Avatrombopag increases platelet count but not platelet activation in patients with thrombocytopenia resulting from liver disease

A. D. MICHELSON, E. SMOLENSKY KOGANOV, E. E. FORDE, S. L. CARMICHAEL and A. L. FRELINGER III
Center for Platelet Research Studies, Dana-Farber/Boston Children’s Cancer and Blood Disorders Center, Harvard Medical School, Boston, MA, USA

To cite this article: Michelson AD, Smolensky Koganov E, Forde EE, Carmichael SL, Frelinger III AL. Avatrombopag increases platelet count but not platelet activation in patients with thrombocytopenia resulting from liver disease. J Thromb Haemost 2018; 16: 2515–9.

Background: The thrombopoietin (TPO) receptor agonist (TPO-RA) avatrombopag has recently been Food and Drug Administration-approved for the treatment of thrombocytopenia in patients with chronic liver disease (CLD) scheduled for a procedure. The TPO receptor c-mpl is expressed on the platelet surface, and TPO lowers the threshold for platelet activation. TPO-RAs may therefore also lead to platelet activation. Objectives: To evaluate the effects of avatrombopag on platelet activation. Patients/Methods: CLD patients with thrombocytopenia participated in a randomized, double-blind, placebo-controlled, parallel-group study. No patient received a platelet transfusion within 10 days of study blood draws. Platelet activation was evaluated with whole blood flow cytometry (which, unlike other methods, is accurate in thrombocytopenic samples). Results: Avatrombopag, but not placebo, increased platelet counts. As measured by platelet surface P-selectin and activated glycoprotein IIb–IIIa: (i) the numbers of circulating activated platelets were not increased in avatrombopag-treated patients as compared with placebo-treated patients; and (ii) platelet reactivity to low and high concentrations of ADP and thrombin receptor-activating peptide was not increased in avatrombopag-treated patients as compared with placebo-treated patients. Conclusions: In this randomized, double-blind, placebo-controlled, parallel-group study of CLD patients with thrombocytopenia, avatrombopag increased platelet counts but did not increase platelet activation in vivo or platelet reactivity in vitro.

Keywords: blood platelets; liver diseases; platelet activation; thrombocytopenia; thrombopoietin.

Introduction

The thrombopoietin (TPO) receptor c-mpl is expressed on the surfaces of megakaryocytes and platelets [1]. In addition to increasing megakaryocyte growth and maturation, TPO lowers the threshold for platelet activation in vitro and in vivo [2–5]. TPO receptor agonists (TPO-RAs) may therefore also lead to platelet activation, perhaps to different extents, because TPO-RAs have different binding sites on c-mpl [6]. The Food and Drug Administration (FDA)-approved TPO-RA romiplostim is a TPO peptide mimetic, consisting of four identical 14-amino acid peptides (IEGPTLRQWLAARA) with no sequence homology with TPO, which avidly binds c-mpl and is inserted into a dimerized immunoglobulin Fc domain at a site close to that of TPO [6]. In contrast, the FDA-approved TPO-RA eltrombopag and the novel, recently FDA-approved TPO-RA avatrombopag are TPO non-peptide mimetics that bind to c-mpl near the transmembrane region at sites different from TPO [6–10]. However, eltrombopag and avatrombopag could induce differing
degrees of conformational change in the receptor–ligand complex, downstream signaling, and platelet activation. We previously demonstrated that eltrombopag, unlike TPO [2–5], does not lower the threshold for platelet activation in vivo or platelet reactivity in vitro, at least in patients with immune thrombocytopenia [11].

The objective of this study was therefore to evaluate the effects of avatrombopag, a novel orally administered small-molecule TPO-RA, on platelet activation in vivo and in vitro. To accomplish this objective, we evaluated the effects of avatrombopag on platelet activation as part of two phase 3 randomized, double-blind, placebo-controlled, parallel-group studies of the efficacy and safety of avatrombopag for the treatment of adults with thrombocytopenia associated with chronic liver disease (CLD). Platelet activation was evaluated with whole blood flow cytometry, which, unlike other methods, evaluates activation on individual platelets, and was therefore the optimal method for these thrombocytopenic samples [12]. The activation endpoints were platelet surface P-selectin (a marker of α-granule degranulation) [13], platelet surface activated glycoprotein (GP) IIb–IIIa (also known as integrin αIIbβ3) (as detected by the use of mAb PAC1, a marker of the activation-dependent conformational change in GPIIb–IIIa) [14], and the generation of platelet-derived microparticles [15].

