Impact of Anti-GPIIb/IIIa Antibody-Producing B Cells as a Predictor of the Response to Lusutrombopag in Thrombocytopenic Patients with Liver Disease

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Keywords

Anti-GPIIb/IIIa antibody-producing B cells · Chronic liver disease · Lusutrombopag · Thrombocytopenia · Thrombopoietin receptor agonist

Abstract

Background: To make an accurate estimate of the response to thrombopoietin (TPO) receptor agonists for thrombocytopenia associated with chronic liver disease, we evaluated the influence of antiplatelet autoantibodies on the response to lusutrombopag in thrombocytopenic patients with liver disease. **Methods:** A prospective study was conducted at 2 hospitals. Thrombocytopenic patients with liver disease re-

ceived oral lusutrombopag 3.0 mg once daily for up to 7 days. We analyzed changes in platelet counts from baseline to the maximum platelet count on days 9–14. The definition of clinical response was a platelet count of $\geq 5 \times 10^4/\mu\text{L}$ with an increased platelet count of $\geq 2 \times 10^4/\mu\text{L}$ from baseline. We assessed the correlation between the response to treatment drug and antiplatelet autoantibodies measured by anti-GPIIb/IIIa antibody-producing B cells. **Results:** Thirty patients received the trial drug. There were 25 responders and 5 non-responders. The median change in platelet counts was $3.9 \times 10^4/\mu\text{L}$ (95% CI 2.8–4.6, $p < 0.0001$). The correlation between change in platelet counts and the frequency of the anti-glycoprotein IIb/IIIa antibody-producing B cells was moderate ($r = 0.414$, 95% CI 0.064–0.674, $p = 0.023$). In multivariate

analysis of factors affecting the change in platelet counts, the anti-GPIIb/IIIa antibody-producing B cells were identified as an independent factor (regression coefficient [B] = 0.089; CI 0.021–0.157, p = 0.013). **Conclusion:** Anti-GPIIb/IIIa antibody-producing B cells may be a predictor for TPO receptor agonists in patients with chronic liver disease.

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Introduction

Thrombopoietin (TPO), which is produced by functional liver cells, is the major controller of circulating platelet production by TPO receptors on the megakaryocyte cell surface [1–3]. One of the reasons for the thrombocytopenia in end-stage liver disease is decreased TPO production [4, 5].

Lusutrombopag is a TPO receptor agonist (TPO-RA) for thrombocytopenic patients who have chronic hepatitis and liver cirrhosis [6–8]. For thrombocytopenic patients with inadequate TPO production, TPO-RAs are an ideal treatment drug aimed to increase megakaryocytes through interactions with the TPO receptors on the surface of bone marrow cells [9, 10].

We reported that spleen size is a good predictor for clinical response of lusutrombopag in terms of platelet count [11]. Historically, thrombocytopenia in chronic liver disease has been caused by sequestration and/or increased destruction in a spleen enlarged by congestive splenomegaly secondary to portal hypertension [12]. Therefore, if TPO-RAs had a good clinical response, severe splenomegaly could reduce the efficacy because of increased splenic destruction and sequestration, resulting in reduced platelet counts. However, one phase 3 study showed different results in different cohorts [6]. This is because there are variations from person to person in the multiple factors that contribute to the thrombocytopenia associated with chronic liver disease. Immune-mediated destruction involving antiplatelet antibodies is frequently present in chronic liver disease and is one of the causes of thrombocytopenia [13].

There is extensive evidence that platelets coated by antiplatelet autoantibodies are recognized by macrophages in the spleen, leading to accelerated platelet consumption [14]. To make an accurate estimate of the response to TPO-RAs for thrombocytopenia associated with chronic liver disease, the influence of antiplatelet autoantibodies on the response to TPO-RAs needs to be evaluated in more detail. Therefore, we aimed to assess the influence

of the antiplatelet autoantibodies for the response to TPO-RAs in thrombocytopenic patients with liver disease.

