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Review

Application and investigation of thrombopoiesis-stimulating agents in the treatment of thrombocytopenia

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Lejun Huang, Jianxuan Xu, Huaying Zhang, Mengfan Wang, Yiyue Zhang and Qing Lin

***Abstract*:** Platelets, derived from a certain subpopulation of megakaryocytes, are closely related to hemostasis, coagulation, metastasis, inflammation, and cancer progression. Thrombopoiesis is a dynamic process regulated by various signaling pathways in which thrombopoietin (THPO)–MPL is dominant. Thrombopoiesis-stimulating agents could promote platelet production, showing therapeutic effects in different kinds of thrombocytopenia. Some thrombopoiesis-stimulating agents are currently used in clinical practices to treat thrombocytopenia. The others are not in clinical investigations to deal with thrombocytopenia but have potential in thrombopoiesis. Their potential values in thrombocytopenia treatment should be highly regarded. Novel drug screening models and drug repurposing research have found many new agents and yielded promising outcomes in preclinical or clinical studies. This review will briefly introduce thrombopoiesis-stimulating agents currently or potentially valuable in thrombocytopenia treatment and summarize the possible mechanisms and therapeutic effects, which may enrich the pharmacological armamentarium for the medical treatment of thrombocytopenia.

Keywords: drugs, thrombocytopenia, thrombopoiesis, thrombopoiesis-stimulating agents

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Introduction

Thrombopoiesis, the development of platelets, occurs primarily within bone marrow (BM) and lung.[1] Platelets may be derived from a certain subset of megakaryocytes (MKs) expressing genes associated with proplatelet production and platelet function.[2] Once released by high-ploidy MKs, platelets function as regulators of hemostasis, thrombosis, metastasis, inflammation, and cancer progression.[3] Several factors regulate thrombopoiesis. GATA binding protein 1 (GATA1), friend leukemia virus integration 1 (Fli-1), runt-related transcription factor 1 (RUNX1), and Rap1 GTPases activator C3G play a role in regulating megakaryopoiesis.[4] C-myb (MYB), which adjusts and balances the development of MKs and GATA-1, and Tribbles Pseudokinase 3 gene (TRIB3) are negative modulators of megakaryopoiesis.[5] Thrombopoietin (THPO) is the primary regulating cytokine in thrombopoiesis.[6] Since THPO binds to the myeloproliferative leukemia(MPL) receptor, several downstream signaling pathways consisting of mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinases (ERK), PI3K-Akt, and Janus kinase (JAK)/STAT which participate in MK proliferation, differentiation, maturation, and functional platelet division are activated.[7]

Thrombocytopenia, characterized by platelet counts less than $150 \times 10^3/\mu l$, is related to decreased platelet production, increased destruction, splenic sequestration, dilution, or clumping.[8] Severe thrombocytopenia may lead to low platelet counts and high bleeding risk, and cause life-threatening bleeding events. Thrombopoiesis-stimulating agents are vital in the drug treatment of thrombocytopenia. In this

Correspondence to:
Qing Lin
Division of Cell, Developmental and Integrative Biology, School of Medicine, South China University of Technology, Guangzhou 510006, P.R. China.
qinglin@scut.edu.cn

Lejun Huang
Jianxuan Xu
Huaying Zhang
Mengfan Wang
Yiyue Zhang
Division of Cell, Developmental and Integrative Biology, School of Medicine, South China University of Technology, Guangzhou, P.R. China

review, thrombopoiesis-stimulating agents are classified according to their research progress in treating thrombocytopenia. Some drugs are already in clinical trials for the treatment of thrombocytopenia, such as THPO, thrombopoietin receptor agonists (THPO-RAs), all-trans retinoic acid (ATRA), and interleukin-11 (IL-11)-based agents, which have manifested reliable therapeutic effects in different kinds of thrombocytopenia, including immune thrombocytopenia (ITP), chemotherapy-induced thrombocytopenia (CIT), inherited thrombocytopenia (IT), post-hematopoietic stem cell transplantation (HSCT) thrombocytopenia, presurgical thrombocytopenia, and thrombocytopenia related to chronic liver diseases (CLD), aplastic anemia (AA), radiation, and sepsis (Table 1). Another group of agents includes some drugs and natural products which are found to promote thrombopoiesis but are still not in clinical investigations to deal with thrombocytopenia (Table 2). Their various mechanisms of action in promoting thrombopoiesis (Figures 1 and 2) and other indications apart from thrombocytopenia will be briefly introduced to show the multifunction of these agents (Tables 3 and 4). However, their potential in thrombocytopenia treatment should be highly valued.

Thrombopoiesis agents for clinical treatment of thrombocytopenia

THPO and THPO-RAs

THPO. THPO plays an essential part in thrombopoiesis. Through binding to the c-MPL receptor, it can make a difference in the HSC differentiation and survival, MKs differentiation, and maturation.[9] Given the importance of THPO in promoting thrombopoiesis, PEG-recombinant human MK growth and development factor (PEG-rHuMGDF) and recombinant human THPO (rHuTHPO) are developed for thrombocytopenia treatment. Several clinical trials proved the effect of rHuTHPO in preventing and treating CIT.[10,11] PEG-rHuMGDF has comparatively longer half-life, which is mainly used for CIT in patients with cancer or hematological malignancies as an alternative for rHuTHPO.[12,13] However, the usage of these two recombinant THPO is both limited due to the potential risk of antibody formation to endogenous THPO.[14]

THPO-RAs. THPO-RAs are drugs that bind to THPO receptors, promoting megakaryopoiesis and platelet production.[15] The increasing use of THPO-RAs, such as romiplostim, eltrombopag, and avatrombopag, shows promising effects in ITP, AA, CIT, thrombocytopenia in CLD, IT, post-HSCT thrombocytopenia, and perioperative use for reducing platelet infusion.[16,17] Novel THPO-RAs with better effect and safety, such as lusutrombopag and hetrombopag, are under clinical investigation for thrombocytopenia treatment.

