

Role of romiplostim in splenectomized and nonsplenectomized patients with immune thrombocytopenia

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Abstract: Romiplostim is a thrombopoietin receptor agonist (TPO-RA) used for the treatment of adult primary immune thrombocytopenia (ITP). ITP is an autoimmune condition characterized by low platelet counts due to increased destruction and reduced platelet production. First-line interventions include corticosteroids, anti-D, and intravenous immunoglobulins, while second-line therapies comprise splenectomy, rituximab, cyclosporine A, and TPO-RAs. The recognition that compromised platelet production is a critical part of the pathogenesis of ITP prompted the development of therapeutic strategies based on the stimulation of the TPO receptor. TPO-RAs enhance megakaryocyte proliferation, increase platelet production, and lead to a reduction in bleeding episodes in ITP patients. This review will summarize current data on the TPO-RA romiplostim, with a particular focus on its relation to splenectomy.

Keywords: idiopathic thrombocytopenic purpura, thrombopoietin mimetic peptide, romiplostim, splenectomy, blood platelets

Platelets in immune thrombocytopenia

Werlhof's disease or Morbus Maculosus haemorrhagicus was described by Paul Gottfried Werlhof in 1753.¹ This is likely to be the same condition mentioned in 1557 as "Morbus Pulicaris Absque Febre" by Amatus Lusitanus in his *Curationum Medicinalium Centuriae Quatuor*.² The description by Lusitanus of dark spots without fever is likely to have been a manifestation of immune thrombocytopenia (ITP).³ In 1658, Riverius postulated that the dark spots were due to "thinness of the blood."

The intervening years led to the discovery of blood granules that were later termed platelets. With the description of platelets by Bizzozero in 1882,⁴ it was then possible to associate number of platelets with ITP. In 1887, Denys⁵ observed that the platelet number dropped during the episode of purpura and that there was an increase in platelet count after the hemorrhagic episode.

Why were platelets low in ITP? As documented by Bedson,⁶ the first antiplatelet sera was described by MF Marino in 1905. A decade later, in 1915, JCG Ledingham showed that anti-guinea pig platelet serum was noxious to guinea pigs and produced a condition analogous to ITP in humans. The well-known Harrington-Hollingsworth experiment clearly demonstrated that a factor in plasma was able to significantly reduce platelet number in healthy subjects transfused with plasma from ITP patients.⁷ This evidence appeared to demonstrate that accelerated platelet destruction was the key initiating event in ITP. These platelet-damaging factors in plasma are immunoglobulins that recognize abundant platelet receptors such as GPIIb/IIIa and GPIb/IX. Several

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studies have shown that a large proportion of ITP patients have both platelet-associated and circulating antiplatelet autoantibodies.^{8–10} The coating of platelets by antiplatelet autoantibodies then leads to Fc receptor-mediated phagocytosis.¹¹ There is evidence for additional mechanisms that may cause a reduction in circulating platelets. For instance, destruction of autologous platelets by cytotoxic T-lymphocytes has been observed in chronic ITP patients.^{12,13} Lately, a new mechanism was described that may account, at least in part, for platelet destruction in ITP. The loss of sialic acid moieties from platelet glycoproteins (termed desialylation) due to autoantibody activity increases platelet destruction by liver cells.^{14,15} Thus, several mechanisms are responsible for platelet destruction in ITP. However, as will be discussed (and crucially for the mechanism of action of thrombopoietin receptor agonist [TPO-RAs]), platelet destruction is only a partial explanation, and a reduction in platelet production by megakaryocytes is fundamental for the pathogenesis of ITP.

Role of megakaryocytes in ITP

In 1890, soon after Bizzozzo's description of the platelet, Howell¹⁶ described the megakaryocyte and sometime later in 1906 Wright¹⁷ proposed that these cells were the source of platelets. In the 1940s, it was shown that patients with ITP had normal (or slightly increased) megakaryocyte numbers, but crucially a large proportion of these cells did not form platelets.¹⁸ This suggests that the observed reduction in platelet number is also a consequence of insufficient production. It is now clear that antiplatelet autoantibodies interact with glycoproteins on megakaryocytes.^{19–21} The consequence of this interaction is detrimental to megakaryocytic progenitor cells²² and, as will be described, also to mature megakaryocytes.^{21,23–25} Observations in culture have indicated that ITP autoantibodies have a negative impact on megakaryocyte differentiation, polyploidization, and proliferation.^{26,27} More recently, it was shown that antiplatelet autoantibodies from drug-induced ITP inhibited megakaryocyte maturation, proliferation, and proplatelet formation.²¹ Iraqi et al²³ demonstrated that the treatment of cord blood-derived mature megakaryocytes with IgG purified from ITP plasma inhibited proplatelet formation and platelet release in culture. Both inhibition of proplatelet formation and a reduction in proplatelet complexity have also been observed after treatment of megakaryocytes with anti-GPIIb/IIIa antibodies isolated from ITP patients.²⁴ Together these studies indicate that antiplatelet autoantibodies interact with megakaryocytes and suppress platelet production.