Methods

Adult patients with thrombocytopenia associated with CLD who were enrolled at US sites in the phase 3 ADAPT1 (NC 01972529) and ADAPT2 (NCT01976104) studies (randomized, global, double-blind, placebo-controlled, parallel-group studies to evaluate the efficacy and safety of once-daily oral avatrombopag for the treatment of adults with thrombocytopenia associated with liver disease prior to an elective procedure) [16] provided Institutional Review Board-approved written informed consent to participate in a substudy to evaluate the effects of avatrombopag on platelet function. Patients (n = 30) were randomized in a 1:2 ratio (placebo/avatrombopag) to receive treatment on days 1–5 with placebo or avatrombopag (Dova Pharmaceuticals, Durham, NC, USA): 60 mg daily in patients with baseline platelet counts of < 40 × 10^9 L⁻¹, and 40 mg daily in patients with baseline platelet counts of 40 to ≤ 50 × 10^9 L⁻¹. Samples were collected at baseline (day 0, predrug) and after treatment (day 4 and day 10). No patient received a platelet transfusion within the 10 days prior to these blood draws. Patients had a Model for End-stage Liver Disease score of ≤ 24, and did not have hepatic encephalopathy that could be effectively treated.

Platelet activation was evaluated with whole blood flow cytometry, as previously described [11]. Briefly, citrate-anticoagulated whole blood was incubated (15 min, room temperature) with an antibody cocktail containing fluorescein isothiocyanate-conjugated PAC1, phycoerythrin (PE)-conjugated anti-P-selectin, and PE-Cy5-conjugated anti-CD42b with or without 0.5 μM or 20 μM ADP or 1.5 μM or 20 μM thrombin receptor-activating peptide (TRAP). Samples were fixed with 1% formaldehyde, and then shipped cold together with the remaining blood sample for analysis of platelet-derived microparticles to the Center for Platelet Research Studies at Boston Children’s Hospital. As previously described [15], platelet-derived microparticles were identified by light-scattering properties and positive staining for CD41 and CD42b. Changes from baseline for drug-treated (60-mg and 40-mg avatrombopag cohorts combined) and placebo-treated patients were compared by use of a repeated measures (visits) mixed-effects ANOVA model with factors for subject and treatment. Contrast statements were included to compare treatments and to compare the change from baseline by treatment. A P-value of < 0.05 was considered to be significant. Data analyses were performed with SAS version 9.3 and GRAPHPAD PRISM version 5. The data were analyzed by A.L.F. and A.D.M. All authors had access to the primary clinical trial data.

Results and Discussion

Treatment with avatrombopag, both 60 mg daily and 40 mg daily, but not placebo, resulted in an approximately two-fold increase in platelet counts on day 10 (Fig. 1). The numbers of circulating activated platelets (reflecting platelet activation in vivo) were not increased in avatrombopag-treated patients as compared with placebo-treated patients on day 4 or day 10, as measured according to platelet surface P-selectin and platelet surface activated GPIIb–IIIa (Fig. 2, No agonist). As expected, in vitro activation with both low and high concentrations of ADP or TRAP resulted in increased platelet surface P-selectin and platelet surface activated GPIIb–IIIa in placebo-treated patients (Fig. 2). Platelet reactivity on day 4 or day 10, as measured according to platelet surface P-selectin and platelet surface activated GPIIb–IIIa after activation with low and high concentrations of ADP or TRAP, was not further increased in avatrombopag-treated patients as compared with placebo-treated patients (Fig. 2). Although there were statistically significant reductions in platelet surface P-selectin on day 4 and platelet surface activated GPIIb–IIIa on day 10 in response to 20 μM ADP (Fig. 2), these reductions were quantitatively small, and would not be expected to result in a clinically significant reduction in platelet function. Similar results were obtained irrespective of whether percentage positive or mean fluorescence intensity was used as the readout for platelet surface P-selectin and platelet surface activated GPIIb–IIIa. Similarly, the change from baseline on day 4 in the number of platelet-derived microparticles in avatrombopag-treated patients (235 μL⁻¹, 95% confidence interval [CI] – 93 to 564) was
Fig. 1. Effect of avatrombopag on the platelet count in patients with thrombocytopenia resulting from chronic liver disease. Patients (n = 30) were randomized to treatment on days 1–5 with placebo (n = 10) or avatrombopag: 60 mg daily in patients with baseline platelet counts of < 40 × 10^9 L⁻¹ (n = 10), and 40 mg daily in patients with baseline platelet counts of 40 to ≤ 50 × 10^9 L⁻¹ (n = 10). Samples were collected at baseline (day 0, predrug) and after treatment (day 4 and day 10). The results shown are least squares means ± standard error of the mean generated with the SAS mixed-effects procedure. Asterisks indicate significant (P < 0.05) differences in change from baseline for the drug-treated group as compared with the corresponding placebo-treated group in the mixed-effects model. [Color figure can be viewed at wileyonlinelibrary.com]