Materials and Methods

Eligibility Criteria

A prospective study was conducted at 2 hospitals in Japan. Patients with thrombocytopenia due to chronic liver disease were included. Liver disease in these patients was caused by nonalcoholic fatty liver disease, alcoholic liver disease, viral infection with hepatitis B and C virus, and autoimmune disease. Patients with medical history of splenectomy, history of portal vein thrombosis, Child-Pugh class C liver disorder, other causes of thrombocytopenia, history of thromboembolism, or blood transfusions in the previous 2 weeks were excluded. To exclude hematologic disease, including primary immune thrombocytopenia (ITP), bone marrow puncture and/or biopsy was performed by a hematologist for the enrolled patients.

Study Design

The study design consisted of a screening period followed by a 28-day treatment period. Patients who met the eligibility criteria received oral lusutrombopag at 3.0 mg once daily, starting on day 1 of the treatment period and continuing for up to 7 days. For those patients, we analyzed changes in platelet counts on day 5 to prevent an excessive increase in platelet count reporting and calculated platelet counts after the administration of lusutrombopag on days 9–12. Concomitant drug therapies that influence platelet counts were not changed if the patients were taking them prior to enrollment in the clinical trial.

We analyzed changes in platelet counts and treatment responses after administration of the trial drug from blood samples on day 1 and the maximum platelet count on days 9–14. The definition of clinical response was a platelet count of $\geq 5 \times 10^4/\mu\text{L}$ with an increased platelet count of $\geq 2 \times 10^4/\mu\text{L}$ from baseline after administration of the trial drug [8].

To assess the influence of antiplatelet autoantibodies in the response to TPO-RAs, we evaluated the correlation between the increased platelet counts after administration of the trial drug and antiplatelet autoantibodies. The presence of antiplatelet autoantibodies was proven by the existence of platelet-associated immunoglobulin G (PAIgG) and anti-glycoprotein IIb/IIIa antibody-producing B cells. The PAIgG was measured on intact and lysed platelets using an immunoradiometric assay [15]. The anti-GPIIb/IIIa antibody-producing B cells were detected using the enzyme-linked immunospot (ELISPOT) assay [16]. The details of the ELISPOT assay are given below.

A polyvinylidene difluoride-bottomed 96-well microplate, which was activated with ethanol for incubation, was coated with affinity-purified human GPIIb/IIIa dissolved in PBS containing 0.5 mM CaCl_2 (PBS-Ca). Peripheral blood mononuclear cells (PBMCs) isolated from the enrolled patients were pipetted into the wells and cultured at 37°C with 5% CO_2 for 4 h. During this incubation period, secreted anti-GPIIb/IIIa antibodies were bound to the immobilized GPIIb/IIIa in the immediate vicinity of the secreting cells. After washing away the cells with PBS-Ca containing 0.05% Tween 20, anti-GPIIb/IIIa antibodies that were bound to

Table 1. Baseline clinical characteristics

Clinical characteristics	
<i>N</i>	30
Age, years	68.5 (48–83)
Gender: male, <i>n</i> (%)	19 (63.3)
Height, m	1.61 (1.46–1.88)
Weight, kg	57.2 (46–91)
BMI, kg/m ²	22.6 (18.5–31.1)
Etiology: HCV/HBV/alcohol and NASH/others, <i>n</i>	11/3/12/4
Liver cancer, <i>n</i> (%)	15 (50)
Splenic volume, mL	724 (124–1,499)
Child-Pugh	6 (5–9)
MELD	5 (2–15)
Platelets, ×10 ⁴ /μL	4.51 (1.7–4.9)
Immature platelet fraction, %	5.95 (1.5–10.5)
Megakaryocytes <15/μL, <i>n</i> (%)	21 (70)
TPO <0.4 fmol/mL, <i>n</i> (%)	22 (73.3)
Platelet-associated immunoglobulin, ng/mL/10 ⁷ cells	82 (20–630)
Anti-glycoprotein IIb/IIIa antibody-producing B cells, /10 ⁵ PBMCs	14.1 (0.25–37.7)
WBCs, /μL	3,150 (1,400–4,800)
Hemoglobin, g/dL	12.5 (7.5–15.6)
Prothrombin time, %	68.5 (45–100)
Serum albumin, g/dL	3.6 (2.2–4.5)
BUN, mg/dL	15.2 (6.70–56.5)
Serum Cr, mg/dL	0.72 (0.48–2.29)
Aspartate aminotransferase, IU/L	35.5 (18–133)
Alanine aminotransferase, IU/L	27.5 (9–121)
Total bilirubin, g/dL	1.2 (0.4–2.8)
Ammonia, μg/dL	60 (12–210)
AFP, ng/mL	5 (2–596)
PIVKA-II, mAU/mL	20 (11–476)
Procedures: RFA/IVR/endoscopy/others, <i>n</i>	9/10/6/5

