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Quantitative systems pharmacology model of thrombopoiesis and platelet life-cycle, and its application to thrombocytopenia based on chronic liver disease

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Funding information

This study was supported by Shionogi & Co., Ltd.

Abstract

Platelets are produced by hematopoietic stem cells via megakaryocytes in the bone marrow and play a critical role in hemostasis. The aim of this study was to develop a new platelet model based on the thrombopoiesis and platelet life-cycle by a quantitative systems pharmacology modeling approach, which could describe changes in platelet count profiles in platelet-related diseases and drug intervention. The proposed platelet model consists of 44 components. The model was applied to thrombopoiesis of a thrombopoietin receptor agonist, lusutrombopag. It could well describe the observed platelet count profiles after administration of lusutrombopag for both healthy subjects and patients with chronic liver disease and thrombocytopenia. This model should be useful for understanding the disease progression of platelet-related conditions, such as thrombocytopenia and for predicting platelet count profiles in various disease situations related to platelets and drug administration in drug development.

Study Highlights**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

A comprehensive quantitative systems pharmacology (QSP) model has not been reported for platelets, although several pharmacokinetic/pharmacodynamic models have been proposed.

WHAT QUESTION DID THIS STUDY ADDRESS?

The platelet QSP model, developed based on the mechanisms of thrombopoiesis and platelet life-cycle, describes the platelet count profiles after administration of a thrombopoietin receptor agonist in both healthy subjects and patients with chronic liver disease and thrombocytopenia.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The model constructed in this study would provide mechanistic insights on thrombopoiesis and platelet life-cycle and be useful for prediction of platelet count profiles in situations with missing information.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The model can be applied to simulations for other thrombopoietin receptor agonists or other indications for thrombopoietin receptor agonists.

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INTRODUCTION

Platelets, produced by hematopoietic stem cells (HSCs) via megakaryocytes in bone marrow, play a critical role in hemostasis. As a disease related to platelets, thrombocytopenia increases the risk of bleeding. Thrombocytopenia develops due to various conditions, such as idiopathic thrombocytopenic purpura (ITP) and chronic liver disease (CLD) or chemotherapeutic treatment. The current standard of care for these diseases is platelet transfusion. Recently, thrombopoietin receptor agonists have also been used for treatment of thrombocytopenic patients with ITP and CLD. One such agonist of lusutrombopag (Mylpro; Shionogi & Co., Ltd, Osaka, Japan), which acts selectively on the transmembrane region of the human thrombopoietin receptor (c-mpl), increases the platelet count via the same signal transduction system as that of endogenous thrombopoietin, and promotes thrombopoiesis.¹ The approved dosage for lusutrombopag is 3 mg for 7-day administration to patients with CLD and thrombocytopenia who are scheduled to undergo a procedure to reduce the risk of bleeding.

Approximately $1-4 \times 10^{11}$ platelets are produced daily by megakaryocytes.²⁻⁴ One megakaryocyte produces around 1000–10,000 platelets.²⁻⁴ The average life-span of platelets is 8 to 10 days.⁵ In a healthy state, about one-third of the platelets is pooled in the spleen and two-thirds are distributed throughout the rest of the vascular system.⁶ Thrombopoietin, which is mainly produced in the liver, is a cytokine involved in the production of platelets by activating the thrombopoietin receptor (c-mpl).⁷ It plays an important role in the proliferation and differentiation of megakaryocyte progenitors but has little effect on mature platelets or on the late stage of megakaryocyte development.^{8,9} In addition, thrombopoietin is cleared by platelets by binding to its receptors on the platelets, by which their concentrations in plasma regulate each other.¹⁰

Slow onset of a drug action (i.e., delayed changes in platelet count) is observed after administration of medicines, such as lusutrombopag and chemotherapeutics, that affect platelet count profiles because they influence a part of the process of thrombopoiesis for platelets. Some models have been reported for describing platelet count profiles, which address the slow onset. As one major approach, semimechanistic pharmacokinetic-pharmacodynamic (PK-PD) models¹¹⁻¹⁴ have been reported to describe and understand the PKs and the change of platelet count profiles after drug administration. Harker et al. developed a mathematical model, which included biology of megakaryocyte for describing platelet counts after administration of pegylated recombinant megakaryocyte growth and development factor (PEG-rHuMGDF)¹⁵ for healthy volunteers. Langlois et al. reported a mathematical model for platelets based on pathological dynamics in humans.¹⁶ Their model could

describe the oscillation in platelet profiles for cyclical thrombocytopenia. Although all of these models are useful for describing platelet count profiles, the model structures involved in the underlying thrombopoiesis were different among the reports.¹¹⁻¹⁶ Therefore, it is valuable to construct a comprehensive systems pharmacology model for the platelet life-cycle based on physiological, pathological, and mechanistic information also by utilizing reported model structures and assumptions for understanding the complex processes of thrombopoiesis and platelet life-cycle.

Quantitative systems pharmacology (QSP) modeling can provide mechanistic insights of underlying diseases and contribute to comprehensive understanding of drug efficacy. Thus, using QSP modeling to understand the processes of thrombopoiesis and platelet life-cycle should offer important information on the prediction of platelet count profiles and can be used to investigate factors influencing platelet count profiles in platelet-related diseases. In addition, QSP models could be used to simulate conditions that are not easily tested in clinical settings,¹⁷ and thus could be useful for obtaining missing information for drug development, for example, the prediction of drug responses in patients from healthy subject data at the time of phase transition. The objectives of this study were to develop a new QSP model for thrombopoiesis and platelet life-cycle based on physiological mechanisms and clinical observations and to evaluate its predictability by applying it to clinical data for thrombopoiesis of lusutrombopag in healthy subjects and patients with CLD and thrombocytopenia.