Treatment

Treatment of ITP is initiated if the platelet count is under $30 \times 10^9/L$ or if signs of bleeding are present. Depending on other factors (comorbidities, risk of bleeding), a platelet count of $<50 \times 10^9/L$ may warrant intervention.²⁸ The aim of the intervention is to reduce the likelihood of bleeding by maintaining an adequate platelet level. Corticosteroids (prednisone, dexamethasone) are the first line of therapy²⁸ and act by reducing phagocytosis and by decreasing autoantibody production. Other first-line options are intravenous immunoglobulin (IVIg) and anti-D. The effectiveness of IVIg and anti-D in ITP relate to the suppression of platelet destruction. IVIg may decrease platelet destruction via the inhibition of Fc γ receptors and by exerting a suppressive effect on the production of autoantibodies by B-cells. IVIg may also compete with and promote the clearance of the autoantibody.²⁹ These first-line treatments do not provide a permanent platelet response in most patients.

The second line of ITP therapy includes cyclosporine A, rituximab, splenectomy, and TPO receptor agonists.³⁰ The depletion of B cells with anti-CD20 antibody (rituximab) is effective in ITP. The initial study by Cooper et al³¹ demonstrated a response in 54% of subjects and a lasting response in 32%. An analysis of 72 adults and 66 children who responded to standard rituximab administration found that 26% of patients achieved treatment-free response after 5 years.³² Recently, a new triple therapy given over 4 weeks was developed in our hospital.³³ This intervention consists of high-dose dexamethasone, cyclosporine A, and low-dose rituximab. The trial resulted in relapse-free survival of 76% after 2 years.³³ This indicates that by using current drugs a lasting remission might be attainable in a large percentage of cases and merits further investigation.

Splenectomy

The use of splenectomy as a treatment for ITP was first proposed by Kaznelson. The hypothesis was that ITP was caused by excessive destruction of platelets by the spleen. The first splenectomy performed in an ITP patient was successful and resulted in a rapid increase in platelet number and resolution of the purpura.³ This established the use of splenectomy as the therapy of choice for cases of severe ITP and it remained as the sole therapeutic choice until the 1950s (a second therapeutic option only became available in the middle of the 20th century with the introduction of corticosteroids). To date, splenectomy remains a central option for adults that do not respond to steroid treatment.³⁴ It should be noted that

splenectomy is an effective treatment and is the only intervention that leads to a complete response in a large number of patients.³⁵ A systematic review of the literature (2,623 adults) found that there was a stable complete response in two thirds of ITP patients³⁴ (median follow-up 28 months). Mikhael et al,³⁶ who reviewed 23 studies conducted between 1991 and 2008, reached a similar conclusion. They reported a failure rate in 28% of cases 5 years after splenectomy.³⁶ There are complications associated with the procedure, including the possibility of postoperative bleeding, sepsis, and thrombosis. The rate of splenectomy is influenced by patient choice. Factors that affect decision-making include impact of ITP in the patient's life, personal opinion of the likely success of the intervention, and consideration of splenectomy as a treatment of last resort.³⁷ There may also be some reluctance to splenectomy by practitioners given that it is an invasive procedure that does not eliminate the possibility of relapse.³⁸ It has been proposed that splenectomy could be delayed for up to 3 years due to the increased likelihood of remission in that period.³⁹ Moreover, removal of the organ causes loss of functions such as antibody production and effective elimination of nonfunctional blood cells. In fact, the rate of splenectomy as a treatment for ITP appears to be in decline.⁴⁰