Fig. 2. Effect of avatrombopag on platelet activation in vivo (No agonist) and on platelet reactivity in vitro (in the presence of the indicated concentrations of ADP or thrombin receptor-activating peptide [TRAP]) in patients with thrombocytopenia resulting from chronic liver disease. Platelet activation was measured according to platelet surface P-selectin (top row) and platelet surface activated glycoprotein IIb–IIIa (as determined by the use of mAb PAC1, bottom row). Patients were randomized to treatment on days 1–5 with placebo or avatrombopag: 60 mg daily in patients with baseline platelet counts of < 40 × 10^9 L⁻¹, and 40 mg daily in patients with baseline platelet counts of 40 to ≤ 50 × 10^9 L⁻¹. Samples were collected at baseline (day 0, predrug) and after treatment (day 4 and day 10). Results shown are for both doses of avatrombopag. Data are mean ± standard error of the mean. Changes from baseline for drug-treated (60-mg and 40-mg avatrombopag cohorts combined, n = 20) and for placebo-treated patients (n = 10) were compared by use of a mixed-effects model with P < 0.05 being considered to be significant. [Color figure can be viewed at wileyonlinelibrary.com]

not significantly different from the change from baseline in placebo-treated patients (− 35 µL⁻¹, 95% CI = 474 to 404) (P = 0.32), and nor was there a significant change from baseline on day 10 in the number of platelet-derived microparticles in avatrombopag-treated patients (165 µL⁻¹, 95% CI = 84 to 415) as compared with placebo-treated patients (33 µL⁻¹, 95% CI = 301 to 368) (P = 0.52).

These data also address the functionality of platelets released from the bone marrow by avatrombopag. Despite the nearly two-fold increase in platelet counts in the avatrombopag-treated patients as compared with
placebo-treated patients on day 10 (Fig. 1), avatrombopag-treated patients and placebo-treated patients showed similar magnitudes of increase in surface P-selectin and surface activated GPIIb–IIIa on individual platelets in response to both low and high concentrations of ADP and TRAP (Fig. 2). These data provide evidence that the newly released platelets in avatrombopag-treated patients function normally.

As reported elsewhere [16], both the ADAPT1 and ADAPT2 phase 3 studies demonstrated the superiority of avatrombopag over placebo in increasing the proportion of thrombocytopenic CLD patients not requiring a platelet transfusion or any rescue procedure for bleeding. Consistent with the lack of avatrombopag-induced platelet activation demonstrated in the present substudy of these ADAPT1 and ADAPT2 studies, only one thrombotic treatment-emergent adverse event occurred in the 435 patients enrolled in the ADAPT1 and ADAPT2 studies [16]. This patient was in the avatrombopag 40-mg cohort and had a partial right portal vein thrombosis on day 7 postprocedure, 14 days after the last avatrombopag dose [16], by which time no circulating platelets would have been exposed to avatrombopag (because normal platelet survival is no more than 10 days [17]).

There are several limitations of this study. First, because of the relatively small number of subjects (n = 30) in this platelet function substudy of the ADAPT1 and ADAPT2 studies, the degree of platelet activation could not be compared with the incidence of clinical thrombosis. However, as stated above, there was only one thrombotic treatment-emergent adverse event in the entire 435 subjects enrolled in the ADAPT1 and ADAPT2 studies [16]. Second, this study did not compare avatrombopag with other TPO-RAs.

