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Plasma Thrombospondin in Immune Thrombocytopenic Purpura

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Patients with immune thrombocytopenic purpura (ITP) rarely suffer life-threatening haemorrhages despite significant thrombocytopenia, probably because large numbers of hyperfunctioning platelets are present. Thrombospondin is a platelet α-granule protein and its plasma level may reflect platelet activation. We assessed circulating thrombospondin levels in 12 newly diagnosed ITP patients (one man; 11 women, aged 36 ± 16 years) before they were treated for ITP. Twelve healthy people (four men; eight women, aged 31 ± 11 years) acted as controls. Plasma thrombospondin concentrations were measured using enzyme-linked immunoassays. Thrombospondin concentrations tended to be higher, despite thrombocytopenia, in ITP patients (158.8 ± 28.2 ng/ml) compared with controls (120.7 ± 18.2 ng/ml). The difference was not statistically significant, but the relatively high circulating thrombospondin concentrations we observed suggest that residual platelets could be activated in ITP, thus indicating a more benign clinical course compared with aplastic thrombocytopenia.

KEY WORDS: IMMUNE THROMBOCYTOPENIC PURPURA; THROMBOSPONDIN; PLATELET ACTIVATION; PLASMA THROMBOSPONDIN CONCENTRATION

Introduction

Immune (idiopathic) thrombocytopenic purpura (ITP) is an autoimmune disorder, characterized by auto-antibody-mediated platelet destruction which results in thrombocytopenia. Both platelet destruction and inhibition of thrombopoiesis may be important in the pathogenesis of ITP,[1] which is generally considered to be a benign disease. Thrombocytopenia can be severe but few ITP patients suffer severe haemorrhage,[2,3] which may be attributed to more active residual platelets in ITP patients.[4]

Thrombospondin is a secreted trimeric glycoprotein that affects cell growth, adhesion and migration. The complex biological activity of thrombospondin is ascribed to its ability to bind to cell-surface receptors, growth factors and extracellular-matrix proteins.[5] Like other platelet α-granule proteins (e.g. fibrinogen, albumin and von Willebrand factor) thrombospondin is synthesized in newly formed circulating platelets.[6] A high percentage of young, activated, compound platelets is present in peripheral blood taken from ITP patients and, like P-selectin, plasma thrombospondin levels may reflect platelet activation in patients with thrombocytopenia.[4,7,8]

In the present study, plasma concentrations of thrombospondin were investigated at the initial clinical presentation of ITP, to establish whether this adhesive molecule is a marker of

platelet activation[9] during the pathobiology of the disease.

Patients and methods

PATIENTS

Twelve patients (one man, 11 women; mean age 36 ± 16 years; range, 18 – 76 years) with newly diagnosed ITP were included in this cross-sectional study. Blood samples were obtained from patients at diagnosis, before any ITP treatment was initiated. Platelet counts in two ITP patients ranged between 10 000/mm^3 and 20 000/mm^3 but counts in all other patients were below 10 000/mm^3. Twelve healthy adult subjects (four men, eight women; mean age 31 ± 11 years; range, 18 – 55 years) served as the control group.

BLOOD SAMPLING AND ASSAYS

Venous blood samples were drawn from all subjects into 3.8% trisodium citrate without venous occlusion to determine plasma thrombospondin levels. Plasma was obtained by immediate centrifugation of the samples for 20 min at 3000 ***g*** and then stored at –70 ºC until assayed. Thrombospondin was assayed by sandwich type enzyme-linked immunoassay (ELISA; Asserachrom™ Thrombospondine, Diagnostica Stago, France). Each sample was studied in duplicate.

STATISTICAL ANALYSIS

The Mann–Whitney *U*-test was used to compare plasma thrombospondin levels in patients and controls. Results were expressed as mean ± SD. Statistical significance was assigned to *P*-values < 0.05.

Results

Demographic and laboratory characteristics of patients and control groups are shown in Table 1. The mean plasma thrombospondin concentration was 158.8 ± 28.2 ng/ml (range, 95 – 195 ng/ml) in patients with ITP and 120.7 ± 18.2 ng/ml (range, 28 – 212 ng/ml) in the control group. The higher thrombospondin levels in ITP patients did not reach statistical significance.

Discussion

It has been suggested that residual platelets are activated in ITP,[4] which is a condition that has a relatively benign clinical course compared with other thrombocytopenias. Levels of other α-granule proteins, which are also known to reflect platelet activity, have been reported to be elevated in ITP patients,[4,7] which is consistent with our observation. Generally, however, ITP patients do not experience life-threatening haemorrhages, despite having severe thrombocytopenia.[2,3] Augmented residual platelet function in ITP patients has been explained by high interleukin 6 (IL-6) and P-selectin concentrations during thrombocytopenia.[4,7,10] We have also found significantly ($P < 0.01$) lower than normal plasma thrombospondin concentrations in thrombocytopenia secondary to acute leukaemias.[8] All ITP patients in the present study had increased bone-marrow megakaryocytes and, even though they were thrombocytopenic, their pre-treatment plasma thrombospondin concentrations tended to be raised. This implies that circulating thrombospondin is relatively higher in ITP patients with thrombocytopenia who have increased bone-marrow megakaryocytes, compared with thrombocytopenias associated with megakaryocyte deficiency. Thrombospondin has been considered a marker for platelet activation.[9] This glycoprotein regulates the multimeric size (and therefore haemostatic activity) of von Willebrand factor, a multimeric protein that mediates platelet adhesion to sites of vascular injury.[11]

Immune thrombocytopenic purpura is a heterogenous disorder in which platelet count and function can vary greatly between patients.

TABLE 1:
Age, sex and plasma levels of thrombospondin in patients with immune thrombocytopenic purpura and healthy controls

No.	Age (years)	Sex	Thrombospondin (ng/ml)
Patient 1	40	Female	140
Patient 2	32	Male	171
Patient 3	35	Female	148
Patient 4	61	Female	195
Patient 5	18	Female	143
Patient 6	43	Female	177
Patient 7	25	Female	161
Patient 8	18	Female	142
Patient 9	29	Female	192
Patient 10	76	Female	95
Patient 11	29	Female	155
Patient 12	37	Female	186
Mean ± SD	36 ± 16		158.8 ± 28.2
Control 1	34	Female	38
Control 2	55	Female	28
Control 3	25	Male	111
Control 4	33	Female	195
Control 5	20	Male	126
Control 6	24	Female	176
Control 7	42	Female	212
Control 8	23	Male	56
Control 9	26	Female	144
Control 10	18	Female	185
Control 11	25	Male	73
Control 12	44	Female	105
Mean ± SD	31 ± 11		120.7 ± 18.2

There are many unknown factors in the pathogenesis of ITP: immune platelet destruction and reactive megakaryocytopoiesis are key characteristics of this disease, but there are indications that abnormal mega-karyocytopoiesis and ineffective thrombopoiesis may also be significant.[1,12] In addition, thrombospondin is synthesized in newly formed circulating platelets, which could reflect its role in platelet activation.[6,9] The role of thrombospondin, and the effects of ITP treatment on thrombospondin levels during the biological and clinical course of the disease should be investigated further.

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