METHODS

Assumptions for platelet model development

The platelet model was constructed based on the scheme of thrombopoiesis and platelet life-cycle reported by Szilvassy⁴ and Craig,¹⁸ and focused on the changes in cell counts of precursor cells, megakaryocytes, and platelets. Because actual data for cell counts in humans, such as precursor cells and megakaryocytes in bone marrow, were not available, they were assumed based on the observed platelet count data from clinical studies.^{13,14} The platelet model was constructed assuming that cell division of progenitor cells occurs once a day ($k_{\text{out}} = 1/\text{day}$), on average, based on the typical cell proliferation time (24 h)¹⁹ and the typical doubling time of megakaryoblastic leukemia cell lines (24 h),²⁰ and that cells do not die in the process of megakaryocyte formation from progenitor cells. For megakaryocyte maturation, k_{out} was used to describe the daily maturation process. Based on typical values for blood volume of 5 liters and a platelet count per blood volume at steady-state of 200,000/ μl in adults,¹³ the total platelet count in the whole body could be assumed

as $\sim 1.0 \times 10^{12}$ in the blood ($\sim 1.5 \times 10^{12}$ in the whole body), which was consistent with the reported values^{16,21} of 2.2×10^{12} and $0.75\text{--}2.0 \times 10^{12}$. To maintain 200,000/ μl of platelet count per blood volume with a platelet life-span of ~ 9 days, $\sim 1.7 \times 10^{11}$ platelets are assumed to be produced per day, which is consistent with the reported values of $1\text{--}4 \times 10^{11}$.²⁻⁴ If one megakaryocyte produces ~ 2500 platelets,^{2,3} 6.8×10^7 megakaryocytes are required to produce platelets, which are almost equivalent to 2^{26} megakaryocytes. Therefore, 26 times cell divisions were assumed in the proliferation step from a progenitor cell as megakaryocytes do not divide and their cell count does not increase during the maturation and marrow reservoir steps (for 5 days).^{2-4,22}

Theoretical description of the platelet model

Figure 1 presents the structure of the platelet model and its parameters are shown in Table 1. Details of the platelet model are presented in the Supplementary Model Code and Supplementary Text S1.

The cell proliferation step of progenitor cells, the maturation step for megakaryocytes, and the daily change of platelets were described by the following Equations 1–10;

$$k_{\text{out}1} = k_{\text{out}} \times E_{\text{max}} \times (C_{\text{TPO}} / (EC50_{\text{TPO}} + C_{\text{TPO}})) \quad (1)$$

$$dPC_1/dt = k_{\text{out}1} \times 1 - k_{\text{out}1} \times PC_1 \quad (2)$$

$$dPC_i/dt = 2 \times k_{\text{out}1} \times PC_{i-1} - k_{\text{out}1} \times PC_i \quad (3)$$

$$dMK_1/dt = k_{\text{out}1} \times PC_{27} - k_{\text{out}} \times MK_1 \quad (4)$$

$$dMK_j/dt = k_{\text{out}} \times MK_{j-1} - k_{\text{out}} \times MK_j \quad (5)$$

$$dPLT_1/dt = PP \times k_{\text{out}} \times MK_5 - k_{\text{out}} \times PLT_1 \quad (6)$$

$$dPLT_k/dt = k_{\text{out}} \times PLT_{k-1} - k_{\text{out}} \times PLT_k \quad (7)$$

$$\text{Total platelet count} = \sum PLT \quad (8)$$

$$\text{Plasma platelet count} = \text{Total platelet count}/\text{blood volume} \times (1 - \%SPS) \quad (9)$$

$$\%SPS = 1 - PLT_0/\text{Initial total platelet count} \quad (10)$$

where $k_{\text{out}1}$ is the rate constant for cell division with a thrombopoietin effect (/day), k_{out} is the rate constant for cell

