Thrombopoietin

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ABSTRACT

Thrombopoietin is synthesized in hepatocytes and the kidney. After it enters the blood stream, thrombopoetin is transported to the bone marrow. The receptor for thrombopoietin is known as c-mpl, which is expressed on the surface of platelets, megakaryocytes and Pluripotent Stem Cells (PSCs). The function of thrombopoietin is as a regulator of Colony Forming Unit (CFU)-Meg proliferation and as a stimulator for megakaryocyte maturation and platelet production. The plasma concentration of thrombopoietin is regulated by the platelet number. During thrombocytopenia, the plasma concentration of thrombopoietin is increased, and the platelet production is stimulated. On the other hand, during thrombocytosis, a large number of platelets will remove thrombopoietin from the circulation and the plasma concentration of thrombopoietin will decrease. The clinical application of thrombopoietin is to stimulate the number of platelets in thrombocytopenia induced by chemotherapy. Two forms of recombinant thrombopoietin have been developed for clinical use, i.e.: recombinant Human Megakaryocyte Growth and Development Factor (rHuMGDF) and Polythylene Glycol (PEG)-rHuMGDF. Administration of rHuMGDF is preferred to PEG-rHuMGDF, because the later can stimulate an antibody reaction. In addition, thrombopoietin is also required for the maintenance of PSC, stimulation of PSC proliferation, and mobilization of PSC to peripheral tissues. The concentration of thrombopoietin can be determined by a highly sensitive enzyme-linked immunosorbent assay using a monoclonal antibody. Under normal conditions, the plasma concentration of thrombopoietin ranges from 12 pg/mL to 61 pg/mL.

Keywords: Thrombopoietin, c-mpl, Trombocytopenia

INTRODUCTION

Thrombocytopenia is a common clinical problem requiring treatment to prevent or deal with the bleeding that it may cause. Every year, approximately 8 million units of platelet concentrate are transfused into patients with thrombocytopenia to reduce the risks of severe bleeding. Platelet transfusion is not an ideal form of treatment. At least 30% of transfusion create complications, fever being one of them. Now, there is another way to deal with post-chemotherapy thrombocytopenia, which is administration of thrombopoietin to stimulate the production of megakaryocytes in bone marrow.[1,2]

Thrombopoietin was first found by Kelemen in 1958. In 1993, Wendling found cellular myeloproliverative leukemia (c-mpl), as a thrombopoietin receptor.[3,4] In 1994, de Sauvage et al, Lok et al, Bartley et al, Kuter et al, and Kato et al were able to isolate the thrombopoietin molecule. This paper discusses the structure, receptor, synthesis, thrombopoiesis, function, clinical use, and evaluation of thrombopoietin.

THE STRUCTURE OF THROMBOPOIETIN

Thrombopoietin is a polypeptide consisting of 353 amino acids. Thrombopoietin is made up of two different domains. The first domain is the erythropoietin-like domain, which binds the thrombopoietin receptor and rims the biological function of thrombopoietin. The second domain is a carbohydrate-rich domain. Carbohydrates are bound to several amino acids, which are serine (S), threonine (T) dan asparagine (N).[1,3]

There are two forms of recombinant thrombopoietin, the full length thrombopoietin (recombinant human megakaryocyte growth and development factor = rHuMGDF) and the truncated form produced by *E. coli*, which does not have a carbohydrate (non-glycocylation). This form only contains the receptor-binding region and contains an additional polyethylene glycol (PEG), thus known as pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF).[1,3]

Thrombopoietin Receptor

The receptor for thrombopoietin is c-mpl. The gene for c-mpl is located on the short arm of chromosome 1 region 34 and consists of 12 exons. This receptor is expressed on the surface of the thrombocytes, megakaryo-

