Relativity study of thrombopoietin and transforming growth factor-β_1 in children with idiopathic thrombocytopenic purpura

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Background: **Thrombopoietin (TPO), the major hormone controlling platelet production, and transforming growth factor-beta1 (TGF-β_1), a kind of growth suppressor acting on megakaryopoiesis, have been measured in thrombocytopenias with discordant results. The aim of this sudy was to explore the relationship between TPO, TGF-β_1 and idiopathic thrombocytopenic purpura (ITP) in children.**

Methods: **TPO and TGF-β_1 levels in the serum and bone marrow of 45 children with ITP were measured using the enzyme-linked immunosorbent assay (ELISA) method. Twelve healthy children were enrolled as controls.**

Results: **The serum level of TPO was higher in ITP children than in the controls, but no significant difference was observed between them ($P > 0.05$). The serum level of TGF-β_1 was significantly higher in ITP children than in the controls ($P < 0.01$). The serum level of TPO after therapy was lower than that before treatment, but there was no significant difference between them. Some ITP children having a poor response to steroids had a significantly higher serum TPO level than those having good response and the controls. The bone marrow level of TPO was higher in ITP children than in the controls and also higher than the serum level. There was a positive correlation between the serum level and bone marrow level of TPO ($r = 0.99$, $P < 0.01$). The bone marrow level of TGF-β_1 was higher than the normal serum level. There was a positive relation between serum level and bone marrow level of TGF-β_1 ($r = 0.80$, $P < 0.01$). Before treatment, ITP children had a low platelet count but a high level of TPO. After treatment, when the platelet count increased, the level of TPO reduced. There was a negative correlation between TPO and platelet count ($r = -0.649$, $P < 0.05$) and between TPO and megakaryocyte count ($r = -0.519$, $P < 0.05$).**

Conclusions: **In the pathogenesis of ITP, TGF-β_1 is a feedback regulating factor. The levels of TPO and TGF-β_1 in serum and bone marrow could help evaluate ITP children's conditions, estimate prognosis, and enact treatment regimens.**

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Introduction

The injury or dysfunction of megakaryocytes will lead to thrombocytopenia.[1,2] During the maturity of megakaryocytes, cytokines play important roles. The stimulating cytokines include thrombopoietin (TPO), interleukin (IL)-3, IL-6 and IL-11,[3-8] whereas the inhibiting cytokines include transforming growth factor-beta1 (TGF-β_1), heparin-combined protein, polypeptide and others.[9,10] In this cytokine network which affects the maturity of megakaryocytes, TPO and TGF-β_1 are the key modulating factors. Idiopathic thrombocytopenic purpura (ITP) is the most common hemorrhagic disease in children.[11,12] The aim of this study was to investigate the relationship between TPO, TGF-β_1 and thrombocytopenic disease immunologically and molecule biologically for the diagnosis and treatment of ITP children.

Methods

Patients and treatment

We studied 45 children with acute ITP from January

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2000 to December 2001, of whom 26 were boys and 19 girls, aged from 5 months to 12 years (mean 5.8 years). These patients were treated with prednisone at a dose of 1.5-2.0 mg · kg^{-1} · d^{-1}, tid, po. When their platelet count was normal, the dose was reduced gradually. The period of treatment lasted 4-6 weeks. Twelve healthy children served as controls, 7 boys and 5 girls, aged from 10 months to 11 years (mean 6.5 years).

Assay of TPO and TGF-β_1

The specimen of peripheral venous blood was taken at early morning on an empty stomach and bone marrow through sternal puncture. The levels of TPO and TGF-β_1 in serum and bone marrow were assayed using the eneyme-linked immunosorbent assay (ELISA). The kits of TPO and TGF-β_1 were purchased from Jingmei Biology Company, Beijing, China. The number of peripheral blood platelets and megakaryocytes in bone marrow slides were counted at the same time.

Statistical analysis

All analyses were performed using Statistical Package for the Social Science Software (SPSS, Inc., Chicago, USA). A P value of less than 0.05 was considered statistically significant.

Results

The serum levels of TPO and TGF-β_1 before therapy in ITP patients and controls

The serum level of TPO was higher in the ITP children than in the controls, but no significant difference was noted (Table 1). The serum level of TGF-β_1 was significantly higher in the ITP children than in the controls.

Table 1. The serum cytokines concentration before therapy in ITP children and controls (mean ± SD, μg/L)

Group	n	TPO	TGF-β_1
Controls	12	21.16 ± 1.78	46.17 ± 4.57
ITP patients	45	24.56 ± 5.98*	89.98 ± 2.87**

Compared with controls, *: $P>0.05$, **: $P<0.01$.