Thrombopoietin receptor agonists

The rebound in platelet levels following thrombocytopenia was ascribed to a substance termed thrombopoietin (TPO or THPO) in the 1950s (see review by Kaushansky⁴¹). Isolation and eventual cloning of TPO was a demanding undertaking and took decades to accomplish. Cloning and biological evaluation of murine TPO in 1994⁴² was followed by the demonstration that TPO sustained megakaryocyte colony formation and promoted differentiation and polyploidization both in vitro and in vivo.⁴³ TPO activity is mediated via activation of its receptor, c-Mpl. The cloning and characterization of TPO led immediately to proposals regarding its potential therapeutic use. The first recombinant preparations to be evaluated were human thrombopoietin (rhTPO) and PEGylated human megakaryocyte growth and development factor (PEG-rhMGDF). The former was the full-length protein produced in mammalian cell culture and the latter was expressed in bacteria and contained the TPO receptor binding domain (these compounds are now called first-generation TPO-RAs). These agents were promising in their capacity to increase platelet counts in conditions such as ITP,⁴⁴ myelodysplastic syndrome,⁴⁵ cancer,^{46,47} and HIV-associated thrombocytopenia.⁴⁵

The observation of severe thrombocytopenia in some subjects treated with PEG-rhMGDF ended the clinical application of the first generation of TPO-RAs. The drop in platelet counts was caused by the development of antibodies against PEG-rhMGDF that cross-reacted with and neutralized endogenous human TPO.⁴⁸

To circumvent potential detrimental immune reactions, efforts were directed toward the development of molecules structurally unrelated to TPO. Several TPO-RAs underwent development and two of these, romiplostim and eltrombopag, were approved for clinical use in 2008.^{49,50} Here, we will look specifically at studies using romiplostim.

Romiplostim is a peptibody consisting of four c-Mpl binding peptides (14 amino acids long) linked to two Fc domains of IgG1. The peptide has no sequence homology to TPO but competes for binding to its receptor.⁵¹ Engagement of romiplostim with c-Mpl leads to receptor activation, intracellular signaling, and increased platelet production.⁵² The lack of sequence homology suggests that if anti-romiplostim antibodies were developed, these would not interact with and neutralize endogenous TPO. Indeed, a study of 225 ITP patients treated with romiplostim found that 11% developed anti-romiplostim antibodies, but only one patient had neutralizing antibodies.⁵³ Importantly, none of these antibodies cross-reacted with endogenous TPO.⁵³ Of note, 17 out of 225 patients investigated had preexisting anti-romiplostim antibodies, but this did not affect the activity of the drug.

The effectiveness of romiplostim (also known as AMG 531, AMP-2, or Nplate®, Amgen Inc., Thousand Oaks, CA, USA) as a potential therapy for ITP was first shown in a dose-finding clinical trial.⁵⁴ AMG 531 was found to be effective in increasing platelet counts in ITP patients and did not produce significant adverse side effects.⁵⁴ In this study, most patients (79% in Phase I and 67% in Phase II) had undergone splenectomy,⁵⁴ but the response data were not collated based on this criterion.

Romiplostim and splenectomy

A study by Kuter et al⁵⁵ examined the activity of romiplostim in both splenectomized and nonsplenectomized ITP patients. The durable platelet response (defined as a platelet count of $\geq 50 \times 10^9/L$ during at least 6 of the last 8 weeks of treatment) was more pronounced in nonsplenectomized patients (56%) versus 38% in the splenectomized cohort.⁵⁵ The overall response (both transient and durable) was 88% and 79% for nonsplenectomized and splenectomized patients, respectively. On the other hand, a Japanese Phase III trial of

10 splenectomized and 12 nonsplenectomized ITP patients found no differential effect on weekly platelet response.⁵⁶

Even though treatment with TPO-RA requires ongoing drug administration and is not a single intervention like splenectomy, the potential adverse effects of splenectomy would argue against its preferential use as a second line of therapy. The adverse effects of romiplostim include headaches, fatigue, and nasopharyngitis, while for splenectomy adverse outcomes may include hemorrhage, infection, sepsis, and, in rare cases, death.³⁰ So far, the adverse effects of prolonged romiplostim use seem minor. An analysis of over 1,000 patients receiving romiplostim for a mean of 76 weeks found no increase in adverse effects including thrombosis, bone marrow reticulosis, or malignancy.⁵⁷ The fact that no increase in thrombosis was reported is important since ITP, despite low platelet counts, could be considered to be a prothrombotic condition.⁵⁸ In fact, splenectomy would be more concerning in this case since it is associated with an increase in thrombotic events.⁵⁹ Analysis of 13 clinical trials (653 patients) treated with romiplostim for extended periods (up to 5 years) found that the treatment was tolerated and there were no salient safety concerns in terms of hematopoietic malignancies, bone marrow reticulosis or thrombotic events.⁶⁰ A study of 234 nonsplenectomized patients receiving either romiplostim or standard of care reported better platelet response, improved quality of life, and lower rates of splenectomy (9% versus 36%) in patients receiving romiplostim.⁶¹ It should be noted, however, that a meta-analysis of 15 studies with 3026 thrombocytopenic patients concluded that there was an increased risk of thromboembolism in patients treated with TPO-RAs relative to controls (frequency 3.69% versus 1.46%, respectively).⁶² However, the statistical significance was principally obtained from non-ITP thrombocytopenic patients such as those with chronic liver disease. The authors did not present subgroup analysis of splenectomized and nonsplenectomized subjects. This study lends some support to the European guidelines, which allow romiplostim treatment in nonsplenectomized patients only if splenectomy is not an option.⁶³ The guidelines recommend the use of romiplostim in splenectomized patients if they are refractory to standard treatments.