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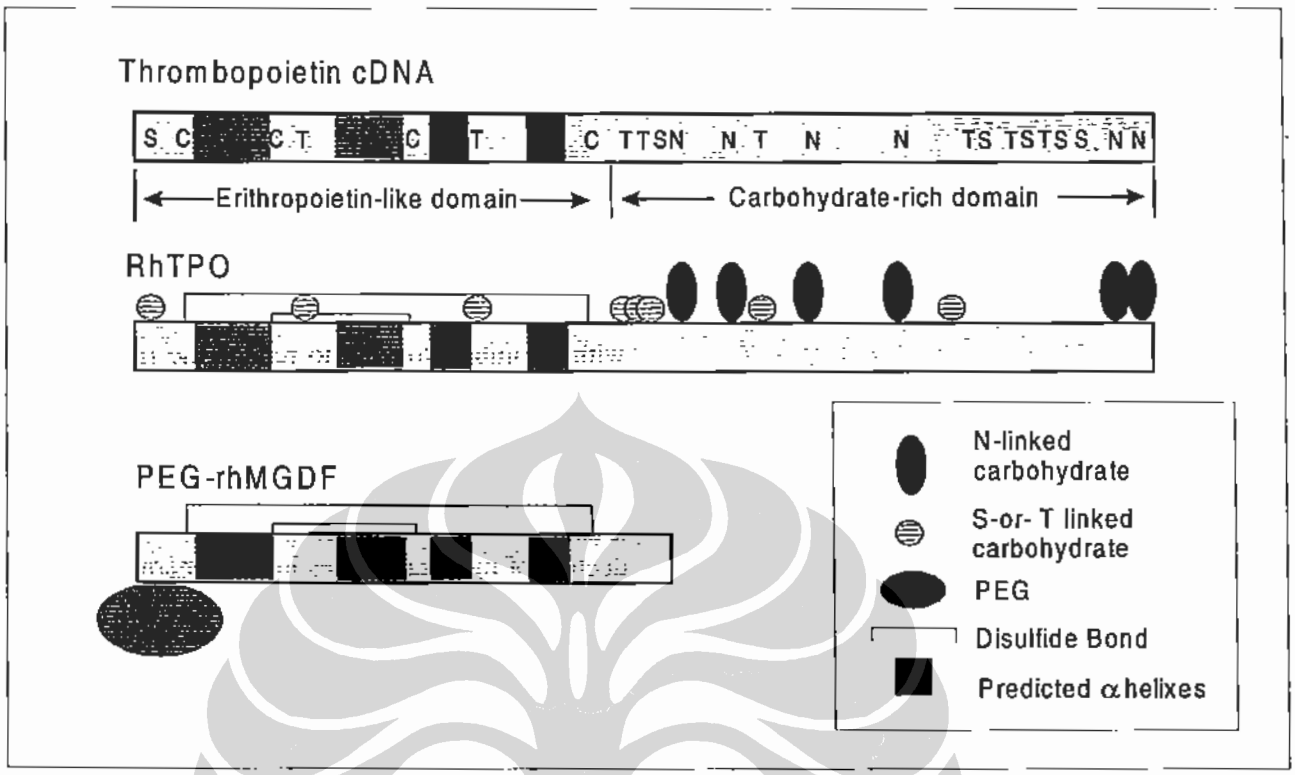

Figure 1. The Structure of Thrombopoietin[1]

cytes, and the surface of Pluripotential Stem Cell (PSC).[5,6] According to Drachman, thrombopoietin and its receptor are important for thrombopoiesis and maintenance of PSC hematopoiesis.[7]

Thrombopoietin Synthesis

Thrombopoietin is synthesized inside hepatocytes and in the kidneys, and is then secreted into the bloodstream.[1,4,6] Thrombopoietin synthesis by hepatocytes is influenced by γ interferon and tumor necrosis factor - α (TNF-α),[4] and is coded by a gene located on the long arm of chromosom 3, region 27. The gene is 6.2 kb long and contains six exons and five introns.[3,6]

In the bloodstream, thrombopoietin has half-life of approximately 30 hours. Under normal conditions, the plasma thrombopoietin level ranges between 12 to 61 pg/mL. The plasma thrombopoietin level is regulated by the platelet count.[6]

Thrombopoiesis

The development process from PSC to CFU-GEMM is influenced by steel factor and thrombopoietin. The development process from CFU-GEMM to CFU-Meg is influenced by steel factor, interleukine-3 (IL-3), interleukine-6 (IL-6), interleukine-11 (IL-11), leukemia inhibitory factor (LIF), granulocyte-colony stimulating factor (G-CSF) and thrombopoietin (Figure 2).[1,8,9,10]

CFU-Meg development into immature megakaryocytes is influenced by steel factor, IL-3, IL-6, IL-11, LIF, erythropoietin and thrombopoietin. The development of immature megakaryocytes into mature ones is influenced by IL-6, IL-11 and thrombopoietin[1] (Figure 2).

