71.5	29	25
Trauma		12	74	4	8
Other diagnoses	Endocrine diseases	7	64	3	4
	Pregnancy	4	31	0	4
	Shock	5	81	2	3
Negative control group		21	77	16	5
Total		156	65.5	84	72

Table 1: Overview of patients analyzed.

Results and Discussion

The mean PTN serum level in the normal control group was 2.63 ng/ml, with no value being above 5.00 ng/ml. Thus, levels ≤ 5 ng/ml were defined as normal. Patients with chronic inflammation showed pleiotrophin serum levels within the normal range (<5 ng/ml). In contrast, highly elevated PTN serum levels were observed in patients with acute disorders, in particular acute inflammation prior to therapy, soft tissue trauma and burn, and acute cardiovascular disease (Table 2 and Figure 1).

	Median pleiotrophin levels	P value
Acute inflammation	24.9	≤ 0.05
Trauma	14.9	≤ 0.05
Acute vascular disease	13.3	≤ 0.05
Pregnancy	15.6	n.s.
Endocrine diseases	10.9	n.s.
Shock	4.6	n.s.
n.s. = non significant		

Table 2: PTN serum levels in patients.

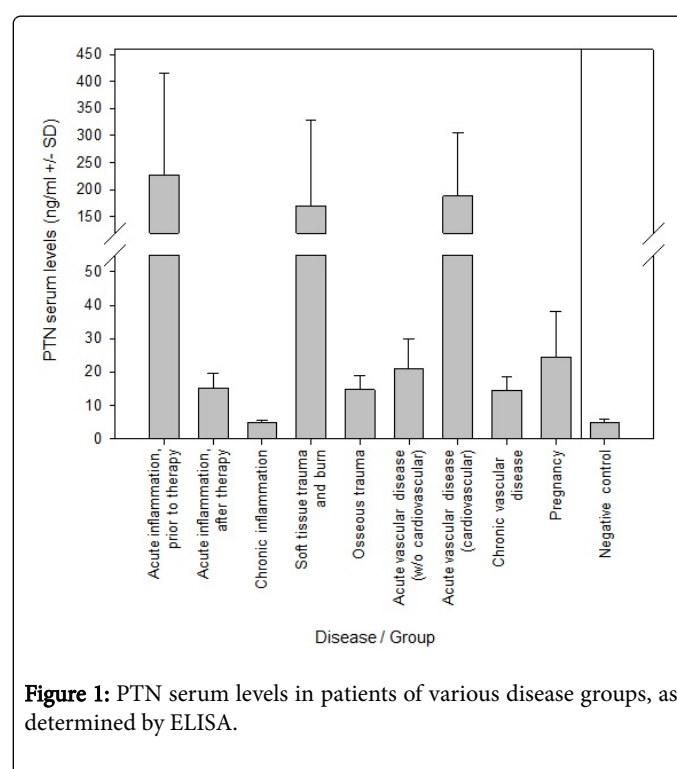


Figure 1: PTN serum levels in patients of various disease groups, as determined by ELISA.

For acute inflammation, serum samples were available from patients without treatment as well as from treated patients. One patient without treatment was diagnosed with erysipiel, four with acute gastrointestinal disorders and one with acute pneumonia. The mean pleiotrophin level in serum was 228 ng/ml, which was ~50-fold higher than in normal serum and >10-fold higher than in patients with acute inflammation after treatment (15 ng/ml). In the latter group, eight patients had been diagnosed with acute gastro-intestinal disorders and seven with pneumonia. From patients with trauma or acute vascular disorder, blood samples had been obtained within 2 days after the invasive treatment procedure. Three patients had been diagnosed with abdominal trauma and one with burns. These four patients were categorized as patients with soft tissue trauma, with their mean PTN serum level being >30-fold increased (170 ng/ml). In contrast, in the eight patients with bone traumas, PTN was only ~3-fold above normal (15 ng/ml). Acute vascular disorders were categorized as acute cardiovascular disorders and other acute vascular disorders. In the sixteen patients diagnosed with acute cardiovascular disorders, a very

high mean PTN serum level of 188 ng/ml was determined, while the seven patients with other acute vascular disorders showed only a moderate increase (21 ng/ml). Similar levels were also observed in chronic vascular disease (14.6 ng/ml) and in pregnant women (24.5 ng/ml).

Although the study was focused on non-malignant disorders serum samples were also collected from patients with treatment-refractory malignant disorders, who were at the hospital during the 2 days in which serum samples were collected. The median PTN level was 21.6 in patients with chronic leukemia and 14.52 in patients with solid tumors. The difference to the controls was statistically not significant.

This study was set to determine PTN serum levels in patients with non-malignant disorders and settings, including inflammation, trauma, vascular disorders and pregnancy. The highest PTN levels with mean serum concentrations >30-50 fold above normal were found in acute inflammation, acute ischemia and acute soft tissue trauma. These data indicate that highly elevated PTN levels are not restricted to malignant tumors but can also be found in non-malignant conditions. This is consistent with published data on the role of PTN in non-tumor settings. While in non-malignant processes PTN can exhibit a mechanism of action similar to that in cancer, such as induction of cell growth and angiogenesis in wound healing and bone repair [20,21], other functions have been identified as well in inflammation. It has been shown that PTN induces the expression of inflammatory cytokines in human peripheral blood mononuclear cells [22]. Given the PTN elevation under various inflammatory conditions or even during pregnancy, special caution must be taken when exploring or employing PTN serum levels as tumor marker.

Conclusion

Our data show that, apart from cancer, pleiotrophin levels are markedly increased in various acute non-malignant disorders that are e.g. related to inflammation. While this supports the role of PTN as acute phase protein beyond its functions as growth and angiogenesis factor in tumors, it also identifies profound caveats towards using PTN serum levels as biomarker for tumor detection and progression. Larger studies are warranted, in order to investigate the role of pleiotrophin in different non-malignant diseases and to investigate the courses of pleiotrophin serum levels from baseline to completion of treatment.

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