Romiplostim is a peptide which binds to the extra-cytoplasmic domain of the THPO receptor, activating various signaling pathways in mature MKs, such as JAK2/STAT5, PI3K-Akt, ERK, and STAT3.[15] Since romiplostim does not have amino acid sequence homology to endogenous THPO, it lowers the risk of THPO antibody formation and cross-reactivity. An analysis of data from five clinical trials in pediatric ITP patients and postmarketing registry shows that romiplostim's immunogenicity occurs infrequently and has no significant association with the deficiency of platelet response or other adverse events.[18] Another analysis from nine clinical studies, including 311 patients with ITP for at most 1 year (277 in romiplostim group) and 726 for more than 1 year (634 in romiplostim group), has proved that romiplostim can be effectively applied in ITP treatment. Platelet counts rose and remained stably elevated in most patients in romiplostim group. What is more, platelet response, which was indicated by platelet counts of at least 50×10^9/liter, occurred in 86% of patients with ITP $\leqslant$ 12 months and 87% of patients with ITP $>$ 12 months, reached a significant increase compared with both placebo group and standard of care group.[19] However, the rate of bleeding events was lower in romiplostim group than that of placebo group and standard of care group. The occurrence rates of thrombotic events for all groups were at similar level, independent of ITP duration.[19] Data from these clinical studies have proved the definite and safe effect of romiplostim in different phases of ITP treatment. Romiplostim also has potency in treating various types of thrombocytopenia, including CIT, *MYH9*-related IT, AA, acute radiation syndrome, presurgical thrombocytopenia, post-HSCT thrombocytopenia, and thrombocytopenia related to CLD.[20–22]

Table 1. Thrombopoiesis-stimulating agents for clinical treatment of thrombocytopenia.

Class of agents	Name	Suggested mechanism(s) in stimulating thrombopoiesis	Suggested effective type(s) of thrombocytopenia
THPO	PEG-recombinant human MK growth and development factor (PEG-rHuMGDF)	Activates the THPO–MPL signaling	CIT
	Recombinant human THPO (rHuTHPO)	Activates the THPO–MPL signaling	CIT
THPO-RAs	Romiplostim	Activates the THPO–MPL signaling	ITP, CIT, IT, post-HSCT thrombocytopenia, thrombocytopenia related to CLD, AA, acute radiation syndrome, presurgical use
	Eltrombopag	Activates the THPO–MPL signaling	ITP, IT, post-HSCT thrombocytopenia, AA
	Avatrombopag	Activates the THPO–MPL signaling	ITP, thrombocytopenia related to CLD, presurgical use
	Lusutrombopag	Activates the THPO–MPL signaling	Thrombocytopenia related to CLD, presurgical use
	Hetrombopag	Activates the THPO–MPL signaling	ITP
IL-11-based agents	Recombinant human IL-11 (RhIL-11)	Activates the gp130 signaling, JAK/TYK, and MAPK signaling	ITP, CIT, thrombocytopenia related to sepsis
	Hyper-IL-11 (H11)	Activates the gp130 signaling, JAK/TYK, and MAPK signaling	In development
	PEGylated IL-11	Activates the gp130 signaling, JAK/TYK, and MAPK signaling	In development
Retinoids	ATRA	Respond to thrombin receptor-activating peptide, may be dependent with THPO	ITP

AA, aplastic anemia; CIT, chemotherapy-induced thrombocytopenia; CLD, chronic liver diseases; ITP, immune thrombocytopenia; THPO, Thrombopoietin.

Eltrombopag is a small molecule and functions within the transmembrane and juxtamembrane domains of the THPO receptor through associating with specific amino acids and metal ions, so that the THPO receptor is activated.[23] Unlike romiplostim that mainly acts in mature MK, eltrombopag usually acts in earlier MK and late MK, stimulates MK precursor and MK differentiation, activates JAK2/STAT5, PI3K-Akt, and ERK1/2 pathways by increasing phosphorylation, and promotes thrombopoiesis.[15,24] It is found that eltrombopag is able to maintain platelet counts and make critical adverse events in ITP treatment less frequent. As a real-world study in Japan has demonstrated, when eltrombopag treatment, – no matter monotherapy or combination with other medicine, such as corticosteroids – was applied, the risk of bleeding-related episodes was about 30% lower than that of corticosteroid monotherapy in patients with ITP.[25] Apart from ITP, a phase II clinical trial investigated that eltrombopag was capable of increasing platelet counts while reducing bleeding events in the treatment of IT. Among 24 patients with different forms of IT with baseline platelet counts of 40.4×10^9/liter, including *ITGB3*-related thrombocytopenia, monoallelic Bernard–Soulier syndrome, X-linked thrombocytopenia/Wiskott–Aldrich syndrome, *ANKRD26*-related thrombocytopenia, or *MYH9*-related disease, 11 patients realized a major response of

Table 2. Thrombopoiesis-stimulating agents with potential value of clinical use.

