THE EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN ON PLATELET FUNCTION IN CHRONIC HAEMODIALYSIS PATIENTS

Thesis

Sumited for partial fulfillment of
the master degree of Internal Medicine

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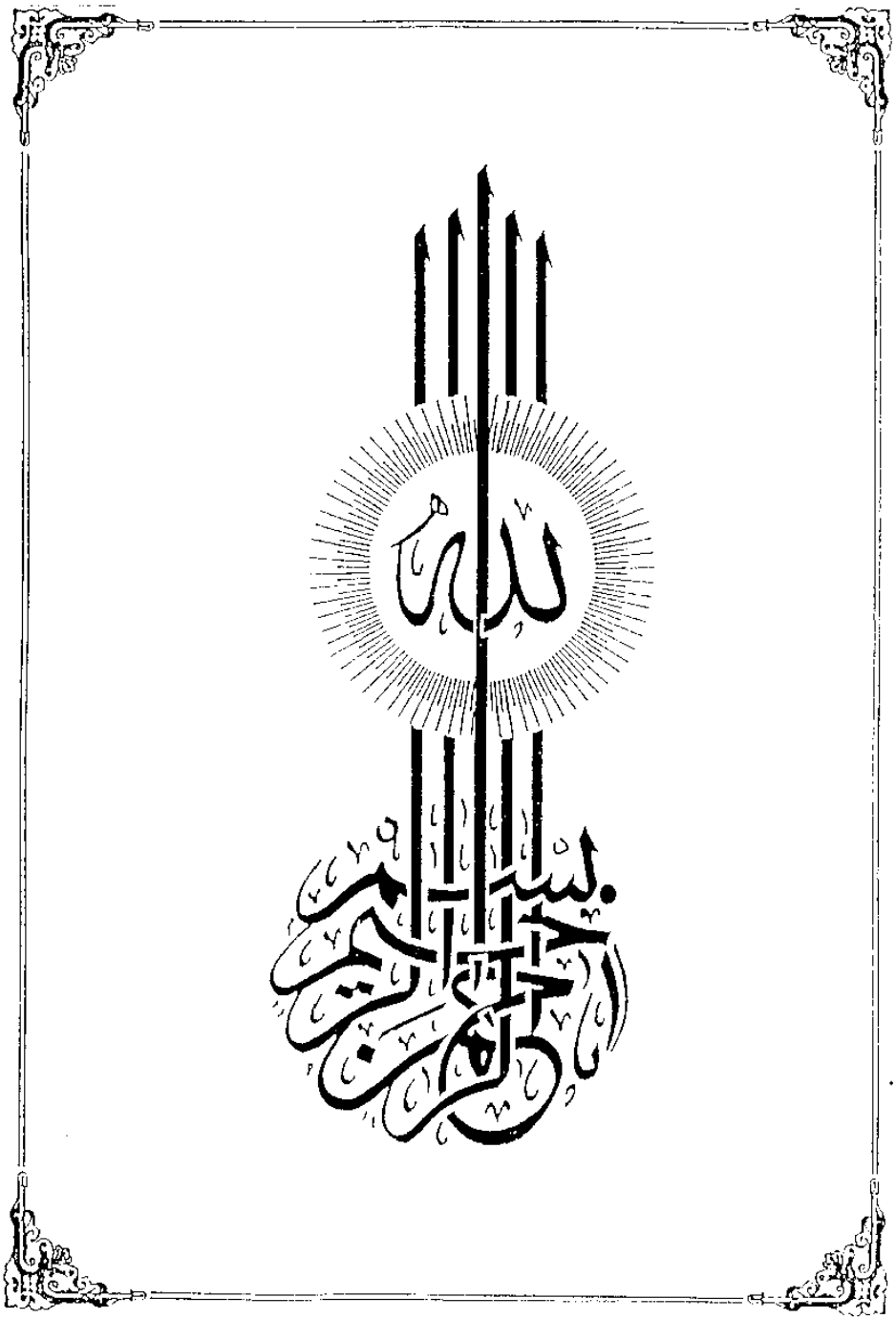
1995



ACKNOWLEDGEMENT

I wish to express my deepest gratitude and appreciation to professor Dr. Essam Mohamed Khedr , Professor of internal Medicine & Nephrology , Ain Shams University to whom I am greatly indebted and grateful for his supervision and valuable suggestions that enabled me to carry out this work .