The Functions of Thrombopoietin

Thrombopoietin functions to stimulate the proliferation of CFU-Meg, the maturation and proliferation of megakaryocytes, as well as platelet production by megakaryocytes.[1,4]

Plasma thrombopoietin levels are regulated by the number of platelets. Within the bloodstream, thrombopoietin will bind with its receptor on the surface of platelets. During thrombocytopenia, there is relatively less thrombopoietin bound to its receptor on the surface of platelet, thus increasing its plasma levels. Subsequently, more thrombopoietin reaches the bone marrow. The increase in thrombopoietin in the bone marrow stimulates platelet production, thus increasing its number in the peripheral bloodstream. On the other hand, during thrombocytosis, there is a great number of thrombopoietin

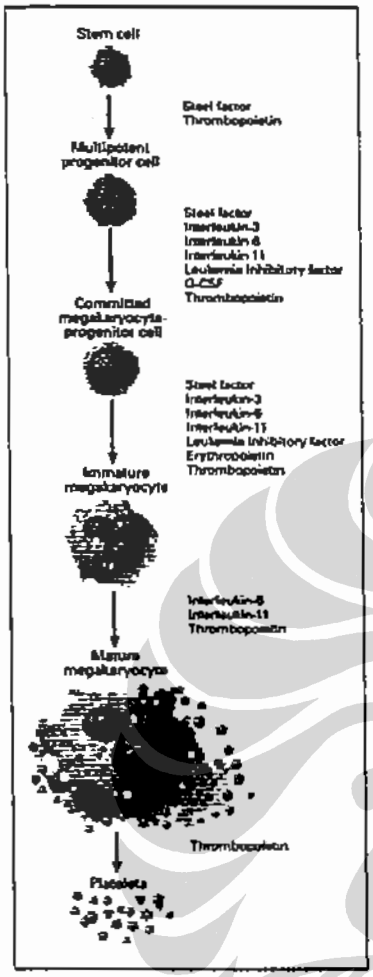

Figure 2. The Influence of Cytokine in Thrombopoiesis.[1]

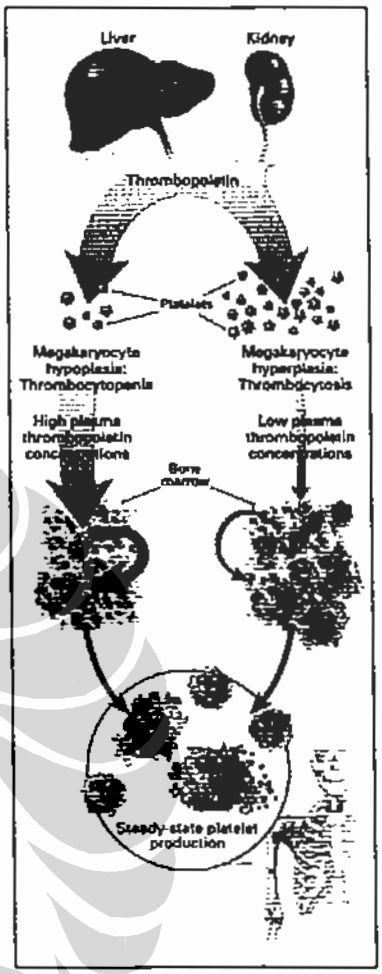

Figure 3. Thrombopoietin Regulation

bound to its receptor on the surface of platelets, thus reducing its plasma levels. Subsequently, less thrombopoietin reaches the bone marrow, thus inhibiting platelet production and reducing the number of platelets in the peripheral bloodstream (Figure 3).[1,11,12,13]