Class of agents	Name	Suggested mechanism(s) in stimulating thrombopoiesis
Catecholamine	Dopamine (DA)	Activates the p38 MAPK, JNK/SAPK, and caspase-3 pathways
	Norepinephrine (NE)	Activates the α2-adrenoceptor-mediated ERK1/2 signaling, then activates the RhoA GTPase signaling
	Epinephrine (EPI)	Activates the α2-adrenoceptor-mediated ERK1/2 signaling, then activates the RhoA GTPase signaling
Hormones	Estrogen	Activates the ERβ-dependent pathway, activates GATA-1 and STAT1
	Melatonin	Activates the ERK1/2 and Akt signaling
	Recombinant human growth hormone (rhGH)	Activates the ERK1/2 and Akt signaling
Benzodiazepines	Clozapine	Activates the IL-6-induced gp130 signaling
Yes-associated protein (YAP) inhibitors	Dobutamine	Inhibits YAP in the Hippo pathway, upregulation of Fli-1
	Verteporfin	Inhibits YAP in the Hippo pathway, upregulation of Fli-1
Natural agents	Ingenol	Activates the PI3K-Akt signaling
	3,3′-di-O-methylellagic acid 4′-glucoside (DMAG)	Activates the PI3K-Akt signaling

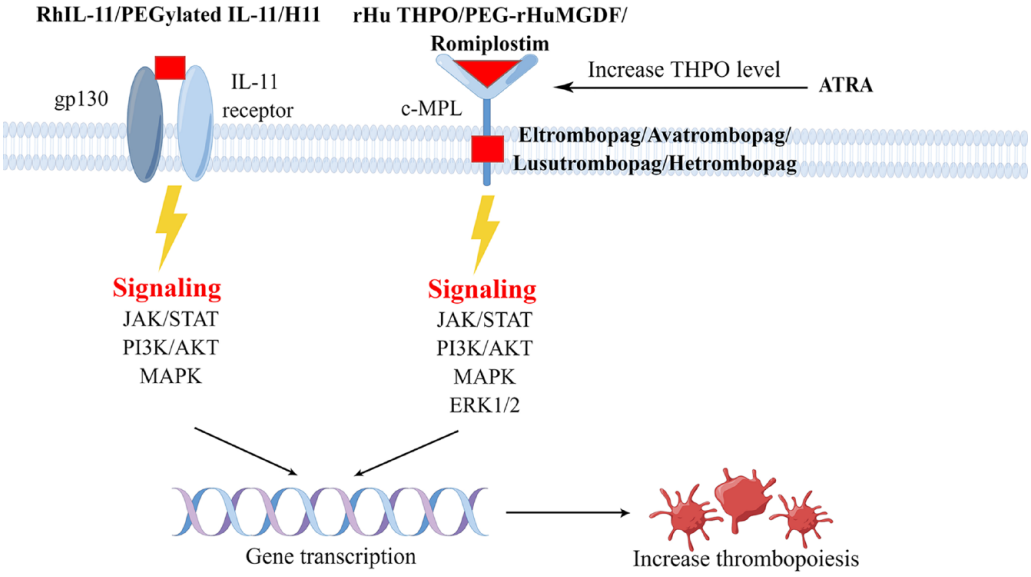

Figure 1. Mechanisms of thrombopoiesis-stimulating agents for clinical treatment of thrombocytopenia. THPO and THPO-RAs bind to different sites of the c-MPL receptor on the MK. IL-11-based agents activate the gp130 signaling. ATRA may increase the THPO level. Downstream activation of the various signaling pathways regulates gene transcription and ultimately increases thrombopoiesis (see also Table 1). Figdraw is used for figure plotting.