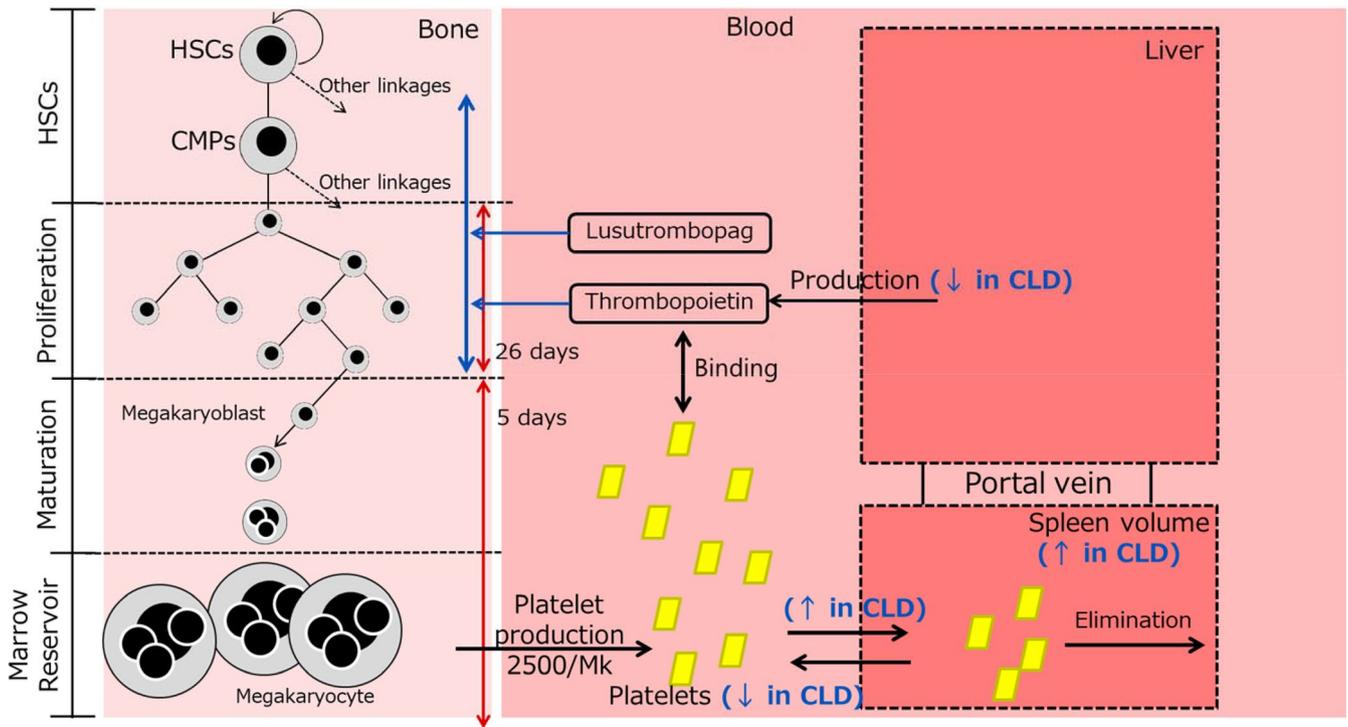


FIGURE 1 Scheme of the platelet model. CLD, chronic liver disease; CMP, common myeloid progenitor; HSC, hematopoietic stem cell; Mk, megakaryocyte. The model includes the components of proliferation, maturation, marrow reservoir of megakaryocytes, platelet production, distribution, and elimination step. The CLD state is assumed to entail decreased production of thrombopoietin and increased distribution to spleen according to an increase of splenic platelet sequestration. The ratio of splenic platelet sequestration (%SPS) were defined as “%SPS = 1-initial platelet count (PLT_0)/initial total platelet count”

TABLE 1 Kinetic parameters for the platelet model

Parameter	Unit	Value	Reference
Pharmacokinetics and pharmacodynamic parameters of thrombopoietin			
Initial concentration of thrombopoietin (TPO ₀) for			
Healthy subjects	(pM)	1.4	10
Thrombocytopenic patients with CLD	(pM)	0.78 ^a	-
Thrombopoietin binding to its receptor on platelet (k_{on})	(pM*day)	1.3	10
Thrombopoietin dissociation from its receptor on platelet (k_{off})	(/day)	60	10
Thrombopoietin nonspecific binding rate (k_{nf})	(/day)	1.2	10
Thrombopoietin nonspecific dissociating rate (k_{fn})	(/day)	3.1	10
Thrombopoietin receptor concentration on platelet (R _{p,0})	(pM)	164	10
Maximum effect via thrombopoietin receptor (E _{max})		4.52	14
Lusutrombopag concentration achieving 50% of E _{max} (EC50 _{Lusu})	(ng/mL)	183	25
Thrombopoietin concentration achieving 50% of E _{max} (EC50 _{TPO})	(pM)	4.9 ^b	-
Parameters for platelet production			
Rate constant for cell division without a thrombopoietin effect (k_{out})	(/day)	1	19,20
Platelet production counts from one megakaryocyte (PP)	(count)	2,500	2–4
Platelet life-span	(day)	9	5
Initial platelet counts (PLT ₀) for			
Healthy subjects	(*10,000/μl)	20 ^a	-
Thrombocytopenic patients with CLD	(*10,000/μl)	4 ^a	-
Pharmacokinetic parameters of lusutrombopag			
First-order rate constant of absorption (k_a)	(/day)	7.2	13
First-order rate constant of elimination (k_e)	(/day)	1.4	13
First-order rate constants from central to peripheral 1 compartment (k_{12})	(/day)	1.3	13
First-order rate constants from peripheral 1 to central compartment (k_{21})	(/day)	2.1	13
First-order rate constants from central to peripheral 2 compartment (k_{13})	(/day)	0.034	13
First-order rate constants from peripheral 2 to central compartment (k_{31})	(/day)	0.14	13
Distribution volume in central compartment	(L)	13.7	13
Unbound fraction (fu _{Lusu})	(%)	0.1	32

Abbreviations: CLD, chronic liver disease; E_{max}, maximum effect.

^aIn house data.

^bCalculated by $EC50_{TPO} = (E_{max} - E_{TPO,ss}) \times TPO_0 / E_{TPO,ss}$.

division without a thrombopoietin effect (/day), E_{max} is the maximum effect on thrombopoietin receptors, C_{TPO} is the thrombopoietin concentration (pM) in plasma, %SPS is the ratio of splenic-platelet sequestration, PLT₀ is the initial platelet count, PP is the platelet production count from one megakaryocyte represented as the typical value of PLT₀/%SPS/([2²⁶ × 9/blood volume]) for healthy subjects and 2500 for patients with CLD and thrombocytopenia, PC₁ is the precursor cell count in the initial compartment, PC_{*i*} is the precursor cell count in the *i*-th compartment (*i* = 2 to 27), MK₁ is the megakaryocyte count in the initial compartment, MK_{*j*} is the megakaryocyte count in the *j*-th compartment (*j* = 2 to 5), PLT₁ is the platelet count in the initial compartment for platelets, and PLT_{*k*} is the platelet count in the *k*-th compartment (*k* = 2 to 9). Then, based on the model, the megakaryocyte production is affected by thrombopoietin concentrations, although

the duration of megakaryocyte maturation is not affected by thrombopoietin concentrations. To describe the maturation of megakaryocytes and the aging step of platelets, five and nine-compartment models were selected, respectively.

The PK model of thrombopoietin reported by Jin F et al.¹⁰ was integrated into the platelet model. E_{max} of thrombopoietin for the thrombopoietin receptor was set as 4.52, which was derived from the E_{max} for lusutrombopag (a thrombopoietin receptor agonist) in the PK/PD model¹⁴ because thrombopoietin and lusutrombopag bind to the thrombopoietin receptor in the same manner. The thrombopoietin concentration achieving 50% of E_{max} (EC50_{TPO}) was calculated by the following equation and set as 4.9 pM:

$$EC50_{TPO} = (E_{max} - E_{TPO,ss}) \times TPO_0 / E_{TPO,ss} \quad (11)$$

where TPO_0 is the initial concentration of thrombopoietin (pM), and $E_{TPO,ss}$ is the efficacy of thrombopoietin at steady-state and set as 1.