Data are expressed as median range or *n* (%). AFP, α-fetoprotein; NASH, nonalcoholic steatohepatitis; MELD, model for end-stage liver disease; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, Fibrosis-4 Index; PIVKA, protein induced by vitamin K absence; RFA, radiofrequency ablation; IVR, interventional radiology; TPO, thrombopoietin.

the membrane were incubated with a nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate to facilitate visualization. Each assay was conducted in 4 independent wells, and the mean of 4 values was determined. The frequency of anti-GPIIb/IIIa antibody-producing B cells was expressed as the number/10⁵ per PBMCs, and the cutoff value was defined as 2.0/10⁵ per PBMCs. The inter- and intra-laboratory reproducibility of the ELISPOT assay was evaluated by multiple laboratories [16, 17]. Furthermore, we performed the simulation using peripheral blood collected from 3 ITP patients, 5 liver cirrhosis patients, and 3 healthy controls to evaluate the intra- and inter-laboratory reproducibility of an ELISPOT assay. As a result, the ELISPOT assay demonstrated a good intra- and inter-laboratory reproducibility. Data collected at baseline included demographic information, concomitant medications, physical characteristics, and laboratory tests in the pre-treatment screening period.

Measurement of splenic volume was calculated based on computed tomography during a 6-month period [8]. The laboratory

parameters was WBC count, platelet count, hemoglobin, aspartate aminotransferase, alanine aminotransferase, BUN, Cr, total protein level, albumin, total bilirubin, α-fetoprotein, percent prothrombin time, and TPO. Serum TPO levels from enrolled patients were measured using a quantitative ELISA. The lower limit of quantification was 0.4 fmol/mL.

Statistical Analyses

We analyzed all the enrolled patients who had received lusutrombopag at least once. All statistical analyses were performed using IBM® SPSS® software, version 24.0 (IBM Corporation, Armonk, NY, USA). *p* values <0.05 were considered statistically significant. The paired data for enrolled patients were compared using the Wilcoxon signed rank test. Responders and nonresponders were compared using the Mann-Whitney U test. Differences in frequency between the 2 groups were examined using the χ² test. Correlations between the antiplatelet autoantibodies and change in platelet counts were determined using Pearson's product mo-

Fig. 1. Correlation between change in platelet count and antiplatelet autoantibodies, determined using Pearson's *r* coefficient. PAIgG, platelet-associated immunoglobulin G.

ment correlation coefficient. Multivariate linear regression analyses were performed to assess the correlation between the changes in platelet counts and antiplatelet autoantibodies. Statistical data analyses were performed by Statista (Kyoto, Japan).

Results

Patients' Characteristics

Thirty-four patients were enrolled from January 2017 through November 2019. During the run-in period, 4 patients dropped out of the clinical study (2 patients had hematologic disease and 2 had Child-Pugh class C liver disorder). Table 1 shows the characteristics of the remaining 30 patients.