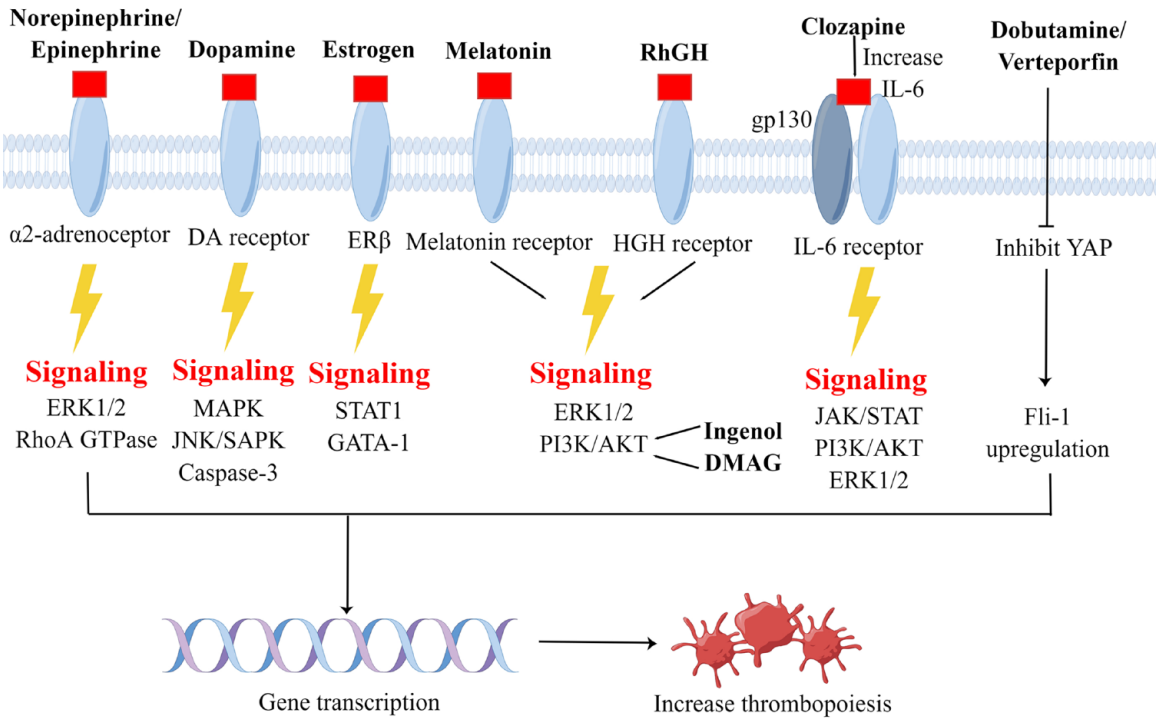

Figure 2. Possible mechanisms of thrombopoiesis-stimulating agents with potential value of clinical use. Catecholamines, estrogen, melatonin, and rhGH bind to their receptors and activate various signaling pathways. Clozapine may increase the IL-6 level and then indirectly promote thrombopoiesis. Dobutamine and verteporfin could inhibit YAP, and then upregulate Fli-1. The two natural products, ingenol and DMAG, are found to activate PI3K-Akt signaling (see also Table 2). Those signaling pathways regulate gene transcription and ultimately increase thrombopoiesis. Figdraw is used for figure plotting.

Table 3. Other indications and mechanisms of action of some thrombopoiesis-stimulating agents for clinical treatment of thrombocytopenia.

Name	Indication(s)	Mechanism(s)
RhIL-11	Tissue protector in anticancer therapy (enhance the recovery of BM, oral epithelium, and intestinal crypt cells after cytotoxic insult by anticancer drugs or ionizing radiation)	Activates the gp130 signaling, JAK/TYK, and MAPK signaling; Upregulates anti-apoptotic gene BCL-2, downregulates apoptotic gene BAX; Induces IEC-6 cell cycle arrest
ATRA	Psoriasis, acne, and ichthyosis	Stimulates FOXO1 gene expression
	Bronchial asthma, inflammatory bowel disease (IBD), periodontitis	Regulates the cellular function of Th17/Treg
	Acute promyelocytic leukemia (APL)	Activates the transcription factor FOXO3A in APL cells

platelet counts over 100×10^9/liter, 10 patients achieved a minor response with platelet counts being no less than two times the baseline value.[26] Eltrombopag is also valid and safe for raising the platelet counts for post-HSCT thrombocytopenia in adult or pediatric patients. There may be a positive correlation between the number of MKs in the BM before treatment and therapeutic effect.[27,28] Combining eltrombopag and anti-thymocyte globulin (ATG) resulted in better safety and effect than using ATG alone to treat severe AA.[29] Vitro colony-forming units of MKs assay

Table 4. Other indications and mechanisms of action of some thrombopoiesis-stimulating agents with potential value of clinical use.

Name	Indication(s)	Mechanism(s)
DA	Cardiovascular diseases (hypertrophy, myocardial ischemia, hypertension, and arrhythmia), acute renal failure	D1R (activates cAMP/PKA signaling, Gs/PLC/PKC signaling); D2R (activates Gi-Go/AC, suppressing cAMP)
NE	Neurogenic shock, hypotension after pheochromocytoma resection or drug intoxication, hemostatic	Activates α-R
EPI	Cardiac arrest, anaphylactic disease (anaphylactic shock, Bronchial asthma, angioneuroedema), glaucoma, hemostatic	Activates α-R/β-R
Estrogen	Perimenopausal syndrome; osteoporosis, ovarian dysfunction, breast cancer (post-menopause), prostate cancer, functional uterine bleeding, acne	Activates ER
Melatonin	Dyssomnia	Activates MT1 and MT2
	Neurological diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, epilepsy, headache, etc.)	Free-radical scavenger, antioxidant, anti-inflammation, immune suppression
	Different types of cancer (reduction in tumor growth and metastases, reduction in the side effects associated with chemotherapy and radiotherapy, decreasing drug resistance in cancer therapy, and augmentation of the therapeutic effects of conventional anticancer therapies)	Antioxidant, antiproliferation (downregulates NF-κB/iNOS, PI3K, and MAPK signaling pathways, inhibits SPHK1) Promoting cell apoptosis (activates BAX/BAK, Apaf-1, caspases, and p53)
	Cardiovascular risk factors and diseases (diabetes, hypertension, hyperlipidemia, obesity, myocardial ischemia–reperfusion injury, pulmonary hypertension, and atherosclerosis)	Antioxidant, anti-inflammation, regulates metabolism
	Bone-related diseases (fractures, osteoporosis, and osteoarthritis)	Antioxidant, anti-inflammation, promoting wound healing and tissue regeneration, regulation of bone mass accumulation and loss
rhGH	Growth hormone deficiency (GHD), short stature (SS)	Activates GHR
Clozapine	Antipsychotics	Activates DR4 and serotonin-DA receptor
Dobutamine	Heart failure, septic shock	Activates β1-R
Verteporfin	Age-related macular degeneration (AMD), central serous chorioretinopathy (CSC), ocular histoplasmosis syndrome, and pathologic myopia	Photodynamic therapy
	Different types of cancer (retinoblastoma, gliomas, breast cancer, etc.)	Inhibits YAP in the Hippo pathway
Ingenol	Warts, skin keratoses, and skin cancer	Activates PKC-α/δ
DMAG	Hepatoprotective	Antioxidant