The relationship between effect of steroids therapy and cytokines mass concentration

The serum level of TPO in ITP patients was slightly lower after therapy (23.44 ± 6.41 μg/L) than before treatment. The serum TPO level in ITP patients with a poor response to steroids was significantly higher (35.29 ± 3.29 μg/L) than in those with a good response (21.67 ± 1.47 μg/L) and the controls.

After treatment, the serum TGF-β_1 mass concentration (89.42 ± 2.60 μg/L) was still significantly higher than normal or was not decreased significantly than that before treatment.

The TPO and TGF-β_1 levels of bone marrow and serum before treatment

The TPO and TGF-β_1 levels of serum and bone marrow in 15 ITP children and 6 controls were detected (Table 2). There was a positive correlation between serum level and bone marrow level of TPO ($r=0.99$, $P<0.01$). There was a positive relation between serum level and bone marrow level of TGF-β_1 ($r=0.80$, $P<0.01$)

Table 2. Comparison between TPO, TGF-β_1 levels of ITP patients and controls (mean ± SD, μg/L)

Group	n	TPO		TGF-β_1	
		Bone marrow	Serum	Bone marrow	Serum
ITP	15	47.63 ± 8.09*#	29.53 ± 1.68	150.90 ± 3.76*#	84.53 ± 3.22
Controls	6	23.56 ± 4.61	21.16 ± 1.79	91.86 ± 5.47	49.17 ± 4.26

Compared with normal, *: $P<0.01$; compared with serum, #: $P<0.01$.

The correlation analysis between the level of serum TPO and blood platelet count, megakaryocyte count in bone marrow

Before treatment, ITP children had a low platelet count (38 ± 23 × 10^9/L) but a high level of TPO. After treatment, when platelet count increased (133 ± 83 × 10^9/L, the level of TPO reduced. A negative correlation was observed between TPO and platelet count ($r=-0.649$, $P<0.05$) and between TPO and megakaryocyte count in bone marrow ($r=-0.519$, $P=0.041$). The serum TPO level was significantly higher in some children with a poor response to steroids than in those with a good response. The megakaryocyte count (131.14 ± 120.06/slide) was lower in the former than in the latter (389.44 ± 317.60/slide).

Discussion

TPO as one of the most potent stimulators of platelet production is a kind of glycoprotein mainly synthesized by hepatocyte, proximal and distal renal tubule cell, and marrow stromal cell. It has been reported that TPO can startup the formation of megakaryocyte clone, stimulate maturity of megakaryocyte, and support generation of functional platelet when megakaryocyte is cultured *in vitro* with combined cytokines.[13-15]

The level of TPO is mainly modulated by peripheral blood platelet and bone marrow megakaryocyte. This modulation depends on the intake and disruption of C-MPL, the receptor of TPO in the platelet. The intake of TPO is reduced when serum platelet count is

low, then the serum level of TPO rises, thus promoting the proliferation, differentiation, maturation of megakaryocyte and the generation of platelet.[16-18] Eventually, this will result in accelerating the intake and degradation of TPO to gain a new balance as a negative feedback effect.

In the present study, we found that before treatment, ITP children had a low platelet count but a high level of TPO. After treatment, however, when the platelet count increased, the level of TPO decreased. There was a negative correlation between TPO and platelet count. The results of this study demonstrated that the number of platelets is an important factor modulating the serum level of TPO. This study also showed a negative correlation between TPO and megakaryocyte count.

TGF-β_1 as a polypeptide growth factor has extensive biological effects including immunosuppression, anti-inflammation, and injury repair.[19,20]

TGF-β_1 negatively modulates hematopoiesis. Inhibition of CFU-Meg by TGF-β_1 has been previously proved by several investigators. By inhibiting the karyokinesis of megakaryocyte, TGF-β_1 suppresses the maturity of megakaryocyte and generation of platelet.[21,22] Our study showed that the serum level of TGF-β_1 in ITP children was significantly higher than that of controls because TGF-β_1 exists in megakaryocyte and α granules of platelet, and the disruption of megakaryocyte and platelet may result in the release of TGF-β_1.

Sakamaki et al[23] discovered that TGF-β_1 not only directly inhibited the formation of CFU-Meg, but also acted on megakaryocyte indirectly through modulating the TPO formation of stromal cells. Bone marrow stromal cell was affected by increased TGF-β_1 and produced plentiful TPOmRNA, which was translated into TPO. Hematopoietic stem cells were affected by TPO and differentiated into megakaryocyte. TGF-β_1 combined with the surface receptor of megakaryocyte to inhibit the function of megakaryocyte. A feedback modulating cycle was then formed.

The relation of steroids therapy to the levels of TPO and TGF-β_1 proved that the assay of TPO is important for assessing the prognosis of ITP patient. If the result of steroids treatment is not satisfactory, other management should be adopted to avoid the repeated use of steroids and their side-effects.[24]

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