The model was developed using MATLAB R2016a (MathWorks, Natick, MA). The model evaluation was performed by NONMEM version 7.3 (ICON Development Solutions, Ellicott City, MD),²³ and Perl-speaks NONMEM version 4.2.0 was used for execution of the NONMEM run.²⁴

Application to platelet count profiles in healthy subjects after lusutrombopag administration

To examine the applicability/predictability of the platelet model, the model was applied to the prediction of platelet count profiles after lusutrombopag administration, and the simulated values were compared with the available data. In the clinical studies with healthy subjects, a single dose (1, 2, 4, 10, 25, or 50 mg)¹³ in Japanese, 14-day multiple doses (1 mg)¹³ in non-Japanese, and 14-day multiple doses (2 mg) in Japanese¹³ were assessed (Table S1). Regarding multiple doses, simulation results were compared with the observations at 1 and 2 mg, respectively, as doses close to the clinical dose of 3 mg. The PK and PK/PD models developed based on phase I studies¹³ were integrated into the platelet model. The E_{max} model was selected as the PD model and E_{max} of lusutrombopag was assumed to be 4.52, as mentioned above. The lusutrombopag concentration achieving a 50% effect of E_{max} ($EC50_{Lusu}$) was assumed as 183 ng/ml based on in vitro data using human bone marrow-derived CD34-positive cells.²⁵ The effect of lusutrombopag was incorporated into k_{out1} of the model with the E_{max} model, as shown in the following equation because the mechanism of action of lusutrombopag can be assumed to be the same as that of thrombopoietin.

$$k_{out1} = k_{out} \times E_{max} \times \left(\frac{C_{TPO}}{EC50_{TPO} + C_{TPO}} + \frac{C_{Lusu} \times fu_{Lusu}}{EC50_{Lusu} \times fu_{Lusu} + C_{Lusu} \times fu_{Lusu}} \right) \quad (12)$$

where C_{Lusu} is the lusutrombopag concentration in plasma, fu_{Lusu} is an unbound fraction of lusutrombopag, and $EC50_{Lusu}$ is the lusutrombopag concentration achieving a 50% effect of E_{max} . The k_{out1} was restricted to be the initial value (i.e., 1/day) or higher so that the platelet production rate would be higher than or equal to the initial state. This is assumed based on the observation that downregulation of platelet production was not confirmed in clinical studies.

The platelet profiles after a single dose and multiple doses of lusutrombopag were simulated using the platelet model and compared with the observed data. The simulated platelet counts were calculated as sums of platelet counts in nine platelet compartments (Equations 8 and 9).

Application to platelet count profiles after lusutrombopag administration in patients with CLD and thrombocytopenia

PK parameters in patients with CLD and thrombocytopenia were assumed to be the same as those of healthy subjects because PK exposure was only modestly different between these groups.¹⁴ The model structure was also assumed to be the same as healthy subjects. The TPO_0 and initial platelet count (PLT_0) were set to the specific values for patients with CLD and thrombocytopenia, as shown in Table 1, and the other parameters were set to the same as healthy subjects. Based on the difference in TPO_0 between healthy subjects and patients with CLD, k_{out1} was restricted to be 0.62/day or higher for patients with CLD and thrombocytopenia so that the platelet production rate would be higher than or equal to the initial state. The PP was also set as 2500 count/megakaryocyte, which was assumed to be almost the same as for healthy subjects because thrombopoietin does not promote the platelet release from terminally differentiated megakaryocytes and accumulation of megakaryocytes in bone marrow.^{4,21}

Two hundred (200) virtual patients with platelet count profiles were simulated with NONMEM by resampling patient demographic data from the clinical data.¹⁴ The simulations were conducted for 7-day multiple dose administration of 3 mg lusutrombopag in Japanese patients with CLD and the simulated values were compared with the observed data after multiple doses (3 mg for 7 days) in Japanese patients with CLD from phase II study of lusutrombopag (Table S1). In the simulations, the PK parameters and intra-individual variability were derived from the literature,¹⁴ as shown in Table 1, and interindividual variability (IIV) for PLT_0 , TPO_0 , PP, and k_{out} were set at 40% as arbitrary values based on the IIV values 17.7%, 65.0%, 53.6%, and 26.5% for PD parameters estimated in the population PK/PD modeling for lusutrombopag¹⁴ because detailed information regarding variability was not available for them.

Global sensitivity analysis

Global sensitivity analysis was implemented via a GSAT package²⁶ based on MATLAB and was used to determine the sensitivity of maximum platelet counts to changes in the interested parameters. The sensitivity indices were conducted for PLT_0 , $EC50_{Lusu}$, TPO_0 , and k_{out} in healthy subjects and for PLT_0 , $EC50_{Lusu}$, TPO_0 , PP, and k_{out} in patients with CLD and thrombocytopenia. The PP in healthy subjects was not evaluated in the sensitivity analysis because it was not a variable but was calculated from $PLT_0 / \%SPS / ([2^{26} \times 9 / \text{blood volume}])$. In the global sensitivity analyses, the lower and upper bounds for PLT_0 ,