suggested that myelodysplastic syndrome (MDS) patients may benefit from eltrombopag combined with azacitidine.[30] These research broaden the vision of its potential in thrombocytopenia and other hematopoietic diseases.

Avatrombopag is a small-molecule THPO-RA. The different binding site of avatrombopag from endogenic THPO could avoid competitive inhibition and contribute to the additivity result of promoting thrombopoiesis.[31] In contrast to

eltrombopag, avatrombopag is incapable of chelating iron or cations.[32] A network meta-analysis for patients with chronic ITP revealed that avatrombopag is associated with statistically significant improvements in either platelet response (platelet counts ⩾ 50,000/μl) or complete response (platelet counts ⩾ 100,000/μl) in different stage of treatment, including day 8, day 28, and month 6, compared with the placebo group.[33] In addition, 64.0% of patients of avatrombopag group were observed to have durable clinically relevant response which means platelet counts of ⩾ 30,000/μl will last for 6 weeks during the final 8 weeks of the core study, while the number of placebo group is 0%.[33] It is found that concomitant ITP medication, such as corticosteroids, was less applied in patients with avatrombopag treatment.[33] Avatrombopag is also effective for treating adult patients who suffer from CLD since it is capable of increasing platelet levels in thrombocytopenia, significantly reducing platelet transfusions or rescue procedures.[34] A lower occurrence of bleeding events has been shown compared with romiplostim and eltrombopag in patients with chronic ITP, indicating avatrombopag's better safety in clinical use.[34] Avatrombopag is also approved for using before surgeries, such as splenectomy, radiofrequency ablation of the spleen, and partial splenic artery embolization.[34]

Lusutrombopag is a small-molecule THPO-RA on the human THPO receptor, which can act selectively and promote the proliferation and differentiation of BM cells into MKs, increasing the platelet levels in thrombocytopenia patients.[35] Lusutrombopag could be used to deal with mild-to-moderate thrombocytopenia in CLD patients. Its therapeutic effect was proved by a clinical trial of CLD patients with different severity of thrombocytopenia. The effect was the same for mild thrombocytopenia (platelet counts ⩾ 50,000/μl) and moderate thrombocytopenia (platelet counts < 50,000/μl).[36] However, as reported from a multicenter clinical study of 139 patients with thrombocytopenia caused by CLD, lusutrombopag has better effect on mild thrombocytopenia than on severe thrombocytopenia.[37] Lusutrombopag is safe and productive for increasing platelet counts, reducing bleeding events and decreasing the need for platelet transfusion of patients with or without hepatocellular carcinoma (HCC), which is a secondary disease for CLD, according to an analysis of data from two phase III clinical trials.[38] It is approved for presurgical use with indications similar to avatrombopag, as alternatives to platelet transfusion.[34]

Hetrombopag is a novel, active, small-molecule THPO-RA. It promotes MKs proliferation and differentiation, and offers an additive thrombopoiesis-stimulating effect in combination with rHuTHPO.[39] A multicenter, randomized phase III trial of hetrombopag showed its efficacy and good tolerance in treating ITP patients who had no response or relapse after previous treatment with realizing a platelet response (58.9% in once-daily 2.5 mg hetrombopag group and 64.3% in once-daily 5 mg hetrombopag group) and bringing down the risk of bleeding and the requirement of rescue therapy through 8 weeks' treatment, compared with placebo.[40]

IL-11-based agents

IL-11 is a cytokine which belongs to interleukin-6 (IL-6) family with hematopoietic proliferative properties. It is crucial for promoting MK colony formation and plays a stimulating effect on thrombopoiesis.[41] Various IL-11-based agents, such as recombinant human IL-11 (RhIL-11), hyper-IL-11 (H11), and PEGylated IL-11, are currently or potentially used to treat thrombocytopenia.

The recombinant form of IL-11, RhIL-11, signals through the gp130 subunit of the receptor. Co-expression of the IL-11 receptor α-chain and gp130 results in high-affinity binding, initiating signal transduction of the JAK/TYK and the MAPK.[42] Due to RhIL-11's inducing MKs maturation and differentiation, and normalizing Th1/Th2 and T-bet/GATA-3 ratios close to healthy states,[43] it shows therapeutic effect in ITP, CIT, and thrombocytopenia in sepsis patients.[44–46] RhIL-11 also acts as a tissue protector in anticancer therapy because it activates the gp130 signaling, JAK/TYK, and MAPK signaling, downregulates apoptotic gene BAX, upregulates anti-apoptotic gene BCL-2, and induces IEC-6 cell cycle arrest,[47,48] although the main indication of RhIL-11 is treating thrombocytopenia.