A total of 19 patients (63.3%) were male. The median age of the patients was 68.5 years (range 48–83 years), and the median body weight was 57.2 kg (range 46.0–91.0 kg), and the median BMI was 22.6 (range 18.5–31.1). Liver disease was caused by viral and nonviral hepatitis ($n = 14$ and 16, respectively). Fifteen patients (50.0%) had liver cancer. TPO levels with the lower limits of quantification were observed in 22 patients (73.3%). A bone marrow examination showed a decreased production of megakaryocytes. The frequency of anti-GPIIb/IIIa antibody-pro-

ducing B cells was $14.1/10^5$ PBMCs (range, 0.25–37.7). The median PAIgG level was 82 ng/ 10^7 cells (range, 20–630).

Clinical Response after Administration of the Trial Drug

All enrolled patients received oral lusutrombopag at 3.0 mg daily for 7 days. The platelet count was significantly increased after administration of a trial drug. The median change in platelet counts was $3.9 \times 10^4/\mu\text{L}$ (95% CI 2.8–4.6, $p < 0.0001$).

There were 25 (83.3%) responders and 5 (16.7%) non-responders. Although 3 nonresponders received platelet transfusions before the invasive treatment, there were no instances of spontaneous bleeding after the procedures.

Correlation between Antiplatelet Autoantibodies and the Change in Platelet Counts

Regarding the correlation between antiplatelet autoantibodies and the increased platelet counts after administration of the trial drug using Pearson's *r* coefficient, the correlation coefficients of anti-GPIIb/IIIa antibody-producing B cells and PAIgG were 0.414 and 0.206, respectively (Fig. 1). Anti-GPIIb-IIIa antibody-producing B cells were associated with a change in platelet count (95%

Table 2. Univariate analyses and multivariate analysis of factors affecting the change in platelet counts

Variable	Univariate analysis					Multivariate analysis				
	regression coefficient	95% CI	standard β error	p value		regression coefficient	95% CI	standard β error	p value	
Age (per 1 year)	0.030	−0.061 0.121	0.044	0.684	0.500					
Body weight (per 1 kg)	−0.063	−0.137 0.011	0.036	−0.314	0.091					
Spleen size (per 1 mL)	−0.003	−0.005 −0.001	0.001	−0.453	0.012	−0.003	−0.005 −0.001	0.001	−0.446	0.007
Serum albumin (per 1 g/dL)	0.202	−1.073 1.477	0.622	0.061	0.748					
Total bilirubin (per 1 mg/dL)	−0.064	−0.824 0.696	0.371	−0.033	0.865					
Platelet counts (per $1 \times 10^4/\mu\text{L}$)	−0.198	−1.052 0.656	0.417	−0.089	0.638					
Child-Pugh score (per 1)	−0.132	−0.600 0.337	0.229	−0.108	0.570					
ALBI score (per 1)	−0.182	−1.466 1.101	0.627	−0.055	0.773					
Prothrombin time (per 1%)	0.007	−0.046 0.060	0.026	0.052	0.786					
Anti-GPIIb/IIIa antibody-producing B cells (per 1)	0.091	0.014 0.168	0.038	0.414	0.023	0.089	0.021 0.157	0.033	0.407	0.013

CI 0.064–0.674, $p = 0.023$). However, PAIgG was not associated with a change in platelet count (95% CI −0.207 to 0.555, $p = 0.326$).

We also evaluated the relation between change in platelet counts and the antiplatelet autoantibodies in multivariate linear regression analysis (Table 2). Regarding the candidates for multivariate analysis, we selected variable factors which have a significant univariate test result based on p value cutoff point of 0.25. Therefore, body weight, spleen size, and anti-GPIIb/IIIa antibody-producing B cells were identified according to the univariate analyses. Multivariate analyses confirmed that spleen size and anti-GPIIb/IIIa antibody-producing B cells were independent of the responder (regression coefficient $[B] = -0.003$, 95% CI −0.005 to −0.001, $p = 0.007$ and regression coefficient $[B] = 0.089$, 95% CI 0.021–0.157, $p = 0.013$, respectively).

