SAFETY AND EFFICCACY OF PLASMID MEDIATED THROMBOPOIETIN GENE THERAPY IN *THE TREATMENT OF THROMBOCYTOPENIA SECONDARY TO CHRONIC LIVER DISEASE*

Thesis submitted in partial fulfillment of the M.D degree

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Abstract

Key words: Thrombopoietin- TPO gene therapy-Hypersplenism.

Thrombocytopenia is a common complication in patients with chronic liver disease, it occurs in about 76% in cirrhotic patients.

Thrombocytopenia secondary to chronic liver disease is mainly due to; Thrombopoiein deficiency, hypersplenism, bone marrow suppression, and autoimmune mechanism.

Plasmid mediated Thrombopoietin gene therapy was effective in inducing thrombopooisis in cirrhotic patients however this effect was only transient, and there was no major side effects recorded in treated patients.

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Khalid El-Sherif

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LIST OF ABBREVIATIONS

ADP	Adenosine Deaminase
ATG	Antithymocyte Globulin
ATP	Adenosine Triphosphate
BM	Bone Marrow
BSS	Bernard Soulier Syndrome
CLD	Chronic Liver Disease
DIC	Disseminated Intravascular Coagulopathy
DMS	Demarcation Membrane System
ELISA	Enzyme Linked Immunosorbant Assay
EPO	Erythropoietin
FOG	Friend of GATA
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
Gp	Glycoprotein
Gp-Ib	Glycoprotein I beta
GTP	Guanosine Triphosphate
5-HT	5 Hydroxy Tryptamine

HBcAb	Hepatitis B Core Antibod
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HLA	Human Leucocytic Antigen
HSCs	Hematopoietic Stem Cells
Ig	Immunoglobulin
IL	Interleukin
IV Ig	Intravenous Immunoglobulin
ITP	Idiopathic Thrombocytopenic Ppurpura
LCF	Liver Cell Failure
MGDF	Megakaryocyte Growth and Development Factor
MK-CSF	Megakaryocyte Colony Stimulating Factor
MPV	Mean Platelet Volume
NO	Nitric Oxide
OLT	Orthotopic Lliver Transplantation
PAF	Platelet Activating Factor
PAIGs	Platelet Associated Immunoglobulin

PEGrhMGDF	Pegylated Recombinant Megakayocyte Growth and Development Factor
PDGF	Platelet Derived Growth Factor
PKC	Protein Kinase C
PNH	Paroxysmal Nocturnal Haemolobinuria
Rh TPO	Recombinant human thrombopoietin
Rh IL 11	Recombinant Human Interleukin 11
RFA	Radiofrequency Ablation
SCF	Stem Cell Factor
TGF-B	Transforming Growth Factor Beta
TPO	Thrombopoietin
vWF	Von-Willbrand Factor

INTRODUCTION

The platelet count in peripheral blood is controlled to maintain normal haemostasis. Thrombocytopenia is observed in various diseases, including chronic viral hepatitis especially in liver cirrhosis (LC). Earlier studies of platelet dynamics in chronic liver disease accompanying thrombocytopenia showed that splenic platelet pooling and accelerated destruction are the main causes of thrombocytopenia, ***(Aster, 1988).***

However, consistent improvement of thrombocytopenia was not obtained by portal decompression procedure Indeed, thrombocytopenia can persist after splenectomy. These findings suggest that other factors are involved in thrombocytopenia observed in liver disease, ***(Crane, 1982).***

Studies showed that decreased production of platelets by megakaryocytes due to low thrombopoietin concentration could be a possible cause of thrombocytopenia in liver cirrhosis, ***(Shimodaira el al, 1996).***

Since thrombopoietin (TPO), a regulator of thrombopoiesis, is produced mainly in the liver, decreased production of TPO may account for thrombocytopenia in liver diseases. TPO has been reported to be expressed in several organs, such as liver, kidney and muscle. Of these, liver is the major organ that produces TPO. Hepatoma cells also express TPO mRNA **,** ***(Hino et al., 995).***

It is reported that serum TPO was low in liver cirrhosis however, it was increased after orthotopic liver transplantation, which was followed by an increase in platelet count. These observations may support the hypothesis

that impaired production of TPO in liver disease contributes to thrombocytopenia associated with chronic viral hepatitis, also Serum TPO levels were positively correlated with prothrombin time and serum albumin ($P < 0.05$, in each case), and negatively correlated with indocyanine green test and Pugh score ($P < 0.01$ and $P < 0.05$ respectively). However, RT-PCR and immunohistochemistry showed that expression of TPO mRNA is similar in the different liver diseases, suggesting that serum TPO is a reflection of the total mass of functional liver***, (Michiko et al., 2ooo).***