As a fusion protein comprising IL-11, H11 targets the gp130 signal-transducing subunit.[49] Compared with rhIL-11, H11 shows more effectiveness in strengthening early progenitors' expansion and directing them to MKs, inducing

the maturation of MKs and the release of platelet-like particles (PLPs). Therefore, H11 may be more productive for stimulating the MK colony formation and PLPs production than RhIL-11, that may be beneficial for thrombocytopenia treatment and an *ex vivo* expansion of MKs.[50] Compared with IL-11, PEGylated IL-11 not only has a longer half-life but also displays its ability of inducing longer-lasting increases in hematopoietic cells.[51] H11 and PEGylated IL-11 may have better potent effects on stimulating thrombopoiesis, but further clinical evidence of H11 and PEGylated IL-11 in thrombocytopenia treatment is needed compared with RhIL-11.

ATRA

ATRA is commonly used to treat skin diseases, inflammatory diseases, and acute promyelocytic leukemia (APL).[52] It relieves psoriasis, acne, psoriasis and ichthyosis by stimulating FOX1 gene expression,[53,54] regulates the cellular function of Th17/Treg to treat bronchial asthma, inflammatory bowel disease (IBD), periodontitis,[55–57] and activates the transcription factor FOXO3A in APL cells.[58] Concentration of 10^{-12} M ATRA could stimulate thrombopoiesis from hematopoietic progenitors in the presence of THPO or granulocyte-macrophage colony-stimulating factor (GM-CSF) plus IL-3.[59] ATRA could stimulate thrombopoiesis in MEG-01 cell by promoting MK differentiation and generation of functional PLPs. These functional PLPs could be activated by thrombin receptor-activating peptide 6 (TRAP-6) with increased externalization of P-selectin.[60] An increasing level of serum THPO was observed in APL patients with ATRA treatment, resulting in an increase in platelet counts.[61] A phase II clinical trial of 66 patients who were newly diagnosed with primary ITP reported that the proportion of patients with a sustained response (maintaining platelet counts at the level of at least 30×10^9/liter and at least twice higher than the baseline counts, without bleeding events and no rescue medication being required in 6 months) in the ATRA plus high-dose dexamethasone group (68%) was significantly higher than that of high-dose dexamethasone monotherapy group (41%), representing a novel therapeutic regimen for ITP.[62] Compared to low-dose rituximab (LD-RTX) monotherapy, ATRA plus LD-RTX increased the overall and sustained response for corticosteroid-resistant or relapse ITP patients (80% *versus* 59% of overall response and 61% *versus* 41% of sustained response).[63] These clinical investigations demonstrated ATRA might stimulate thrombopoiesis through a THPO-dependent manner, which may be beneficial in relieving thrombocytopenia, and drug combination is necessary.

Thrombopoiesis agents with potential value of clinical use

Catecholamine

Catecholamines are a group of tyrosine-derived neurotransmitters, including dopamine (DA), norepinephrine (NE), and epinephrine (EPI). DA has therapeutic effects on cardiovascular diseases (hypertrophy, myocardial ischemia, hypertension, and arrhythmia) and acute renal failure.[64] NE and EPI are commonly used to deal with severe allergic reactions, septic shock, and cardiopulmonary resuscitation.[65–67] Animal experiment demonstrated that EPI and NE in response to sympathetic stimulation could promote platelet production by the activation of α2-adrenoceptor-mediated ERK1/2 signaling, which induces MKs adhesion and migration, and the ERK1/2 activation-mediated RhoA GTPase signaling could stimulate proplatelet formation (PPF), platelet release, and platelet activation.[68] However, the insufficient evidence of the effect of exogenous NE and EPI on platelet production requires further investigation to clarify the association between medicinal NE and EPI with thrombopoiesis. As the precursor of NE and EPI, DA cannot accelerate the early stages in thrombopoiesis proceedings but promote production of platelet from MKs in the final stages of thrombopoiesis, relating to oxidative stress-mediated signaling pathways which consist of p38 MAPK, JNK/SAPK, and caspase-3 pathways in human primary MKs. In addition, administration of DA in mice could elevate platelet counts.[69] These findings of catecholamines on stimulating thrombopoiesis provide new insights into drug treatment of thrombocytopenia.

Estrogen

Estrogen, one of the primary sex hormones, plays various effects in relieving perimenopausal syndrome, osteoporosis, ovarian dysfunction, functional uterine bleeding, breast cancer (post-menopause), prostate cancer, and acne.[70–72] Estrogen receptors (ER), including ERα and

ERβ, which express on MKs, could mediate the promoting effect of MKs differentiation, PPF, and platelet production.[73,74] Estrogen-induced platelet production may be regulated by the ERβ-dependent pathway. Through detecting BM-derived MKs and Meg-01 cells of estrogen-treated mouse, it could be found that estrogen acts as a potential activator of GATA-1, then promotes the activation of STAT1 for MKs polyploidization, maturation, and platelet production. That is consistent with the result that platelet counts in estrogen-treated mice had a significant increase.[75] Evidence from clinical cases in postmenopausal women receiving estrogen replacement therapy showed an increase in MK numbers,[76] but the stable effect of estrogen on elevating platelet counts in human, especially thrombocytopenia patients still require further investigation.