Analyses of the Antiplatelet Autoantibodies in Responders

The frequency of anti-GPIIb-IIIa antibody-producing B cells in responders after administration of lusutrombopag was significantly higher than that in nonresponders (15.0 vs. $2.5/10^5$ PBMCs, $p = 0.004$) (Fig. 2). However, there was no significant difference between responders and nonresponders in the frequency of PAIgG (51.7 vs. $87.5 \text{ ng}/10^7$ cells, $p = 0.315$).

Analyses of the Antiplatelet Autoantibodies in Adverse Events

The incidences of adverse events are summarized in Table 3. There were no treatment-related deaths or grade ≥ 3 treatment-related adverse events. There were 6 patients with any grade of AEs who suffered fever, aspartate aminotransferase increase, gastrointestinal disorders, and hypertension during treatment. No significant differences between patients with and without adverse events were found with respect to the frequency of anti-GPIIb-

Fig. 2. Comparison of antiplatelet autoantibodies and response after administration of the trial drug. Data are expressed as mean $\pm$ SD. Separate analyses of complications were compared using the Mann-Whitney U test. PAIgG, platelet-associated immunoglobulin G.

Table 3. Adverse events

Adverse events	Grade	N (%)
Fever	Any ≥ 3	2 (6.6) 0
Aspartate aminotransferase increased	Any ≥ 3	2 (6.6) 0
Gastrointestinal disorders	Any ≥ 3	1 (3.3) 0
Hypertension	Any ≥ 3	1 (3.3) 0
Portal thrombosis	Any ≥ 3	0 0

IIIa antibody-producing B cells (13.9 vs. 14.3/ 10^5 PBMCs, 95% CI -1.01 to 4.52 , $p = 0.717$).

The Scatter Plot of Antiplatelet Autoantibodies and Spleen Volume in Nonresponders

There was also no correlation between the frequency of anti-GPIIb/IIIa antibody-producing B cells and spleen volume. However, high frequency of anti-GPIIb/IIIa an-

tibody-producing B cells and smaller spleen size were associated with a lack of clinical response (Fig. 3). Three nonresponders received platelet transfusions before invasive treatment. All patients who received platelet transfusions had low frequencies of anti-GPIIb/IIIa antibody-producing B cells.

Discussion

To our knowledge, this is the first report that suggests that the level of antiplatelet autoantibodies may play a role as a predictor for response to TPO-RAs. This clinical study showed that the frequency of anti-GPIIb/IIIa antibody-producing B cell levels was correlated with the response for lusutrombopag in severe thrombocytopenic patients due to chronic hepatitis and liver cirrhosis. In patients who had chronic hepatitis, autoantibodies directed against platelet surface antigens lead to accelerated platelet consumption by the spleen and triggered their rapid destruction, as observed in chronic ITP [18, 19]. In terms of thrombocytopenia, the key difference between chronic liver disease and chronic ITP is the plasma TPO levels [20]. Plasma TPO levels in ITP patients are normal

Fig. 3. Scatter plot of antiplatelet autoantibodies and spleen size in responders and nonresponders. White mark, responders; blue mark, nonresponders; red circle, patients who received platelet transfusions.

or minimally increased as a result of increased clearance of the hormone and binding to an expanded megakaryocyte mass [21]. On the other hand, chronic liver disease has very low TPO levels as a result of reduced TPO production by a severely damaged liver cell mass. Previously, researchers reported that TPO levels correlated with response to TPO-RAs in ITP patients, with higher TPO levels predicting a poor clinical response [22]. Therefore, low TPO levels in patients with chronic liver disease had a significant effect after administration of TPO-RAs.