The American Society of Hematology (ASH) guidelines regard splenectomy as an intervention that achieves remission in most patients. Therefore, the ASH guidelines would consider splenectomy as an option following failure of corticosteroid therapy.⁶⁴ The international consensus group, on the other hand, gives equal consideration to splenectomy as to other second-line treatments such as rituximab, cyclosporine A,

and TPO-RAs.³⁰ Splenectomy remains the only option for patients that are refractory to both first- and second-line therapies, but the procedure is advisable only if satisfactory platelet counts are not obtained for some time.²⁸

Refractoriness

Refractoriness refers to the proportion of ITP patients that does not respond to treatment. In the case of splenectomized patients, it refers to persistent low platelet counts and the requirement for additional therapy to maintain adequate levels.⁶⁵ Why would refractory patients respond to TPO-RAs? The dual nature of ITP, ie, increased platelet destruction and decreased platelet production, suggests that if platelet production is sufficiently suppressed, then interventions that prevent platelet destruction, such as corticosteroids, IVIg, rituximab, and splenectomy, would not be adequate to overcome the platelet deficit. Therefore, only patients with higher levels of platelet production would be expected to be more responsive to inhibition of platelet destruction. TPO-RAs are the only option that deals with suboptimal platelet production and are likely to benefit preferentially patients with impaired platelet formation. The fact that splenectomized patients responded well to romiplostim treatment^{55,56} indicates that lack of platelet production was a major underlying cause in responders. Dual therapy with IVIg and romiplostim in patients unresponsive to either therapy has been successfully used to raise the platelet levels prior to splenectomy.⁶⁶ It is likely that in these patients splenic platelet destruction was severe and could not be overcome by the activity of romiplostim alone. An unexpected benefit of TPO-RA administration is the sustained remission achieved by certain patients after cessation of therapy.^{67,68} Remission seems to be independent of splenectomy status, sex, or age.⁶⁵ A potential mechanism is the restoration of immune tolerance by Tregs.⁶⁹

Why are not all ITP patients responsive to TPO-RAs? Nonresponsiveness to TPO-RAs may be due to intrinsic properties of the antiplatelet autoantibodies. It is clear that the known activity of TPO-RAs (ie, induction of megakaryopoiesis) still occurs in nonresponders, but platelet release is somehow blocked by the effect of the autoantibody.⁷⁰ There might be several reasons for deficient platelet release by megakaryocytes, for instance, defective signaling pathways or mutations; however, it appears that the activity of different classes of autoantibodies, such as anti-GPIIb/IIIa antibodies, might prevent platelet release.²⁵ Experimental evidence using cultured megakaryocytes indicates that, in the presence of ITP autoantibodies, TPO-RAs are capable of increasing the proportion of megakaryocytes producing

proplatelets from an existing population.²³ This implies that there is an additional mechanism in which TPO-RAs operate on mature megakaryocytes, overcome the harmful activity of the antiplatelet antibody, and promote proplatelet formation. Importantly, TPO-RAs were not effective in the presence of some of the autoantibodies tested,²³ suggesting that the nature of the autoantibody (specificity, binding site, affinity) is a substantial determinant of the outcome. Future work will substantiate this proposed property of TPO-RAs.

Conclusion

For ITP patients refractory to other treatments, splenectomy remains the only therapy that provides the prospect of complete remission. The development of romiplostim has seen the addition of a platelet-boosting drug to the treatment of ITP. Romiplostim administration generates a stable platelet response in a majority of patients without significant adverse effects and results in a reduced number of patients undergoing splenectomy. Ongoing observation is required to establish its safety and effectiveness after long-term use.

Disclosure

The author reports no conflicts of interest in this work.

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