Melatonin

Melatonin is a hormone synthesized and secreted mainly from the pineal gland.[77] It is mainly used as a drug to treat sleep disorders, such as insomnia and circadian rhythm disorders.[78] It could be potentially beneficial to the treatment of neurological diseases, cardiovascular diseases, bone diseases, and cancers.[79–82] Therapeutic potential of melatonin in thrombocytopenia is under development. Melatonin could promote thrombopoiesis by increasing HSCs and MKs expansion, PPF, and platelet formation.[83] From a molecular perspective, melatonin-induced thrombopoiesis might be mediated by ERK1/2 and Akt signaling.[83] Besides stimulating thrombopoiesis, melatonin could inhibit MKs apoptosis by activating Akt signaling through melatonin receptors.[84] Recent clinical evidence supported that compared with placebo, melatonin treatment could significantly enhance platelet counts (from 175.67 ± 92.84 to 191.10 ± 98.82) in patients who were affected by liver disease, reflecting its potential in thrombocytopenia treatment.[85] Further clinical evidence on patients with different kinds of thrombocytopenia is required, and melatonin is hopeful to become novel choice in the drug treatment of thrombocytopenia.

Recombinant human growth hormone

Recombinant human growth hormone (rhGH) is used for replacing treatment of growth hormone deficiency.[86] It also acts as the thrombopoiesis-stimulating agent which promotes various steps in platelet development, including the late stage of MK differentiation, PPF, and production of platelet.[87] Treatment of rhGH could activate STAT5. In an early phase, activation of ERK1/2 proceeds slowly but in a prolonged period in M07e megakaryoblastic cells and primary MKs treated with rhGH. However, activation of Akt was only observed in mature MKs but not in M07e megakaryoblastic cells, implying that the terminal differentiation of MKs induced by rhGH may be attributed to the Akt pathway.[87] Moreover, through significantly accelerating and promoting platelet production, rhGH was manifested to strengthen the effect of a tandem dimer of thrombopoietin mimetic peptide (dTMP) on thrombopoiesis in M07e cells and mice model of radiation-induced thrombocytopenia (RIT), denoting that rhGH and c-MPL may achieve thrombopoiesis complementarily and synergistically.[87] More clinical evidence is required to confirm the promoting effect of rhGH on relieving thrombocytopenia, especially in combination with THPO and THPO-RAs.

Clozapine

Clozapine is an antipsychotic drug effective for resistant schizophrenia.[88] However, its stimulating effects on the hematopoietic system, such as agranulocytosis, leukocytosis, and thrombocytosis, are recognized as significant side effects.[89,90] A follow-up study about clozapine's stimulating effect on patients' thrombopoiesis of a 1-year period demonstrated that since the beginning of clozapine treatment in the first week, platelet counts increased synchronically but transiently, and the early spike in platelet counts reflected raised IL-6 levels, which induced thrombopoiesis through the gp130 signaling and interaction with IL-3 and THPO.[91,92] This result suggests that IL-6 might be the crucial factor in clozapine-induced thrombocytosis. However, the direct evidence of clozapine's interactions with IL-6 or other important factors such as THPO to promote thrombopoiesis is required for affirming the relevance of clozapine to the platelet development process.

Yes-associated protein inhibitors

Yes-associated protein (YAP) is an effector molecule in the Hippo pathway, which plays an

essential role in MKs proliferation, differentiation, and production of platelet.[93] Dobutamine and verteporfin are two Food and Drug Administration (FDA)-approved YAP inhibitors. Dobutamine is commonly used for septic shock and heart failure,[94,95] and verteporfin for cancer, age-related macular degeneration (AMD), central serous chorioretinopathy (CSC), ocular histoplasmosis syndrome, and pathologic myopia.[96–98] *In vitro* experiment revealed that dobutamine and verteporfin could induce megakaryocytic cell maturation and generate thrombopoiesis.[99] Dobutamine phosphorylates YAP, inhibits YAP translocation into the nucleus, and interacts with its target genes.[99] Verteporfin inhibits the interaction between YAP and transcriptional enhanced associate domain (TEAD) and downregulates the expression of their downstream target genes.[99] Treatment with dobutamine or verteporfin on MEG-01 cells results in an increasing level of MKs maturation, differentiation, and production of platelets, and their thrombopoiesis-stimulating effects may be related to upregulation of Fli-1,[99] indicating this group of agents' potential effect in the treatment of thrombocytopenia.

Natural agents that promote platelet production

Ingenol. Ingenol is a natural product with potential effect in the treatment of warts, skin keratoses, and cancers by activating PKC-α/δ.[100,101] Drug screening found that ingenol could promote differentiating MK and producing platelet. Specifically, it could stimulate differentiation of MK in K562 and HEL cells, and accelerated thrombopoiesis in RIT mice model, as an increasing platelet counts and MKs in BM and spleen was seen.[102] RNA-sequencing revealed that the thrombopoiesis-stimulating process of ingenol may foster through PI3K-Akt signaling.[102] Especially, using c-MPL knock-out mice or deleting THPO or c-MPL by their neutralizing antibodies in K562 cells could not suppress the thrombopoietic effect of ingenol, indicating that ingenol may be a THPO-independent thrombopoiesis-stimulating agent, which provides a potential medicine for thrombocytopenia treatment.[102]