**Conclusions**

The therapeutic objective of the use of TPO-RAs is to increase platelet counts in thrombocytopenic patients. Because TPO lowers the threshold for platelet activation in vitro and in vivo [2–5], TPO-RAs may also lead to platelet activation, perhaps to different extents because TPO-RAs have different binding sites on c-mpl [6–10]. However, in this randomized, double-blind, placebo-controlled, parallel-group study of patients with thrombocytopenia caused by CLD, treatment with the novel TPO-RA avatrombopag resulted in increased platelet counts but did not increase either platelet activation in vivo or platelet reactivity in vitro.

**Addendum**

A. D. Michelson and A. L. Frelinger III designed the study, analyzed and interpreted data, and prepared the manuscript. E. Smolensky Koganov, E. E. Forde, and L. Carmichael collected and analyzed data. All authors granted final approval of the manuscript for submission.

**Acknowledgements**

This study was supported in part by research funding from Eisai to Boston Children’s Hospital (principal investigators A. D. Michelson and A. L. Frelinger).

**Disclosure of Conflict of Interests**

A. D. Michelson and A. L. Frelinger have been principal investigators or coinvestigators on research grants to Boston Children’s Hospital from Anelixis, Baxalta, GE Global Research, Ionis, Ironwood, Medtronic, Megakaryon, National Institutes of Health (2P01HD036379-16A1), Pfizer and Sysmex. A. D. Michelson has received royalties from Elsevier, and been a consultant to Alynylam and Instrumentation Laboratory and a member of an advisory committee for AstraZeneca, Dova (the manufacturer of avatrombopag), and Janssen. The other authors state that they have no conflict of interest.

**References**

1. Debili N, Wendling F, Cosman D, Titeux M, Florindo C, Dusant-Fourt I, Schooley K, Methia N, Charon M, Nador R. The Mpl receptor is expressed in the megakaryocytic lineage from late progenitors to platelets. Blood 1995; 85: 391–401.
2. Harker LA, Marzec UM, Hunt P, Kelly AB, Tomer A, Cheung E, Hanson SR, Stead RB. Dose-response effects of pegylated human megakaryocyte growth and development factor on platelet production and function in nonhuman primates. Blood 1996; 88: 511–21.
3. Peng J, Friese P, Wolf RF, Harrison P, Downs T, Lok S, Dale GL, Burstein SA. Relative reactivity of platelets from thrombopoietin- and interleukin-6-treated dogs. Blood 1996; 87: 4158–63.
4. Rodriguez-Linares B, Watson SP. Thrombopoietin potentiates human megakaryocyte growth and development factor on platelet activation, perhaps to different extents because TPO-RAs have different binding sites on c-mpl [6–10]. However, in this randomized, double-blind, placebo-controlled, parallel-group study of patients with thrombocytopenia caused by CLD, treatment with the novel TPO-RA avatrombopag resulted in increased platelet counts but did not increase either platelet activation in vivo or platelet reactivity in vitro.

A. D. Michelson and A. L. Frelinger III designed the study, analyzed and interpreted data, and prepared the manuscript. E. Smolensky Koganov, E. E. Forde, and L. Carmichael collected and analyzed data. All authors granted final approval of the manuscript for submission.

**Acknowledgements**

This study was supported in part by research funding from Eisai to Boston Children’s Hospital (principal investigators A. D. Michelson and A. L. Frelinger).

**Disclosure of Conflict of Interests**

A. D. Michelson and A. L. Frelinger have been principal investigators or coinvestigators on research grants to Boston Children’s Hospital from Anelixis, Baxalta, GE Global Research, Ionis, Ironwood, Medtronic, Megakaryon, National Institutes of Health (2P01HD036379-16A1), Pfizer and Sysmex. A. D. Michelson has received royalties from Elsevier, and been a consultant to Alynylam and Instrumentation Laboratory and a member of an advisory committee for AstraZeneca, Dova (the manufacturer of avatrombopag), and Janssen. The other authors state that they have no conflict of interest.