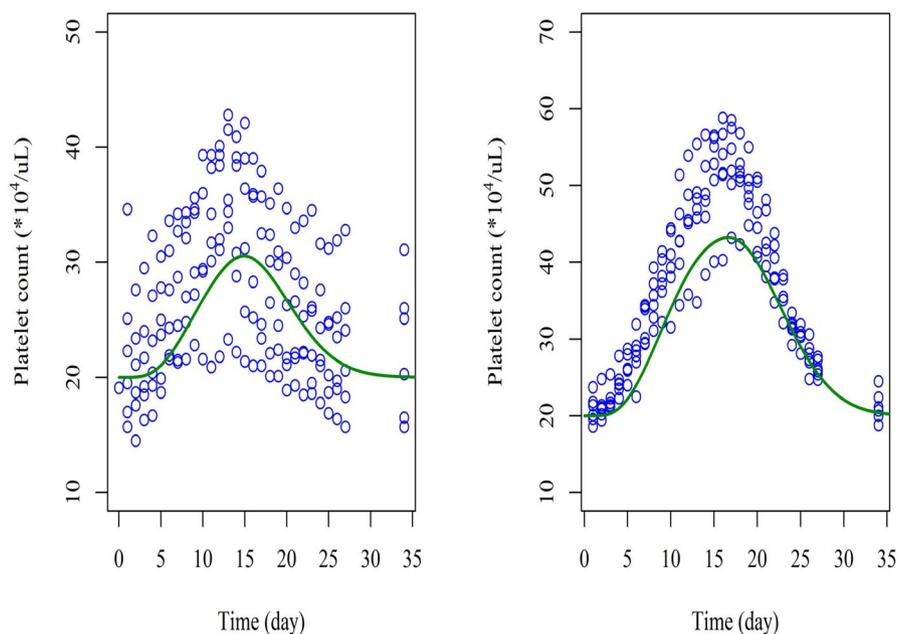


FIGURE 2 Simulated and observed platelet count profiles for healthy subjects after multiple administrations of lusutrombopag. Left: The 14-day multiple dose (1 mg) in non-Japanese. Right: The 14-day multiple dose (2 mg) in Japanese. The plots show the observed data and the lines show the predicted data from the platelet model

were set to 15 and 45, respectively, for healthy subjects as the normal range and 1 and 5, respectively, for patients with CLD and thrombocytopenia as the typical range at pretreatment for the target patient population.¹⁴ The lower and upper bounds for PP were set to 1000 and 10,000, respectively, as one megakaryocyte was reported to produce around 1000–10,000 platelets.^{2–4} The lower and upper bounds of k_{out} were set to 0.6 and 1.4, which were 0.6-fold and 1.4-fold of the typical value based on IIV for KM (41.8%) in population PK model of lusutrombopag.¹³ The lower and upper bounds of $EC50_{Lusu}$ and TPO_0 were set at 1/3 to 3 times of the typical values, respectively. The number of samples for the quasi-random Monte Carlo simulation was 1000.

RESULTS

Model structure of the platelet model

The model consists of 44 components (Figure 1), including those for describing the proliferation, maturation, marrow reservoir steps from the progenitor cell, and platelet life-cycle steps. The distribution of platelet to spleen was described using %SPS. The model also includes the components of PK and PD of thrombopoietin. All pathways for the compartments were described by a series of ordinary differential equations (Supplementary Text S1).

Application to platelet count profiles in healthy subjects after lusutrombopag administration

The simulated and observed platelet count profiles for healthy subjects are shown in Figure S1 for a single dose and in Figure 2 for multiple doses. In the multiple dose cases, the model-predicted maximum increase of platelet count and the time to peak platelet count were $10.5 \times 10,000/\mu\text{l}$ and 14.9 days after the first dose, respectively, after 14-day multiple doses of 1 mg; $23.2 \times 10,000/\mu\text{l}$ and 16.6 days after the first dose, respectively, after 14-day multiple doses of 2 mg. The model-simulated profiles of concentrations or counts of the components (thrombopoietin, precursor cells, megakaryocytes, and platelet) are shown in Figure 3 for 14-day multiple doses of 2 mg. Administration of lusutrombopag increased counts of megakaryocytes and platelets in all compartments with a time-delay depending on the compartment, but did not change the counts of precursor cells in any compartment, which is consistent with the model assumption that lusutrombopag stimulates the proliferation and differentiation of megakaryocyte progenitors as thrombopoietin.^{4,8} The plasma concentration of thrombopoietin was decreased by administration of lusutrombopag, which was due to enhanced binding with the increased platelets. The model-simulated profiles in platelet counts were consistent with the observations for both a single dose (Figure S1) and multiple doses (Figure 2) without parameter estimation.

