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PDGF-A, PDGF-B, TGFβ, and bFGF mRNA Levels in Patients With Essential Thrombocythemia Treated With Anagrelide

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Plasmatic levels of PDGF-AB, TGFβ$_1$, and bFGF are increased in patients with essential thrombocythemia (ET) while intraplatelet levels are low for PDGF, normal for TGFβ, and elevated for bFGF. To evaluate the contribution of gene expression to the dysregulated cytokine levels, we studied platelet PDGF-A, PDGF-B, TGFβ$_1$, and bFGF mRNA in ET patients before and during anagrelide treatment. We found decreased PDGF-A and PDGF-B, increased TGFβ$_1$, and normal bFGF mRNA levels. During treatment, mRNA levels remained decreased for PDGF-A, were increased for PDGF-B and normal for TGFβ$_1$. In untreated patients, protein expression of PDGF paralleled its mRNA levels while different patterns of RNA and protein were found for TGFβ$_1$ and bFGF. Am. J. Hematol. 78:155–157, 2005. © 2005 Wiley-Liss, Inc.

Key words: PDGF; TFGβ; bFGF; essential thrombocythemia; anagrelide

INTRODUCTION

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder characterized by thrombocytosis and increased number of megakaryocytes in the bone marrow.

Platelet-derived growth factor (PDGF), transforming growth factor β$_1$ (TGFβ$_1$), and basic fibroblast growth factor (bFGF) are synthesized by megakaryocytes and stored in platelet α-granules. These cytokines have several biological effects, including the development of myelofibrosis and the regulation of megakaryopoiesis [1,2].

We previously showed that plasma levels of these cytokines were increased in ET patients while intraplatelet levels of PDGF were low, normal for TGFβ, and high for bFGF [3]. The aim of this study was to evaluate platelet mRNA levels of PDGF-A, PDGF-B, TGFβ$_1$, and bFGF in ET patients before and during anagrelide treatment in order to assess the contribution of cytokine gene expression to the dysregulation of protein levels.

DESIGN AND METHODS

We studied nine patients with ET diagnosed according to the PVSG criteria [4] before and during anagrelide treatment; median age, 50 years; median follow-up, 6 years. Written consent was obtained. RNA was isolated by TRIzol (Gibco BRL, Grand Island, NY) [5] from leukocyte-depleted platelets, leukocyte/platelet ratio

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$< 1{:}10^6$. cDNA was synthesized using SuperScript Preamplification System (GIBCO). Semiquantification of PDGF-A, PDGF-B, TGFβ_1, and bFGF mRNA was performed by RT-PCR after coamplification and normalization with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or β_2-microglobulin (β_2M) with the following upstream/downstream primers: PDGF-A, 5′-CCTGAC GTATTCCACCT-3′/5′-CTGGAGATAGACTCCGT-3′; PDGF-B, 5′-CCGGAGTCGGCATGAA-3′/5′-TTT CTCACCTGGACAGGTCG-3′; TGFβ_1, 5′-TAAAAG TGGAGCAGCACGTG-3′/5′-GAACCCGTTGATG TCCACTT-3′; bFGF, 5′-AAGAGCGACCCTCA CAT CAA-3′/5′-TGCCACATACCAACTGGTGT-3′; GAP DH, 5′-TGCACCACCAACTGCTT-3′/5′-TACTCCT TGGAGGCCAT-3′; β_2M, 5′-AAAGATGAGTAT GCCTGCCG-3′/5′-ACTCAATCCAAATGCGGC-3′. Conditions were set to ensure a linear range of amplification. A ratio between each cytokine and its internal control was calculated by densitometry. Each result represents the mean of three measurements.

Statistical Analysis

Data are expressed as median and range. Paired samples from each patient obtained before and during treatment and a normal control were assayed in the same experiment and analyzed by the Newman-Keuls test.

RESULTS

Decreased PDGF-A mRNA was observed before treatment compared to controls; PDGF-A/GAPDH 0.28 (range 0.25–0.44) and 0.59 (range 0.35–2.15), respectively, $P = 0.013$. These levels increased slightly during treatment, 0.37 (range 0.12–0.55), although they remained lower than in controls, $P = 0.009$ (Fig. 1A).

PDGF-B mRNA levels before treatment were lower than in controls; PDGF-B/GAPDH 0.35 (range 0.23–0.85) and 0.88 (range 0.47–1.36), respectively, $P = 0.009$, and increased during treatment, 1.33 (range 0.70–2.01), patients versus controls, $P = 0.044$ (Fig. 1B).