I would like to pay a special gratitude to Professor Dr. Neven Ahmed Kasem , Ass. Prof. of clinical pathology , Ain Shams University for her generous help and valuable assistance.

My sincere thanks to Dr. Mohamed Ali Ibrahim , Ass. Prof. of Internal Medicine & Nephrology, Ain Shams University for his trustful help , unfailing advices, and kind guidance in this study.

My thanks are also to Dr. Mohamed Sherif , Lecturer of Internal Medicine & Nephrology , Military Medical Academy for his generous help and advice.

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INTRODUCTION

The presence of bleeding tendency in patients with chronic renal failure is well recognised and contributes to their morbidity and mortality.

Extensive investigations have failed to identify with certainity the exact cause of bleeding tendency in chronic renal failure; it is generally beleived that qualitative and / or quantitative platelet abnormalities are the most significant factors in the pathogenesis of uremic bleeding *(Rabiner, 1983).*

Another factor related to vessel wall endothelium is prostacyclin (PG I_2), which is generated from prostaglandin endoperoxides by vessel wall endothelium , it is the most potent inhibitor of platelet aggregation *(Ylikorkola et al., 1982).*

The anaemia, frequently present in chronic renal failure, has been implicated in the pathogenesis of the hemostatic defect of uremic patients; the prolonged bleeding time was found to be related to the severity of anaemia, and it has been demonstrated that red cell transfusions improve clinical bleeding , shorten the prolonged bleeding time and increase platelet adhesiveness to glass beads in hemodialysed patients *(Livio et al., 1982 ; Fernandez et al., 1985).*

Recombinant human erythropoietin (rHuEPO) has been proven to correct effectively the anaemia of chronic renal failure *(Winnearls et al., 1986 ; Eschbach et al., 1987)* , and also improvement in bleeding tendency in uremic patients after rising the hematocrit with (rHuEPO) have been reported *(Moia et al., 1987 ; Van Geet et al., 1989).*

This hemostatic effect has been related to the increase in red cell mass and the resultant rise in blood viscosity *(Moia et al., 1987)* ; also direct effect of (rHuEPO) on platelet function is suggested *(Castillo et al., 1993).*

However , an increase in platelet count and an improvement in platelet aggregability have also been reported during (rHuEPO) treatment *(Van Geet et al., 1989 ; Akizawa et al., 1992)* . Furthermore , a risk for thrombosis , such as A-V

fistular clotting , has been reported in uremic patients under (rHuEPO) treatment *(Casati et al., 1987 ; Schaefer et al., 1989 ; Sundal et al., 1989)* .

AIM OF THE WORK

The aim of this study is to analyze the possible effect of recombinant human erythopoietin (rHuEPO) treatment on platelet function in uremic patients under regular hemodialysis.

REVIEW OF THE LITERATURE

REVIEW OF THE LITERATURE

HEMOSTASIS & PLATELET FUNCTION :-

The participation of platelets in hemostasis is a fundamental component of this physiologic process . The reactions involved include adhesion to the cut end of a blood vessel , spreading of adherent platelets on the exposed subendothelial surfase , secretion of stored platelet constituants (including molecules involved in hemostasis and wound healing) , and formation of large platelet aggregates *(Johnson et al ., 1979)* .