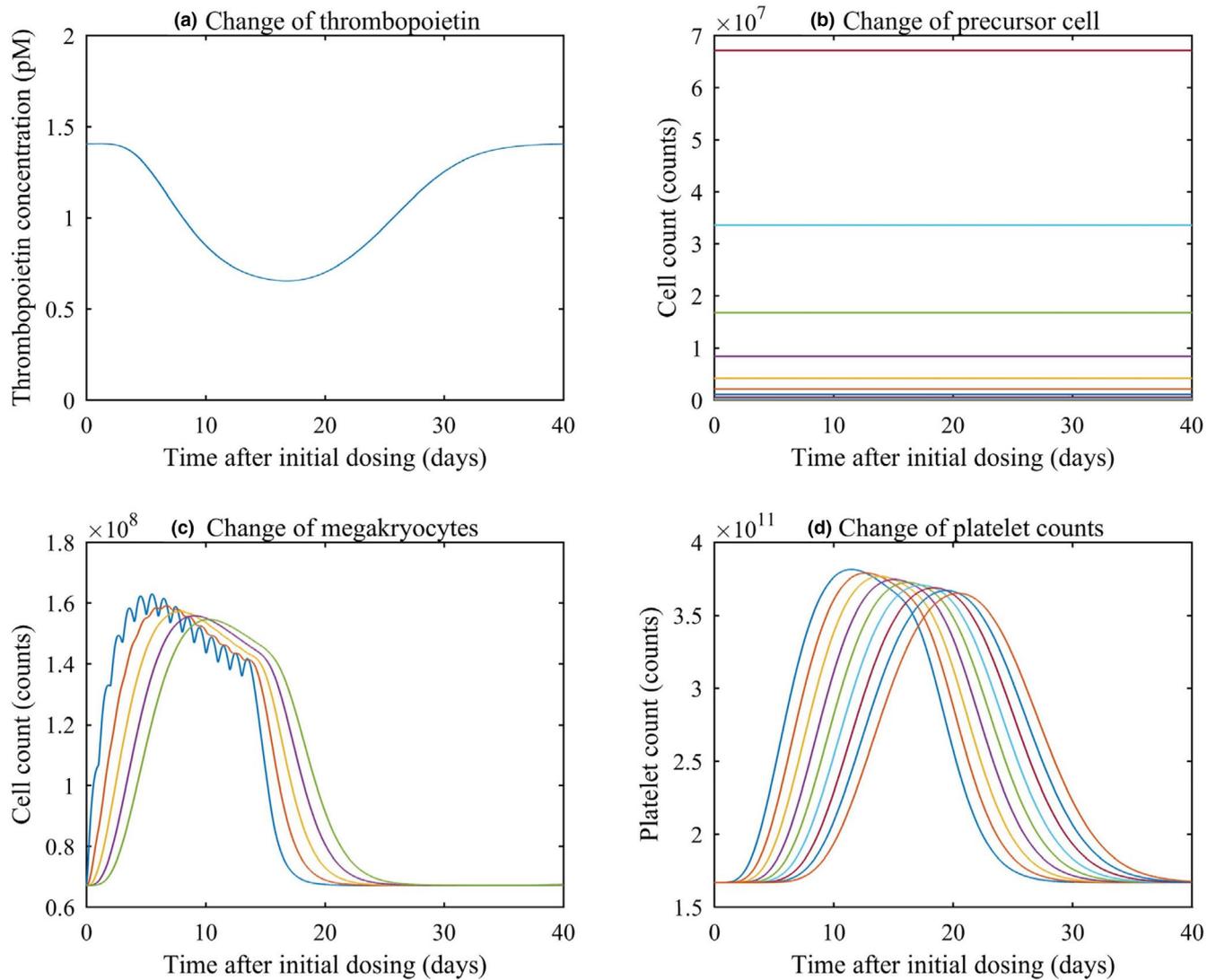


FIGURE 3 Simulated profiles of components in the model for healthy subjects after multiple administrations of lusutrombopag of 2 mg for 14 days. The lines show the simulated profile in each compartment of the model for (a) thrombopoietin concentration (pM) in plasma, (b) cell counts in precursor compartments during the proliferation step, (c) cell counts in megakaryocyte compartments during the maturation and marrow reservoir steps, and (d) counts in platelet compartments

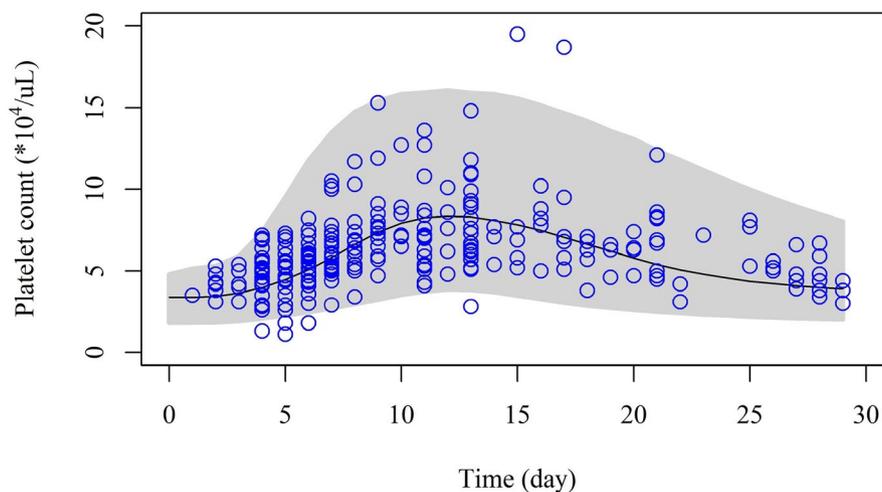


FIGURE 4 Simulated and observed platelet profiles following 7-day multiple doses of 3 mg to patients with chronic liver disease and thrombocytopenia. Open circles show the observed data from the phase II studies, and the solid line shows the predicted median. Shaded area shows 90% prediction interval

Application to patients with chronic liver disease and thrombocytopenia

The model-simulated platelet count profile with 90% prediction interval in patients with CLD and thrombocytopenia is shown in Figure 4 with the observations. The predicted maximum increase of platelet count and time to peak platelet count for the 7-day dose of 3 mg were $3.39 \times 10,000/\mu\text{l}$ and 12.9 days after first dose, respectively, which were consistent with the observed data. The 90% prediction intervals were estimated by 200 simulations, which were performed using patient demographic data in clinical trials. In addition, IIV was set as 40% for PLT_0 , TPO_0 , PP, and k_{out} . Most of the observed data were included in the 90% prediction intervals, suggesting that the platelet model could well predict the platelet count profiles in patients after administration of lusutrombopag. As shown in Figure 4, the platelet model could predict the platelet profiles for patients with thrombocytopenia by adjusting the model parameters for thrombocytopenia in the model.

Global sensitivity analysis

The global sensitivity analysis with the Sobol index was performed for the platelet model to determine pathways or factors with important impacts on the maximum platelet counts (Figure 5). The analysis was performed for 7-day multiple-dose administration of lusutrombopag separately for healthy subjects and patients with CLD and thrombocytopenia. For healthy subjects, the most influential parameter for maximum platelet counts was PLT_0 , followed by TPO_0 and $\text{EC50}_{\text{LUSU}}$ with sensitivity indices of 0.4715,

0.3376, and 0.2632, respectively. The order of the impact was the same for patients with CLD and thrombocytopenia with sensitivity indices of 0.4996, 0.4009, and 0.193, respectively, with the impact of PLT_0 being similar to that in healthy subjects. The sensitivity analyses suggested that PLT_0 was the most important factor on changes in platelet counts after administration of lusutrombopag for both healthy subjects and patients with thrombocytopenia. In contrast, TPO_0 seemed to have a modest impact on maximum platelet count.

