ORIGINAL ARTICLE

Observational retrospective study of vascular modulator changes during treatment in essential thrombocythemia

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Essential thrombocythemia (ET) patients are at risk of developing thrombotic events. Qualitative platelet (PLT) abnormalities and activation of endothelial cells (ECs) and PLTs are thought to be involved. Microparticles (MPs) can originate from PLTs (PMPs), ECs (EMPs), or red cells (RMPs). Previous studies have indicated that MPs contribute to ET pathophysiology. Endothelial modulators (eg, nitric oxide (NO), adrenomedullin (ADM), and endothelin-1 (ET-1)) are also involved in the pathophysiology of this condition. We hypothesized that treatments for reducing PLT count might also indirectly affect MP generation and endothelial activity by altering endothelial modulator production. The rationale of this study was that hydroxyurea (HU), a cytostatic drug largely used in ET, induces the production of a potent vasoactive agent NO in ECs. An observational retrospective study was designed to investigate the relationship between MPs, NO, ADM, and ET-1 in ET patients on treatment with HU, anagrelide (ANA), aspirin (ASA), and a group of patients before treatment. A total of 66 patients with ET diagnosis: 18 on HU + ASA, 15 on ANA + ASA, 19 on ASA only, and 14 untreated patients, and 18 healthy controls were included in this study. Blood samples were analyzed for MP (absolute total values) and functional markers (percentage values) by flow cytometry. PLT-derived MPs were studied using CD61, CD62P, CD36, and CD63, whereas endothelial-derived MPs were studied using CD105,

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CD62E, and CD144. Endothelial modulator markers (NO, ADM, and ET-1) were measured by ELISA. Total MP count was higher in the group treated with ANA + ASA ($P < 0.01$). MP markers modified in ET patients returned to levels of healthy controls following treatment, in particular, in patients on ANA treatment. NO and ADM values were higher in the HU group ($P < 0.001$). HU and ANA treatment also affected MP production in a cell origin–specific manner. HU and ANA, although acting via different pathways, have similar final effects. For instance, HU causes vasodilatation by increasing NO and ADM levels, whereas ANA impairs vasoconstriction by reducing ET-1. In conclusion, therapy with HU cytostatic drugs and ANA can reduce PLT count in ET, and also affect endothelial modulatory agents, with HU sustaining vasodilation and prothrombotic MP concentration, whereas ANA decreases vasoconstriction. (Translational Research 2017;■:1–13)

Abbreviations: ■■■ = ■■■

INTRODUCTION

Essential thrombocythemia (ET) is a hematological disease currently classified within the Philadelphia-negative myeloproliferative neoplasms (MPNs) by the World Health Organization (WHO 2008). For a long time, ET has remained neglected by physicians because formulation of an ET diagnosis was never clear. Specific disease markers (apart from an elevated platelet [PLT] count) have been lacking, allowing the possibility of a diagnosis of reactive thrombocythemia. Due to the discovery of a pathognomonic mutation of the tyrosine kinase JAK2 gene (the JAK2 V617mutation), ET is undergoing a new renaissance in research. Encouraging results obtained using tyrosine kinase inhibitors for the treatment of chronic myeloid leukemia have led to the development of similar drugs for treating ET: the JAK2 inhibitors. These drugs are very promising and change the course of this disease and other similar diseases. Other genetic mutations affecting MPNs have been recently identified including the MPL5 and the CARL mutations,[1,2] and it is likely that future drugs will target these new mutations. The discovery of these mutated genes has helped clinicians to improve ET diagnosis. Nevertheless, the management of ET patients and their disease course remains problematic. In particular, it is still unclear why some patients develop thrombotic or, more rarely, hemorrhagic events. Our study focused on ET patients carrying the JAK2 V617F mutation. Interestingly, JAK2 V617F-induced constitutive activation of JAK2 occurs within endothelial cells (ECs).[3] This suggests that ECs also participate in the pathophysiology of ET and therefore, support our hypothesis that drugs aimed at controlling the risk of developing a thrombotic/hemorrhagic event might also target ECs. Plasma levels of soluble thrombomodulin and P selectin are elevated in JAK2 V617F ET patients. The increased expression of adhesion molecules (selectins) on endothelial cell wall and on circulating cells is one of the triggers of thrombotic events in ET.[4] Current guidelines recommend the use of cytostatic agents and antiaggregant agents. Anagrelide (ANA), an anti-PLT agent, has also been recommended in ET low-risk patients or in those resistant to hydroxyurea (HU). However, the exact disease pathophysiology sustaining thrombotic/hemorrhagic events is still unknown. Similarly, we do not fully understand how ANA and HU reduce the rate of thrombotic or hemorrhagic events. Most physicians simply assume that the risk of developing a thrombotic event directly correlates with the PLT number; however, a linear correlation between PLT number and thrombotic events has never been proven in ET. The risk of developing thrombotic events is also linked to the number of circulating white blood cells[5] and to the prolonged exposure to uncontrolled high blood cell count fluctuation.[6-10] In addition, when PLT levels are very high (usually $> 1{,}200{,}000/\mu L$), patients tend to have a bleeding—rather than a thrombotic—diathesis. In ET, the excess circulating PLTs tend to bind to the ultra-large von Willebrand fragments (ULvWFs); consequently, the number of circulating ULvWFs drops significantly.[11] ULvWFs are key molecules actively involved in the early phase of tissue repairing. The lack of ULvWF in turn impairs the efficacy of PLTs and coagulation pathways, favoring a bleeding diathesis. Overall, little progress has been made on understanding the disease pathophysiology. Vascular endothelial damage and microparticle (MP) generation have been suggested by several authors as key players in ET pathophysiology; however, up to now, studies on MPs and endothelial modulators are limited and are qualitative in nature.[12-16] This study focuses on MPs and their possible association with endothelial modulators and pharmacological interventions in