PRODUCTION AND KINETICS :

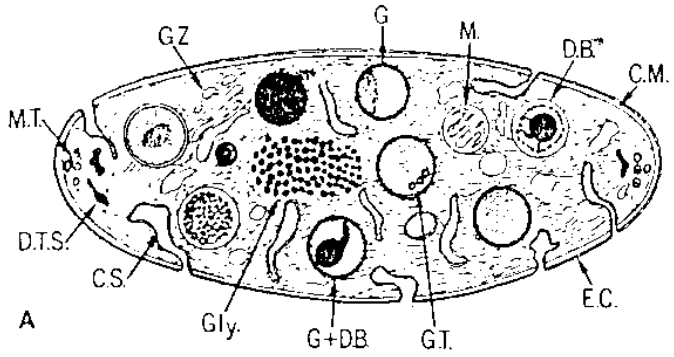
The platelets develop in the inter medullary components of bone marrow . The platelet precursor of megakaryocyte undergoes a number of nuclear divisions leading in general to a 16 lobed cell which then begins to produce platelets .

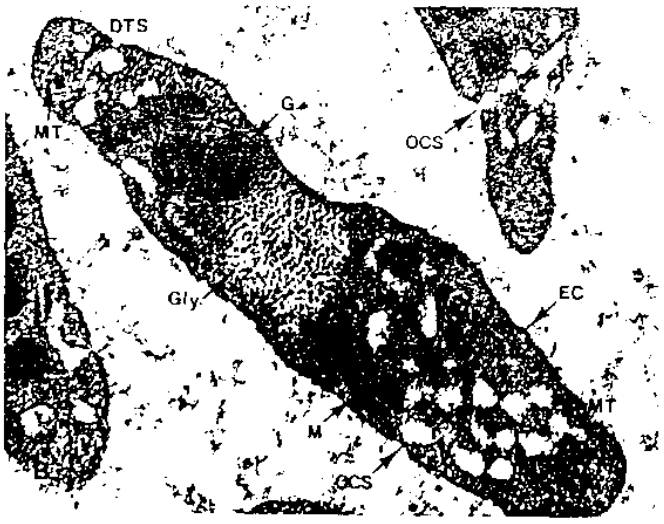
When platelets mature , they circulate for aproximately lo days . During this time , they decrease in size and increase in density primarily because of the loss of plasma membrane . *(George and Lewis , 1978)* .

Studies showed age related changes in platelet survival , and a rough correlation between shortened survival and vascular disease suggesting that increased platelet turnover may result from vascular lesions *(Abrahamsen , 1978)* .

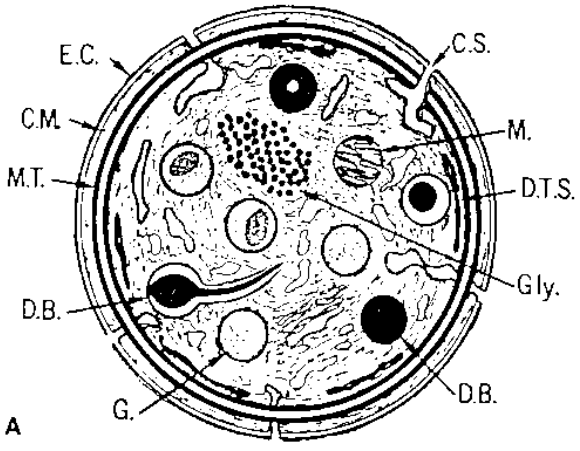
MORPHOLOGY OF BLOOD PLATELETS :-

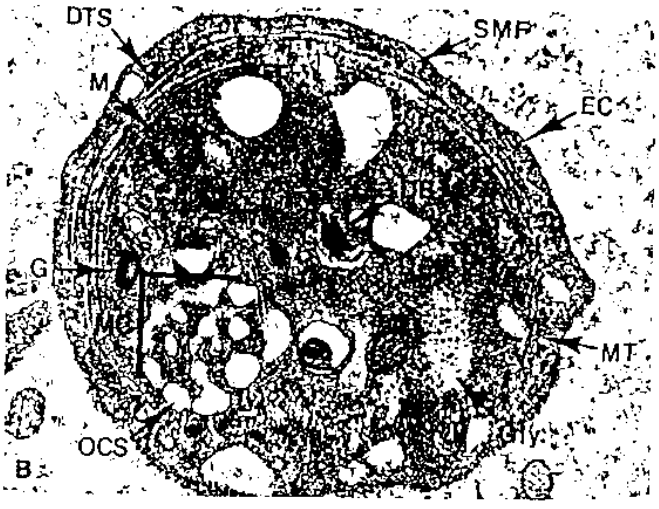
Platelets are small non nucleated discoid cells about 3μ long and 1μ thick . In stained blood films , the clear blue cytoplasm is seen to contain a few granules , but the electron microscope reveals a very complex system of membranes , microtubules and organelles *(Thompson , 1982)* . Under the electron microscope platelets appear to be formed of 4 distinct divisions *(Vermylen et*





