Eltrombopag enhances platelet adhesion by upregulating the expression of glycoprotein VI in patients with chronic immune thrombocytopenic purpura

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Eltrombopag, a thrombopoietin receptor agonist, has been approved for the treatment of patients with immune thrombocytopenia because of its abilities to enhance platelet production and reduce hemorrhage. Both platelet count and platelet adhesion are crucial to stop bleeding. Although eltrombopag is known to improve platelet counts, its effects on platelet adhesion are not yet known. This study aimed to assess the efficacy of eltrombopag on platelet production and platelet adhesive affinity. To evaluate the efficacy of low-dose eltrombopag (25 mg) for patients with chronic refractory immune thrombocytopenic purpura (ITP) and to determine the ex vivo platelet adhesion ability before and after treatment with eltrombopag, we conducted an open-label, multicenter study in which 25 Taiwanese patients with chronic ITP were enrolled. During the 6-month evaluation, the starting and maximum doses of eltrombopag were 25 and 50 mg, respectively, to maintain the platelet count of ≥50,000 per μL. Flow-based adhesion assay was used to detect the percentage of platelets adhering to immobilized von Willebrand factor-collagen on microslides. Of the enrolled patients, 48% achieved a platelet count of ≥50,000 per μL. Interestingly, 83% of all responders required 25 mg of eltrombopag daily to achieve the target platelet count. In addition, the percentage of bleeding patients was significantly reduced in both responders and nonresponders by 50% from the baseline level throughout the treatment period. The ex vivo platelet adhesion capacity was elevated after the 6-month eltrombopag treatment in both responders and nonresponders. Furthermore, glycoprotein VI (*GPVI*) expression was significantly upregulated after treatment with eltrombopag. Low-to-intermediate dose of eltrombopag showed good efficacy to expedite platelet production and augment platelet adhesion. These 2 factors might explain the efficacy of eltrombopag in ameliorating hemorrhage in patients with ITP. (Translational Research 2015;166:750–761)

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Abbreviations: CMFDA = 5-chloromethylfluorescein diacetate; FLI1 = Friend leukemia integration 1; GPIb = platelet membrane glycoprotein Ib; GPIIb = platelet membrane glycoprotein IIb; GPIIIa = platelet membrane glycoprotein IIIA; GPVI = glycoprotein VI; GPIX = platelet membrane glycoprotein IX; HA = healthy adults; ITP = immune thrombocytopenic purpura; MFI = mean fluorescence intensity; RPL19 = ribosomal protein L19; RT-PCR = real-time polymerase chain reaction; SC = surface coverage; siRNA = small interfering RNA; TPO = thrombopoietin; vWF = von Willebrand factor

AT A GLANCE COMMENTARY

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Background

Eltrombopag, a thrombopoietin receptor agonist, has been approved for the treatment of patients with immune thrombocytopenic purpura because of its platelet production enhancement and hemorrhage reduction abilities. Both platelet count and platelet adhesion are crucial for stopping bleeding. However, the effect of eltrombopag on platelet adhesion capacity has not yet been investigated.

Translational Significance

We conducted an open-label multicenter study involving chronic immune thrombocytopenic purpura patients, who were refractory to conventional immunosuppressants. The 6-month eltrombopag treatment improved the ex vivo platelet adhesion capacity both in responders and nonresponders. Glycoprotein VI (*GPVI*) expression was significantly elevated after eltrombopag treatment.

INTRODUCTION

Chronic immune thrombocytopenic purpura (ITP) is characterized by autoantibody- and cell-mediated premature platelet destruction,[1,2] which results in thrombocytopenia and bleeding tendency. In addition to accelerated platelet destruction, suboptimal thrombopoiesis is noted in chronic ITP patients.[3,4] Treatment with recombinant human thrombopoietin (TPO) led to TPO receptor stimulation in some ITP patients.[5] Eltrombopag, a small TPO receptor agonist, has high potency to stimulate proliferation and differentiation of bone marrow precursor cells to produce platelets.[6] Patients with chronic ITP receiving eltrombopag treatment showed significant reduction in bleeding episodes and remarkable increase in platelet counts with admirable safety.[7-9] Successful prevention of bleeding events depends on the platelet count and function in ITP patients.[10] These patients have higher baseline level of platelet activation than healthy donors.[11,12] Maintenance of vascular integrity requires platelet activation via P-selectin, platelet membrane glycoprotein (GP)Ib, and GPIIb–IIIa expression in circulating platelets. These molecules have been found in activated platelets showing minimal changes in counts[11] or even decreases[13] in ITP patients treated with eltrombopag without platelet agonists; furthermore, in the 28-day evaluation, the surface coverage (SC) of platelets at a high shear rate increased but not significantly.[13]

For securing vascular integrity, binding of the GPIb-V-IX complex to subendothelial von Willebrand factor (vWF) is required for the initiation of adhesion of circulating platelets to the extracellular matrix and enabling consolidated adhesion of GPVI to collagen. The adhesion of GPVI to collagen plays a central role in platelet adhesion and aggregation.[14,15] More importantly, GPVI completely activates firm platelet adhesion and aggregation under high shear flow rates to cease bleeding and avoid the rolling of platelet on the vWF layer via GPIb-V-IX.[16,17] These findings suggest that changes in platelet adhesion, particularly the impact of GPVI, needed to be urgently explored in ITP patients receiving long-term (6 months or more) administration of eltrombopag.

Holmes et al[18] suggested that *GPVI* expression was upregulated in megakaryocytes after TPO stimulation. The promoter of *GPVI* is modulated by a transcription factor, Friend leukemia integration 1 (FLI1), which interacts with Sp-1 and GATA-1 to enhance the transcription of *GPVI*. This megakaryocyte-specific expression of *GPVI* is partly regulated by CpG demethylation, which can be directly initiated by TPO.[19] Because eltrombopag does not bind to the same site on the TPO receptor as TPO, competitive binding is prevented, and eltrombopag and TPO can produce an additive cell-signaling effect.[20] Therefore, we hypothesized that eltrombopag could upregulate *GPVI* expression in ITP patients, thereby enhancing their platelet adhesion capacity, leading to reduce bleeding symptoms.

In this study, we attempted to determine the effects of eltrombopag on the expression of platelet adhesion molecules and platelet adhesion at a high shear rate in ITP patients ex vivo. Furthermore, we investigated the efficacy of low-dose eltrombopag in Taiwanese ITP patients to achieve a platelet count of >50,000 per μL. According to the RAISE study[7] and a clinical trial in Japan,[21] Asian patients showed good responses to low