DISCUSSION

Thrombocytopenia, which develops due to various causes, entails a great risk of bleeding. This risk can be decreased by treatment, such as with platelet transfusion or drug administration of thrombopoietin receptor agonists. For comprehensive understanding of drug efficacy and progressions of underlying diseases, QSP modeling can offer mechanistic insights and information that could not be easily tested in clinical settings. Therefore, we aimed at developing a new platelet model for thrombopoiesis and platelet life-cycle based on physiological mechanisms and clinical observations by the QSP approach, and then applied it to thrombopoiesis of lusutrombopag in healthy subjects and patients with CLD and thrombocytopenia.

In the process of the platelet model development in this research, theoretical mechanisms of thrombopoiesis and platelet life-cycle⁴ were considered, and cell cycles were assumed based on the observed platelet counts in healthy subjects from clinical studies.^{13,14} In this modeling, platelet production was initiated from a precursor cell, although

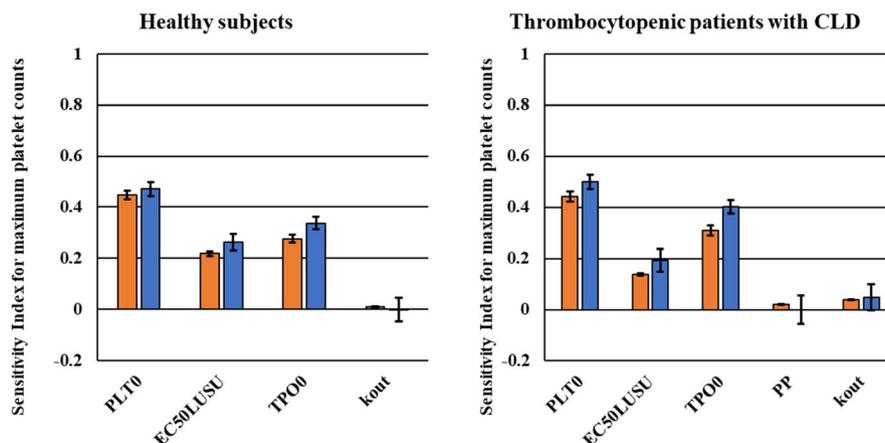


FIGURE 5 Results of global sensitivity analysis for the model parameters. Left, healthy subjects; right, patients with chronic liver disease (CLD) and thrombocytopenia. Orange bars, the first order effect; blue bars, the total order effect. $\text{EC50}_{\text{LUSU}}$, lusutrombopag concentration achieving 50% of E_{max} ; k_{out} , rate constant for cell division without a thrombopoietin effect; PP, platelet production count from one megakaryocyte; PLT_0 , initial platelet count; TPO_0 , initial concentration of thrombopoietin

production of blood cells is initiated by HSC in the model scheme (Figure 1). This is because HSC rarely divides²⁷ and the effect of the HSC growth process is thought to be limited for the change of platelet count by thrombopoietin and lusutrombopag. In addition, the effects of substances other than thrombopoietin were not integrated into the platelet model, although platelet production is also affected by various factors, such as interleukin-6.⁴ This is because thrombopoietin is the most important factor for platelet production and the impacts of other factors were considered to be less than that of thrombopoietin.²⁸ The number of cell cycles of progenitor cell were set to 26 times based on whole platelet counts and the number of platelet counts produced by one megakaryocyte, and 5 compartments were selected to describe the maturation process of megakaryocyte with a transit rate constant of $k_{\text{out}} = 1/\text{day}$, which is consistent with the reports that a typical proliferating human cell divides on average every 24 h and a typical duration of megakaryocyte maturation is 5 days. Regarding platelet lifespan, 9-platelet compartments were used in this research for describing maturation of platelets, whereas platelet lifespan could be more simply modeled as proposed previously.^{11,13,14} In our proposed model, aging steps of platelets were incorporated so that platelets are cleared by reticuloendothelial system after their lifespan (8–10 days)⁵ in the model, which had been also proposed by Herker et al.¹⁵ The duration of the megakaryocyte maturation is not changed by the change of thrombopoietin concentration, although the ploidy is changed by the change of thrombopoietin concentration. This is because thrombopoietin has little effect on the maturation of the platelets or on the late stage of megakaryocyte development.^{4,7} Consequently, the platelet model was developed with 44 compartments, as shown in Figure 1, to provide mechanistic insights on thrombopoiesis and platelet life-cycle. The model structure is reasonable for describing platelet count profiles after administration of a thrombopoietin receptor agonist.

Application of the platelet model to lusutrombopag for healthy subjects showed that it could well describe platelet profiles after single and multiple administration of lusutrombopag. The effect of lusutrombopag was incorporated into $k_{\text{out}1}$ of the model with the same E_{max} model as that for thrombopoietin (Equation 12) because lusutrombopag acts via the same signal transduction system as that of endogenous thrombopoietin. This assumption is supported by the fact that the effect of eltrombopag, which is in the same drug class of lusutrombopag, is additive to the effects of thrombopoietin.^{1,7} The model could also describe the slow onset phase based on assumptions that thrombopoietin and lusutrombopag affect the proliferation from a progenitor cell, an early step in platelet production, but do not stimulate the maturation and reservation of megakaryocytes, which is supported by the literature.⁴ By adjusting the model parameters for thrombocytopenia derived from CLD, the platelet model could also