As a predictor for response to TPO-RAs for thrombocytopenia associated with chronic liver disease, factors related to thrombocytopenia that lead to destruction of platelets within the spleen may be more clinically useful than those that lead to platelet production. This study cohort showed multiple factors related to thrombocytopenia, including decreased activity of the hematopoietic growth factor, bone marrow suppression, immune-mediated thrombocytopenia, and the pooling of platelets in the enlarged spleen caused by liver cirrhosis and portal hypertension. Most of the enrolled patients had low plasma TPO levels and severe thrombocytopenia in the bone marrow. On the other hand, a high frequency of anti-GPIIb/IIIa antibody-producing B cells and smaller spleen size

were associated with good clinical response. Platelets are destroyed by macrophages or platelet antigens degraded to peptides by dendritic cells, depending on the spleen size and the frequency of autoantibodies. If thrombocytopenic patients have small spleens with high frequency of autoantibodies, significant effects may be expected.

In the present study, we evaluated PAIgG and anti-GPIIb/IIIa antibody-producing B cells as antiplatelet autoantibodies. The level of PAIgG was not associated with clinical response because PAIgG is increased in many patients with non-ITP. Anti-GPIIb/IIIa antibody-producing B cells were reported to be highly sensitive and specific, compared with PAIgG, for evaluating the presence or absence of autoantibodies. GPIIb and IIIa, which are major platelet membrane proteins, are the most common targets of autoantibodies in patients with ITP [23, 24]. Although an inter-laboratory standardization of anti-GPIIb/IIIa antibodies has been difficult to achieve, Kuwana et al. [16] established an ELISPOT assay for the detection of circulating B cells secreting IgG anti-GPIIb/IIIa antibodies. They also reported that the circulating B cells producing anti-GPIIb/IIIa antibodies for anti-platelet autoimmunity have a similar profile in patients with liver cirrhosis and ITP [25].

Recently, therapies for primary ITP have been directed to ameliorate the condition of impaired platelet production due to antiplatelet autoantibodies [26]. TPO-receptor agonists are a useful treatment for chronic ITP aimed at increasing megakaryocytes through interactions with the TPO receptors. If the levels of antiplatelet antibodies are similar in patients with ITP and patients with chronic liver disease, TPO-RA therapy is useful for thrombocytopenia due to immune-mediated thrombocytopenia in chronic liver disease. When administering TPO-RAs to patients with thrombocytopenia, clinicians should pay careful attention to the factors related to thrombocytopenia and take into account the destruction of platelets from antiplatelet antibodies.

This study had some limitations. First, the sample size is relatively small to draw definitive conclusions. Second, 4 patients were excluded according to the exclusion criteria. This may have been a sample selection bias. Third, in the present study, no patients received oral lusutrombopag daily for 5 or 6 days to prevent an excessive increase in platelet count. Therefore, we did not assess the impact of the discontinuation of the drug orally.

Conclusion

This clinical study showed the influence of antiplatelet autoantibodies on the response to TPO-RAs in patients with chronic hepatitis and liver cirrhosis. The frequency of anti-GPIIb/IIIa antibody-producing B cells may play a role as a predictor for the response to lusutrombopag.

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Statement of Ethics

This study was approved by the Institutional Review Board Ethics Committee at both institutes, and all patients provided written informed consent. This study is registered in the UMIN Clinical Trials Registry as UMIN 000025776.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Wada N., Uojima H., Satoh T., Okina S., Iwasaki S., Shao X., Takiguchi H., Arase Y., Itokawa N., Atsukawa M., Miyazaki K., Hidaka H., Kako M., Kagawa T., Iwakiri K., Horie R., Suzuki T., and Koizumi W. contributed equally to this work; Uojima H., Wada N., Satoh T., Okina S., and Takiguchi H. collected and analyzed the data; Uojima H. drafted the manuscript; Hidaka H. designed and supervised the study; Iwasaki S., Shao X., Arase Y., Itokawa N., Atsukawa M., Miyazaki K., and Hidaka H. offered technical or material support. Kako M., Kagawa T., Iwakiri K., Horie R., Suzuki T., and Koizumi W. were general supervisors of the study group. All authors discussed the results and commented on and approved the manuscript for publication.

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