Discoid platelets. (A) The diagram summarizes ultrastructural features observed in thin sections of discoid platelets cut in cross-section. Components of the peripheral zone include the exterior coat (EC), trilaminar unit membrane (CM), and submembrane area containing specialized filaments (SMF) that form the wall of the platelet and line channels of the surface-connected canalicular system (CS). The matrix of the platelet interior is the sol-gel zone containing actin microfilaments, structural filaments, the circumferential band of microtubules (MT), and glycogen (Gly). Formed elements embedded in the sol-gel zone include mitochondria (M), granules (G), and dense bodies (DB). Collectively they constitute the organelle zone. The membrane systems include the surface-connected canalicular system (CS) and the dense tubular system (DTS), which serve as the platelet sarcoplasmic reticulum. (B) A platelet sectioned in the equatorial plane, which reveals most of the structures indicated on the diagram. The membrane complex (MC) is a specialized association of the DTS and CS. (Magnification of B, ×28,000.)





Discoid platelets. (A) The diagram summarizes the structures observed in platelets sectioned in the equatorial plane. (B) An example of such a platelet. The designations for the structural features are presented in the legend of Figure 19-1. (Magnification of B, ×28,000.)

al.,1983) :

1- The peripheral zone .

2 - The sol-gel zone.

3 - The organelle zone.

4 - The membrane systems.

Under the peripheral membrane, there are helical coiled bundles of microtubules which are involved in platelet contraction. A gel like matrix embes the organelles and microfilamints.

There is a system of channels by which it is possible that the products of secretory granules reach the organelles. The metabolic requirments of the platelets are maintained by the mitochondria *(Thompson, 1982)* .

1 - THE PERIPHERAL ZONE:

The peripheral zone is made up of an exterior coat, a trilaminar membrane, submembranous filamints, and an open canalicular system *(Vermylen et at., 1983).* The exterior coat is rich in carbohydrate mainly glycoproteins, contains the antigenic characteristics and the properties responsible for platelet adhesiveness *(Elementson 1988)* .

The platelet membrane is similar to other cell membranes and contains the phospholipid substance that accelerate blood coagulation known as platelet factor 3 (pf3) and arachidonic acid which is the precursoer of the various prostaglandins and thromboxanes that are essential for platelet aggregation *(Gresele, 1987).* The submembrane filament ressembles microfilaments and probably serve as a form of stress fibre that produces tension at the cell surface to maintain the discoid shape *(Fox et al., 1988).*

2 - THE SOL-GEL ZONE :

This zone is made up of micro-tubules and microfilaments . The

microfilaments are composed of thrombosthenin (actin - myocin elements) and function in the contractile process during platelet release *(Vermylen et al., 1983)*

3 - THE ORGANELLE ZONE

The organelle zone contains granules of various electron densities, mitocondria, and a dense tubular system *(Nurden et.,1982)*. The dense bodies granules,function as storage granules for adenosine diphosphate, adenosine triphoshate, serotonin and calcium (*Costa and hui.1987)*.

Alpha-granuels contain a number of platelet specific protiens, including platelet factor 4 (PF4), beta-thromboglobulin (BTG), platelet derived growth factor (PDGF), fibrinogen, thrombospondin, von-willebrand factor and fibronectin (*Deugl, 1987*).