TGFβ_1 mRNA levels in patients were increased compared to controls, TGFβ_1/β_2 M 0.86 (range 0.60–1.36) and 0.53 (range 0.29–0.67), respectively, $P = 0.009$, and returned to normal during remission, 0.59 (range 0.27–1.23), $P =$ NS (Fig. 1C).

Basic FGF mRNA levels in untreated patients did not differ from controls, bFGF/GAPDH 0.84 (range 0.45–1.48) and 1 (range 0.39–2.10), nor during treatment, 1.12 (range 0.60–2.76), $P =$ NS (Fig. 1D).

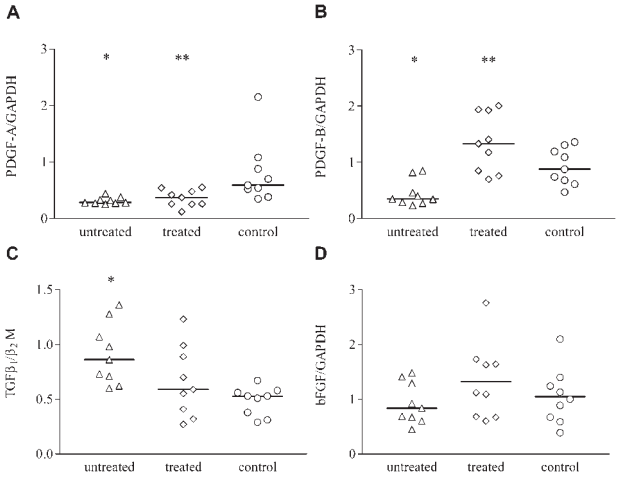

Fig. 1. Platelet gene expression levels of PDGF-A, PDGF-B, TGFβ_1, and bFGF. Semiquantification of gene expression of platelet mRNA was performed by PCR after coamplification and normalization with a housekeeping gene. The expression level for each transcript was evaluated by calculating the ratio between the PCR product of each cytokine and the internal control by densitometric scanning. (Δ) Untreated patients; (◊) patients during treatment with anagrelide; (O) normal controls. Median values are represented as horizontal lines. (A) Ratio of PDGF-A/glyceraldehyde 3-phosphate dehydrogenase (GAPDH). *Untreated versus controls, $P = 0.013$; **during treatment versus controls, $P = 0.009$. (B) Ratio of PDGF-B/GAPDH. *Untreated versus controls, $P = 0.009$; **during treatment versus controls, $P = 0.044$. (C) Ratio of TGF$\beta_1\beta_2$ microglobulin. *Untreated versus controls, $P < 0.009$. (D) Ratio of bFGF/GAPDH.

DISCUSSION

We previously reported that platelet PDGF-AB protein levels were decreased in ET patients [3]. Here, we found low mRNA intraplatelet levels of PDGF-A and PDGF-B, which may contribute to the decreased PDGF protein. However, platelet release of α-granule contents secondary to in vivo platelet activation could be another mechanism [6,7]. During treatment, PDGF-A mRNA levels rose slightly without reaching normal values while PDGF-B mRNA increased over control levels. The rise in PDGF mRNA together with the reduction of PDGF α-granule release [7] represent potential explanations leading to the normalization of PDGF protein during treatment [3].

Although we previously showed normal platelet TGFβ_1 protein expression, mRNA levels were increased. Release of TGFβ_1 to plasma may explain this discrepancy. However, dysregulation in protein translation cannot be ruled out. During anagrelide treatment, TGFβ_1 mRNA decreased to normal. Because TGFβ_1 plays a pivotal role in the pathogenesis of myelofibrosis, normalization of mRNA levels during treatment is of interest regarding that anagrelide does not stimulate progression to fibrosis in ET [8].

We previously showed that platelet bFGF protein levels were increased in ET patients. However, normal

mRNA bFGF suggests protein rise cannot be attributed to increased transcription. Dysregulation in protein translation could be another possibility.

Anagrelide reduces megakaryocyte size and ploidy and is effective in reducing platelet counts in patients with ET [9]. The mechanisms underlying the changes in mRNA levels during anagrelide treatment are not clear. Inhibition of megakaryocyte maturation, normalization of platelet counts, or modulation of intracellular pathways that regulate cytokine expression represent likely explanations.

In conclusion, in untreated patients, protein expression of PDGF paralleled its mRNA levels while different patterns of RNA and protein levels were found for $TGF\beta_1$ and bFGF.

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