describe the platelet profile (Figure 4) well for patients with CLD and thrombocytopenia after lusutrombopag administration as well as the reduced platelet increase in patients compared with healthy subjects. The differences in the platelet model between healthy subjects and patients with thrombocytopenia were the parameter values for PLT_0 , TPO_0 , and PP (a modest difference for PP), but the basic model structure was the same. In the model, low platelet counts in patients with CLD and thrombocytopenia compared with healthy subjects are caused by the change in megakaryocyte production and the change in the splenic-plasma sequestration (%SPS). Regarding %SPS, there have been reports of increased pooling of platelets in the spleen enlarged by congestive splenomegaly due to portal hypertension,²⁹ with the splenic platelet pool reaching 50%–90% under splenomegaly.⁶ In this study, PLT_0 for patients with CLD and thrombocytopenia was approximately one-fifth that of healthy subjects. The %SPS for the patients with CLD was assumed as 78.6% depending on the initial platelet counts (PLT_0), whereas it was 33.3% for healthy subjects, which was consistent with the literature.⁶ In addition, it was also supported by the report that the platelet counts in patients with CLD who underwent splenectomy recovered to the same level as healthy subjects.³⁰ In addition, the reduced increases in the maximum platelet count in the patients with thrombocytopenia compared to healthy subjects could be explained by the difference in %SPS. Thus, the model could adequately describe the platelet count profiles for not only healthy subjects but also patients with CLD and thrombocytopenia by adjusting the model parameters according to the decrease in platelets in CLD to the model.

Global sensitivity analyses of the platelet model revealed that PLT_0 was the most important factor for the maximum platelet count after lusutrombopag treatment in both healthy subjects and patients with CLD and thrombocytopenia. This is consistent with the report in which the platelet increase after lusutrombopag treatment was significantly lower in patients with a baseline platelet count less than or equal to 30,000/ μl compared with those with a baseline platelet count greater than 30,000/ μl .³¹ Because PLT_0 (baseline platelet count prior to treatment) is usually measured in clinical situations for lusutrombopag treatment, the high sensitivity of the platelet increase to PLT_0 indicated that the model would provide a reasonable prediction of platelet count profiles with the measured PLT_0 . As mentioned above, PLT_0 is related to %SPS, and %SPS would be correlated with changes in platelet profiles. In contrast, TPO_0 seemed to have only a modest impact on maximum platelet counts although a decrease of production of thrombopoietin has been reported to be a cause of thrombocytopenia with CLD because thrombopoietin is produced in the liver and plays an important role in platelet production. One of possible explanations on the small impact of TPO_0 is that thrombopoietin and platelet concentrations in plasma regulate each other¹⁰ to the normal conditions in the model (e.g., an increase in thrombopoietin

concentrations would lead to an increase in platelets), whereas the increased platelets would decrease thrombopoietin concentrations, suggesting a change in thrombopoietin concentrations does not simply affect platelet concentrations. Regarding IIV for PLT_0 , TPO_0 , PP, and k_{out} , 40% was arbitrarily selected for simulations because detailed information regarding variability was not available for them. Based on the global sensitivity analyses, the platelet response would be influenced by the variation of PLT_0 , whereas the contribution of TPO_0 , PP, and k_{out} would have a minimal impact on platelet profiles, under the situation that PLT_0 is usually measured in clinical situations. Therefore, the selections for IIV for PLT_0 , TPO_0 , PP, and k_{out} are not expected to markedly impact on platelet responses. Although further studies are needed, the platelet model integrating the PK model of a drug with the adjusted model parameters for thrombocytopenia with CLD adequately predicted the platelet count profiles, suggesting that modifications of disease- or drug-specific parameters can predict platelet count profiles in various states if mechanisms of thrombocytopenia for other diseases or drug administration could be integrated into the platelet model.

Semimechanistic models, such as that proposed by Friberg et al., have been reported for describing platelet counts profiles.¹¹ Population PK/PD models have also been reported by Katsube et al.^{13,14} based on data from clinical trials of lusutrombopag. These models were used to describe platelet count profiles by estimating model parameters based on the clinical data and to discuss dose justification by utilizing post hoc analyses for new drug application. Our platelet model is not suitable for estimating subject's specific parameters for each individual based on clinical trial data because it would be difficult to estimate all the parameters in its current model form due to its complexity. However, this platelet model would be a useful tool for understanding the mechanisms of thrombopoiesis and platelet life-cycle, and for simulating situations with missing information (e.g., no data for dosage, dose frequency, dosing duration, etc.) after administration of lusutrombopag. The model can be applied to other thrombopoietin receptor agonists by incorporating PK parameters and their parameters related to drug susceptibilities. In addition, the model can be applied to other indications related to platelets if the model sufficiently incorporates the mechanisms of the indication and the differences in the model parameters.

In conclusion, the platelet QSP model was constructed based on the mechanisms. The platelet model could adequately describe the platelet count profiles after administration of lusutrombopag for both healthy subjects and patients with CLD and thrombocytopenia. The platelet model constructed in this study should be useful for understanding the processes of thrombopoiesis and platelet life-cycle, the effect of thrombopoietin on platelet production, and the PDs of thrombopoietin receptor agonists. In addition, the model would be applicable for predicting platelet count profiles in thrombocytopenia caused by various diseases.

CONFLICT OF INTEREST

The authors, Ryosuke Shimizu, Takayuki Katsube, and Toshihiro Wajima, are all employees of Shionogi & Co., Ltd.

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

R.S., T.K., and T.W. wrote the manuscript. R.S. designed the research. R.S. performed the research. R.S. analyzed the data.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Shimizu R, Katsube T, Wajima T. Quantitative systems pharmacology model of thrombopoiesis and platelet life-cycle, and its application to thrombocytopenia based on chronic liver disease. *CPT Pharmacometrics Syst. Pharmacol.* 2021;10:489–499. <https://doi.org/10.1002/psp4.12623>