Congenital amegakaryocytic thrombocytopenia is a congenital disorder that is characterized by thrombocytopenia and the absence of megakaryocytes in the bone marrow. Most of this disease is caused by a mutation on both mpl alleles, drastically reducing thrombopoietin.[14] Congenital amegakaryocytic thrombocytopenia can only be treated with PSC transplantation. With the presence of normal hematopoietic PSC, well functioning thrombopoietin receptors become available.[7]

Thrombopoietin on its own or together with G-CSF can increase the mobilization of peripheral PSC. Administration of thrombopoietin combined with G-CSF is better able to increase the mobilization of peripheral PSC compared to administration of G-CSF alone. This can cause a lower rate of apheresis and a reduction in the need for transfusion during bone marrow regeneration.[15]

Thrombopoietin is also able to increase the survival and proliferation of PSC. Thrombopoietin can influence PSC by directly binding with receptors on the surface of PSCs or by stimulating other cells in the bone marrow micro-environment to produce growth factors.[16]

CLINICAL USE

Because of its ability to increase thrombopoiesis, thrombopoietin can be used in clinical conditions accompanied with thrombocytopenia, such as in patients receiving chemotherapy. The thrombopoietin that is commonly used is recombinant thrombopoietin.[2,3]

From a study of lung cancer patients undergoing chemotherapy who received subcutaneous rHuMGDF

for 16 days, it was found that the reduction in platelet count in patients receiving rHuMGDF was less than that in patients receiving placebo. The number of platelets also more rapidly returns to normal compared to those receiving only placebo.[2]

In a study of patients with advanced stage cancer receiving chemotherapy and subcutaneous rHuMGDF for 7-20 days, patients receiving rHuMGDF demonstrated less reduction in platelet count compared to those receiving placebo alone. The development of thrombocytopenia was slow and the return of platelet count to normal was also faster than in those receiving placebo alone.[2]

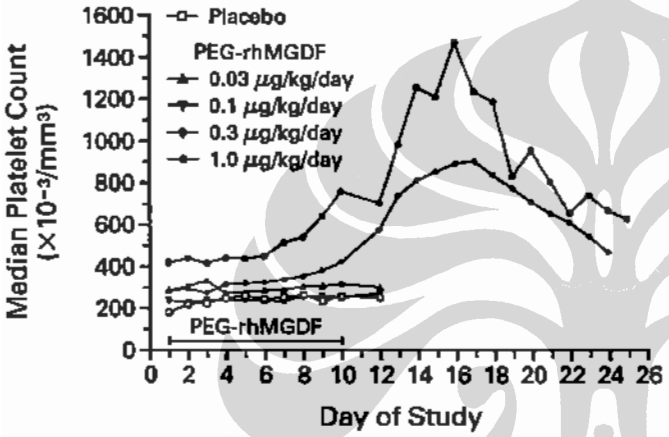

Figure 4. The Effect of PEG-rHuMGDF on the Number of Platelets[3]

In a study of lung cancer patients receiving chemotherapy, those receiving 0.3-1 ug/kg/day of subcutaneous PEG- rHuMGDF for 10 days demonstrated a more rapid return of platelet count compared to those receiving placebo. The platelet count started to increase on the sixth day and in average reached its peak on day 16 (see Figure 4). The platelet count continued to increase for several days after the termination of treatement. Administration of less than 0.3 ug/kg/day of PEG- rHuMGDF does not produce an increase in platelet count.[3,15]

According to Yang, quoted by Drachman, thrombopoietin administration in the form of PEG-rHuMGDF could stimulate the production of anti-thrombopoietin antibodies, while administration in the rHuMGDF form of does not cause antibody production. Thus, rHuMGDF is the form of thrombopoietin routinely used in vivo.[7]

EVALUATION OF THROMBOPOIETIN

Serum thrombopoietin level can be evaluated using the enzyme-linked imunosorbent assay (ELISA) method. In this method, a standard, serum, or blank is inserted into a micro-titer plate that has been coated with IgG monoclonal antibodies for thrombopoietin. horseradish-peroxidase conjugated anti-thrombopoietin antibody is then added. We then add a tetrametilbenzidin substrate, which will be broken down by peroxidase to form color. The reaction is terminated by adding acid and the immersion is read at a 450 nm wave length.

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