4 - THE MEMBRANE SYSTEMS:-

platelets have two discrete membrane systems not found in other blood cells, the open canlicular system derived from the plasma membrane of the megakaryocyte and the dense tubular system representing residual smooth endoplasmic reticulum of the megakaryocyte (*white, 1979*).

A-THE OPEN CANALICULAR SYATEM:-

It consists of tortous invaginations of the surface membrane into the interior of the platelet providing a communication between the plasma and the interior of the platelet. The channels of the open canalicular system serve as channels for substances extroded by platelets during the release reaction *(Vermylen et al., 1983)*.

B- THE DENSE TUBULAR SYSTEM:-

it is made up of network of narrow canaliculi and it is particularely pronounced close to the microtubules, prostaglandin synthesis is localised in the dense tubulsar system *(Gerrard et al., 1986)*

PHYSIOLOGICAL ASPECTS OF PLATELET FUNCTION:-

platelet activation involves:-

A- the interaction of an agonist with a platelet membane receptor.

B- the transfer of a signal to the interior of the platelet.

C- then, specific cell reactions which include:-

a- shape change;

b- adherance;

c- aggregation;

d- release;

e- interaction with blood coagulation; and

f- finally, clot retraction (*Vermylen et al., 1983*).

A- INTERACTION OF AGONIST WITH SPECIFIC MEMBRANE RECEPTOR:-

Some of the various receptors, which are present on platelets are of particular interest for platelet activation, e.g, the receptors for thrombin, adenosine diphosphate, fibrinogrn and prostaglandins *(Steimer, 1987)*..

Thrombine is the most physiolgical activator of platelets, adenosine diphosphate-induced platelet aggregation needs divalent cation (ca^{++}) and intact fibrinogen as a cofactor *(Benner et al., 1983)*. little is known about platelets for collagen, and fibronectin has been proposed as collagen rececptor on platelets *(Laduca,1987)*.

B- TRANSFER OF SIGNALS TO THE INTERIOR OF PLATELETS :-

The signal consists of an increased local concentration of calcium ion and is modulated by cyclic adenosine monophosphate (cAMP) *(Vermylen et al., 1983)*

I- PHOSPHOLILPID METABOLISM :-

Stimulation of platelets produces alteration in membrane phospholipid, phosph- inosoitol is activated and both diacylglycerol and phosphatidic acid are formed *(Majerus et al., 1983)*. Diacylglycerol acts as a co factor for a protein kinase (protein kinase c) which enhances calcium mobilization *(Thiagarajar, 1988)*.

II- CALCIUM METABOLISM :-

Cytoplasmic free calcium increases by many different agonists, through stimulation of the influx from extra-cellular medium and through the dicsharge of calcium from intracellular storage sites, mainly the dense tubular system *(Sage, 1987)*. The mechanisms controling the influx of extacellular calcium is unknown; release of calcium from the dense tubular system is mediated by inositol triphosphate derived from the hydrolysis of phosphatipyl-inositol 4.5-dis phosphate in the inner leaflet of platelet membrane *(Brass, 1985)*.

III - ARACHIDONATE METABOLISM : -

Stimulation of platelets with sufficient powerful stimulus to induce degranulation, leads to activation of arachidonic acid biosynthesis. The activation of phospholipase C and A_2 liberate arachidonate from phosphatidyl-inositol and phosphatidyl-choline respectively *(Feinstein et al., 1988)*.

Arachidonic acid is converted by a membrane bound cyclo-oxygenase into labile intermediate compounds, the endoperoxides which are converted mainly to thromboxane A_2 (TX A_2) which is an extremely potent platelet stimulant and vasoconstrictor *(Gresele , 1987)* .

Small amount of PG D2, PG F_2 and PG E_2 are also formed from endoperoxides . Although all are vasoconstrictive , the only one with a powerful effect on platelets is PG D_2, which inhibits platelet responses by activating adenylcyclase, and thus elevating cAMP level *(Gordon and Henson , 1985)* .