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Research Article

Essential Thrombocythemia and Fibroblast Growth Factor-2: Role of Anagrelide

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Abstract

Evolution into myelofibrosis is part of the natural history of the essential thrombocythemia (ET). The myelosuppressive therapy in ET may itself increase the risk of transformation to myelofibrosis. The challenge in treating ET is to prevent this risk. It is reported that activated thrombocythemic platelets release fibroblast growth factor-2 (FGF-2). Anagrelide (ANA) is a myelosuppressive agent that inhibits the platelet function. The major concern regarding ANA is whether it do not increase the putative risk of transformation to myelofibrosis. In this study we report the results of a randomized group of patients with ET in treatment with ANA that after a follow-up of 5 years showed a reduction in the myelofibrosis. A likely explanation for this finding is the broader activity of ANA which also affects the platelet function.

Keywords: Essential Thrombocythemia; Platelet Activation; FGF-2; Myelofibrosis; Anagrelide

Fibrosis and myeloproliferation complicate the essential thrombocthemia (ET) [1]. Platelet endothelial activation releases fibroblast growth factor-2 (FGF-2) [2,3] inducing fibrosis and myeloproliferation [4]. Anagrelide (ANA) is an platelet activation inhibitor cytoreductive [5,6]. Aim of this investigation is to evaluate the effect of ANA on FGF-2 levels.

Method Section

Platelets, PF4, tissue factor pathway inhibitor (TFPI), tissue factor (TF) and von Willebrand factor (vWF), as markers of platelet endothelial activation , FGF-2, reticulin and white blood cells (WBC) and haemoglobin (Hb) as indicators of fibrosis and myeloproliferation, respectively, were evaluated in randomly enrolled ET patients on ANA. All measurements were performed at the start of ANA and when hematologic complete response, defined as platelets < 400x109/L for more than 3 months was experienced. This study involved 126 patients (main group) (78 man, 48 women; mean age, 52 years; range, 28-77 years) with ET according to WHO [7]. The informed consent according to the Helsinki Protocol was obtained from all patients and the study was approved by the Institutional Ethics Committee. The mean duration of disease was 9 years (range, 4-21 years). None of them had splenomegaly or mutational status. After a mean time from diagnosis of 4 years, all patients started ANA. ANA was initially administered at a dose of 0.5 mg/day. Subsequently, the dose was increased by 0.5 mg/day every week until the platelet count had decreased to below 400x109/L. The average maintenance dose was 2.0 mg/day (range 0.5-6 mg/day). Therapy was well tolerated. All patients received aspirin. None of the patients had acquired or inherited thrombophilia or previous thrombosis. All had bone marrow biopsy at diagnosis and underwent follow-up trephines every 2 years. Sixty subjects (control group) with reactive thrombocytosis age- and sex-matched to the patients served as con-

trols. Platelets, WBC and Hb were determined on the Sysmex XE-21300 (Dasit, Milan, Italy). PF4, FGF-2, TFPI and TF were measured by enzyme-linked immunosorbent assay (Diagnostica Stago, Boehringer Mannheim, Mannheim, Germany; American Diagnostica Inc., Greenwich, CT; Quantikine Human Immunoassay, R&D Systems, Minneapolis, MN, USA). vWF was measured by immunoturbidimetric assay (Dade Behring Marburg GmbH, Marburg, Germany). In order to avoid platelet activation, blood was collected in special iced-tubes (Diatube H. Diagnostica Stago) which contain platelet antiaggregants. Considering that FGF-2 may be produced by platelets, we adjusted FGF-2 per platelet (FGF^{PLT} $pg/10^6$). The statistical methods were 2-tailed Student *t* test and Pearson or Spearman tests (SPSS 17.0; SPSS Chicago, IL, USA).

Result Section

Main Group

Bioclinical data – Pre-treatment

Platelets (1000±300x109/L), PF4 (130±46 IU/ml), TFPI (160±60 ng/ml), TF (230±290 pg/ml), vWF (20±7.0 %), FGF^{PLT} (0.09±0.09 $pg/10^6$), reticulin 1.2, WBC (10.0±2.4x10^9/l), Hb (13.5±1.4 g/dl) (Table).