**References**

1. Debili N, Wendling F, Cosman D, Titeux M, Florindo C, Dusant-Fourt I, Schooley K, Methia N, Charon M, Nador R. The Mpl receptor is expressed in the megakaryocytic lineage from late progenitors to platelets. Blood 1995; 85: 391–401.
2. Harker LA, Marzec UM, Hunt P, Kelly AB, Tomer A, Cheung E, Hanson SR, Stead RB. Dose-response effects of pegylated human megakaryocyte growth and development factor on platelet production and function in nonhuman primates. Blood 1996; 88: 511–21.
3. Peng J, Friese P, Wolf RF, Harrison P, Downs T, Lok S, Dale GL, Burstein SA. Relative reactivity of platelets from thrombopoietin- and interleukin-6-treated dogs. Blood 1996; 87: 4158–63.
4. Rodriguez-Linares B, Watson SP. Thrombopoietin potentiates human megakaryocyte growth and development factor on platelet production and function in nonhuman primates. Blood 1996; 88: 511–21.
5. Pasquet JM, Gross BS, Gratacap MP, Quek L, Pasquet S, Payastrue B, van Willigen G, Mountford JC, Watson SP. Thrombopoietin potentiates collagen receptor signaling in platelets through a phosphatidylinositol 3-kinase-dependent pathway. Blood 2000; 95: 3429–34.
6. Kuter DJ. Thrombopoietin mimetics. In: Michelson AD, ed. Platelets, 3rd edn. San Diego: Elsevier/Academic Press, 2013: 1217–42.
7. Yamane N, Tanaka Y, Ohyabu N, Yamane S, Maekawa K, Ishizaki J, Suzuki R, Itoh T, Takemoto H. Characterization of novel non-peptide thrombopoietin mimetics, their species specificity and the activation mechanism of the thrombopoietin receptor. Eur J Pharmacol 2008; 586: 44–51.
8. Abe M, Suzuki K, Sakata C, Sugawara K, Hirayama F, Koga Y, Kawasaki T, Naganuma S, Itoh H. Pharmacological profile of AS1670542, a novel orally-active human thrombopoietin receptor agonist. Eur J Pharmacol 2011; 650: 58–63.
9. Terrault NA, Hassanen T, Howell CD, Joshi S, Lake J, Sher L, Vargus H, McIntosh J, Tang S, Jenkins TM. Phase II study of avatrombopag in thrombocytopenic patients with cirrhosis undergoing an elective procedure. J Hepatol 2014; 61: 1253–9.
10 Bussel JB, Kuter DJ, Aledort LM, Kessler CM, Cuker A, Pendergrass KB, Tang S, McIntosh J. A randomized trial of avatrombopag, an investigational thrombopoietin-receptor agonist, in persistent and chronic immune thrombocytopenia. *Blood* 2014; **123**: 3887–94.

11 Psaila B, Bussel JB, Linden MD, Babula B, Li Y, Barnard MR, Tate C, Mathur K, Frelinger AL, Michelson AD. In vivo effects of eltrombopag on platelet function in immune thrombocytopenia: no evidence of platelet activation. *Blood* 2012; **119**: 4066–72.

12 Michelson AD. Flow cytometry: a clinical test of platelet function. *Blood* 1996; **87**: 4925–36.

13 Hsu-Lin S-C, Berman CL, Furie BC, August D, Furie B. A platelet membrane protein expressed during platelet activation and secretion. Studies using a monoclonal antibody specific for thrombin-activated platelets. *J Biol Chem* 1984; **259**: 9121–6.

14 Shattil SJ, Hoxie JA, Cunningham M, Brass LF. Changes in the platelet membrane glycoprotein IIb-IIIa complex during platelet activation. *J Biol Chem* 1985; **260**: 11107–14.

15 Michelson AD, Rajasekhar D, Bednarek FJ, Barnard MR. Platelet and platelet-derived microparticle surface factor V/Va binding in whole blood: differences between neonates and adults. *Thromb Haemost* 2000; **84**: 689–94.

16 Terrault N, Chen Y-C, Izumi N, Kayali Z, Mitrut P, Tak W, Allen L, Hassanein T. Avatrombopag before procedures reduces need for platelet transfusion in patients with chronic liver disease and thrombocytopenia. *Gastroenterology* 2018; **155**: 705–18.

17 Josefsson EC, Dowling MR, Lebois M, Kile BT. The regulation of platelet life span. In: Michelson AD, ed. *Platelets*, 3rd edn. San Diego: Elsevier/Academic Press, 2013: 51–66.