3,3′-di-O-methylellagic acid 4′-glucoside. 3,3′-di-O-methylellagic acid 4′-glucoside (DMAG), a natural ellagic acid derived from Sanguisorba officinalis L. (SOL), has potential antioxidant and hepatoprotective effect.[103] DMAG is found to have thrombopoiesis-stimulating effect as increasing differentiation of MKs was observed in the HEL cells with DMAG treatment.[104] According to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, molecular docking simulation, molecular dynamics simulation, and western blot analysis in the human erythroleukemia (HEL) cells, DMAG could bind to TLR2, ITGB3, and ITGA2B and activate the PI3K-Akt signaling pathway.[104] In mice model of RIT, DMAG could increase the number of von Willebrand factor (VWF)-positive MKs and platelets, with no difference in the mean platelet volume (MPV) compared with THPO treatment group.[104] The function of DMAG-induced platelets is assessed by platelet adhesion, platelet aggregation, and tail bleeding time assays, indicating the potential value of DMAG on platelet recovery of thrombocytopenia treatment.[104]

Conclusion

Thrombopoiesis is a complex process that offers various targets for different thrombopoiesis-stimulating agents. THPO–MPL is the primary pathway of regulating thrombopoiesis. Targeting directly the THPO–MPL pathway or its downstream pathways, such as JAK/STAT, ERK1/2, and PI3K-Akt signaling, accounts for many thrombopoiesis agents. Meanwhile, other pathways, such as the ERβ-dependent pathway and the Hippo pathway, are significantly effective in thrombopoiesis, and drugs promote thrombopoiesis independent to THPO signaling may reveal novel regulator pathway of platelet development.

Some thrombopoiesis drugs are in clinical treatment for various kinds of thrombocytopenia. THPO-RAs are the most commonly used thrombopoiesis-stimulating agents in the treatment of thrombocytopenia as an upgrade alternative of recombinant THPOs. IL-11-based agents and ATRA might be beneficial to promoting platelet production, but they usually play a role as an assistant medicine in the combined therapy for ITP, with little clinical evidence in the treatment of other kinds of thrombocytopenia. However, the risk of drug-induced thrombocytosis and thrombosis events should be considered when using these drugs in clinical practice.

Further investigations are needed for other thrombopoiesis-stimulating agents which are

newly found to promote platelet production and may show potential value in supplementing treating regimens for thrombocytopenia. Some agents discussed above such as neurotransmitters – EPI, NE and DA, hormones – estrogen and rhGH, and melatonin, antipsychotics, such as clozapine, two YAP inhibitors and some natural products, their thrombopoiesis-stimulating effects and potential signaling pathways are investigated. Still, more thrombopoiesis agents are investigated, but the underlying mechanisms are unknown, which worth introducing briefly. Valproic acid (VPA) was found to induce MK polyploidy, maturation, PPF, and PLP formation,[60] and upregulation of FLI-1 was detected in VPA-treated MEG-01 cells.[99] The thrombopoietic effect of VPA may be different from most HDACIs that inhibit platelet production and cause thrombocytopenia,[105–107] suggesting the specificity of VPA in stimulating thrombopoiesis. BKT140, a CXCR4 antagonist, could induce the development of MKs and stimulate the production of platelet progenitors, potentially reducing the severity and duration of CIT in mice, however, thrombopoiesis-stimulating effects are not found in other CXCR4 inhibitors.[108] Traditional drug for ITP treatment, such as vinca alkaloids (VAs), may have thrombopoiesis-stimulating effects apart from commonly known immunosuppressive effect, according to animal experiment in the rat.[109–111] The development of novel drug screening models has become an effective way for investigating thrombopoiesis-stimulating agents. Using the b1-tubulin reporter line with imMKCL-derived iPSCs, two agents, Wnt-C59 and TCS-359, which promote MK maturation and PLP release, were successfully found.[112] After investigation, figuring out the definite mechanism of their thrombopoietic effect are vital for developing novel medicine in clinical treatment of thrombocytopenia.

The future of thrombocytopenia treatment is encouraging for many thrombopoiesis-stimulating agents which are in clinical trials or early investigations. Thrombopoiesis-stimulating agents could deal with different types of thrombocytopenia, such as ITP, CIT, CLD, and IT, according to clinical evidence. Another large amount of thrombopoiesis-stimulating agents is not yet in clinical use, but research evidence has proved their potential effects in thrombocytopenia treatment to a certain extent. Most agents play their effects through THPO–MPL or its downstream signaling pathways, and some novel pathways regulating platelet development should be highly regarded. The development of thrombopoiesis-stimulating agents may enrich the pharmacological armamentarium for medical treatment of thrombocytopenia.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contributions

Lejun Huang: Investigation, Resources, Writing – original draft.

Jianxuan Xu: Resources, Writing – original draft.

Huaying Zhang: Resources.

Mengfan Wang: Investigation.

Yiyue Zhang: Writing – review & editing.

Qing Lin: Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

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ORCID iDs

Lejun Huang https://orcid.org/0000-0002-1194-6921

Jianxuan Xu https://orcid.org/0000-0003-1865-1656
Huaying Zhang https://orcid.org/0000-0003-2727-9884
Qing Lin https://orcid.org/0000-0001-8087-9112

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