Control Group

Bioclinical data

Platelets (500±25x109/L), PF4 (4.0±2.0 IU/ml), TFPI (95±10 ng/ml), TF (4.3±2.5 pg/ml), vWF (80±18 %), FGF^{PLT} (0.01±0.001 $pg/10^6$), reticulin 0, WBC (5.0±1.0x109/L), Hb (12±0.4 g/dl) (Table).

Comparison of mean values between main group vs control group was p<.0001 (Table).

Main Group

Bioclinical data – Post-treatment

Platelets (380±50x10^9/L), PF4 (8.0±3 IU/ml ng/ml), TFPI (105±50 ng/ml), TF (9±1 pg/ml), vWF (90±32 %), FGF^{PLT} (0.01±0.0 pg/106), reticulin 0, WBC (7.0±1.0 x10^9/L), Hb (12.4±1.1 g/dl) (Table).

Both pre- and post-ANA measurements were serially repeated and showed concordance. Trephines were assessed by three hematopathologists with knowledge only of the age and sex of the patient. A positive correlation there was between PF4 and platelets (p<.0001) and PF4 and TFPI and TF and vWF (p=.005 and p=0.014 and p=<.0001, respectively) and between PF4 and FGF^{PLT} (p<.0001) and between TFPI and TF and vWF and FGF^{PLT} (p=0.003 and <.0001 and p<.0001, respectively), and between FGF^{PLT} and reticulin (p<.0001), and between FGF^{PLT} and WBC and Hb (p=0.002 and p=0.004).

Discussion

In a cohort of 126 ET patients we performed a correlation study between platelet endothelial activation, fibrosis and myeloproliferation. Activated platelets release PF4 [8]. Platelet activation induces endothelial activation [9] and release of TFPI [10], TF [11], and vWF [12]. Platelet endothelial activation releases FGF-2 [4,13]. FGF-2 is a fibrogenic and myeloproliferative factor [4,13]. On this basis, we evaluated our data in ratios PF4/platelet, PF4/TFPI, PF4/TF, PF4/vWF, PF4/FGF-2, TFPI/FGF-2, TF/FGF-2, vWF/FGF-2, FGF-2/reticulin, FGF-2/WBC, FGF-2/Hb.

Table. Bioclinical Data and Statistics of ET patients.

	Main Group Pre - ANA	Control Group	Statistics P	Main Group Post - ANA	Statistical Variables P
Plt (x10^9/L)	1000±300	500±25	<.0001	380±50	PF4/Plt <.0001
PF4 (IU/ml)	130±46	4.0±2.0	<.0001	8.0±3	PF4/TFPI 0.005
TFPI (ng/ml)	160±60	4.0±2.0	<.0001	105±50	PF4/TF 0.014
TF (pg/ml)	230±290	4.3±2.5	<.0001	9±1	PF4/vWF <.0001
vWF (%)	20±7.0	80±18	<.0001	90±32	PF4/FGF^{PLT} .0001
FGF^{PLT}(pg/10^6)	0.09±0.09	0.01±0.001	<.0001	0.01±0.0	TFPI,/$FGF^{PLT,}$ 0.003
Reticulin	1.2±0.1	0	<.0001	0	TF/ FGF^{PLT} <.0001
WBC (x10^9/L)	10.0±2.4	5.0±1.0	<.0001	7.0±1.0	vWF,/FGF^{PLT} <.0001
Hb (g/dL)	13.5±1.4	12±0.4	<.0001	12.4±1.1	FGF^{PLT}/reticulin <.0001
					FGF^{PLT} /WBC .002
					FGF^{PLT}/Hb .004.

Sahni et al. [3] reported elevated FGF-2 and increased endothelial function. We reported normal FGF-2 and normal platelet endothelial function after ANA. Campbell et al. [14] observed no increase in biopsy associated reticulin after HU. We found no FGF-2 associated reticulin after ANA. Passamonti et al. [15] found a fibrotic risk at 5 years after HU. We found no fibrotic risk at 5 years after ANA. In the report of Burger et al [13] CD34 count was a marker of increased FGF-2 associated myeloproliferation. In our report, WBC and Hb were markers of normalized FGF-2 associated myeloproliferation. These results are novel data not previously reported about the effect of ANA on FGF-2 and confirm previous reports about the effect of ANA on platelet endothelial activation.

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