TPO is synthesized primarily in the liver as a single 353-amino acid precursor protein. On removal of the 21-amino acid signal peptide, the mature molecule consists of 2 domains: a receptor-binding domain that shows considerable homology to erythropoietin and a carbohydrate-rich carboxy-terminus of the protein that is highly glycosylated and important in maintaining protein stability, ***(Foster et al., 1997).***

Since TPO was first cloned, several recombinant TPOs have been developed for clinical evaluation. There are 2 of these preparations, rhTPO and PEG-rHuMGDF, that have undergone considerable preclinical and clinical evaluation. The amino acid sequence of rhTPO is identical to that of endogenous TPO. rhTPO is produced in mammalian cells and is glycosylated. Nonetheless, its molecular weight is 90 kd, less than the 95 kd of the native molecule. PEG-rHuMGDF (Amgen, Thousand Oaks, CA), is produced in Escherichia coli and consists of the receptor-binding, 163 arnino-terminal amino acids of native TPO. It is conjugated to a 20-kd polyethylene glycol moiety to increase its circulatory half-life and possesses all the biologic activity of native TPO. These 2 recombinant thrombopoietins have similar pharmacologic characteristics and show profound in vitro and in

vivo effects on megakaryocyte development and platelet production, ***(Begley and Basser 2000).***

Recently, thrombopoietin (TPO), the ligand for the c-mpl receptor, has been isolated by several groups. It is the principal regulator of megakaryo-thrombopoiesis. It increases the number of megakaryocytes and platelets, and expands the number of megakaryocytic progenitor cells in vivo, ***(Kaushansky, 1995).***

TPO is a physiological factor mediating the feedback loop between circulating platelets and bone marrow megakaryocytes. Although the regulatory mechanisms of TPO are not fully understood, the level of TPO has been reported to be determined by the ability of platelets to remove TPO from the circulation, ***(Kuter et al., 1994)***. This hypothesis was confirmed by the reports that TPO mRNA is modulated by platelet count ***,(McCart et al., 1995),*** and serum TPO levels are directly regulated by c-mpl-mediated binding to platelets, ***(Fielder et al., 1996).***

A study was done to evaluate the efficacy and safety of recombinant human thrombopoietin (rhTPO) on chemotherapy-induced severe thrombocytopenia and it was concluded that administration of rhTPO after chemotherapy significantly reduces the degree and duration of thrombocytopenia and the need for platelet transfusions. In the same study the recorded side effects were transient fever, Knee arthralgia, dizziness, headache and chill, these side effects were mild and tolerable, ***(Bai et al., 2004).***

Another study to evaluate the efficacy and safety of human recombiriant thrombopoietin rhTPO) in chronic refractory idiopathic thrombocytopenic purpura and they found that, Consecutive subcutaneous

injection of rhTPO for a maximum of 14 days was associated with a temporary elevation in platelet counts in patients with chronic refractory ITP ***,(Zhao et al., 2004).***

AIM OF THE WORK

The aim of this study was to assess the safety and efficacy of plasmid mediated gene therapy for the treatment of patients with thrombocytopenia secondary to chronic liver disease, and to adopt this as DNA based therapy.

PLATELET FORMATION

Megakaryocyte development:

Megakaryocytes are rare myeloid cells (constituting less than 1% of these cells) that reside primarily in the bone marrow, **(Ogawa, 1993).** but are also found in the lung and peripheral blood. In early development, before the marrow cavities have enlarged sufficiently to support blood cell development, megakaryopoiesis occurs within the fetal liver and yolk sac. Megakaryocytes arise from pluripotent HSCs that develop into 2 types of precursors, burst-forming cells and colony-forming cells, both of which express the CD34 antigen, **(Briddlle et al., 1999).**

Development of both cell types continues along an increasingly restricted lineage culminating in the formation of megakaryocyte precursors that develop into megakaryocytes, **(Ogawa, 1993).** Thrombopoietin (TPO), the primary regulator of thrombopoiesis, is currently the only known cytokine required for megakaryocytes to maintain a constant platelet mass**, (Kaushansky, 2005).** TPO is thought to act in conjunction with other factors, including IL-3, IL-6, and IL-11, although these cytokines are not essential for megakaryocyte maturation, **(Kaushansky and Drachman. 2002).**

Megakaryocytes tailor their cytoplasm and membrane systems for platelet biogenesis. Before a megakaryocyte has the capacity to release platelets, it enlarges considerably to an approximate diameter of 100 μm and fills with high concentrations of ribosomes that facilitate the production of platelet-specific proteins, **(Long et al., 1982).**

Cellular enlargement is mediated by multiple rounds of endomitosis, a