Platelet Secretory Functions In Health & Disease

Essay
Submitted in partial fulfillment of the requirements of the
MS Degree in Internal Medicine

By

Sally Mahmoud Ismail Moussa

MB.Bch

Under Supervision of

Professor Dr. Abdel Rahman Abdel Hamid Soliman
Professor of Internal Medicine and Hematology
Faculty of Medicine
Ain Shams University

Dr. Amal Mostafa El Afify
Assistant Professor of Internal Medicine and Hematology
Faculty of Medicine
Ain Shams University

Dr. Hany Mohamed Abd-Allah Hegab
Lecturer of Internal Medicine and Hematology
Faculty of Medicine
Ain Shams University

Ain Shams University
2008

Introduction:

The hemostatic system consists of platelets, coagulation factors, and the endothelial cells lining the blood vessels. The platelets arise from the cytoplasmic fragmentation of megakaryocytes in the bone marrow and circulate in blood as disk-shaped anucleate particles Platelets are known to contain a number of performed morphologically distinguishable storage granules, the alpha granules, the dense granules, and lysosomes. The alpha granules contain platelet specific proteins, cationic proteins, coagulation factors and glycoproteins. The dense granules contain proaggregatory factors such as adenosine-diphosphate (ADP), calcium, and 5-hydroxytryptamine (serotonin). Lysosomes are morphologically similar to alpha granules and contain a number of acid hydrolases; they are analogous to lysosomes in other cells and are involved in protein and lipid degradation (*Fukami et al., 2000*)

During activation, the granules are centralized and their contents are discharged into the lumen of the open canalicular system, from which they are then released to the exterior (the release reaction). Following activation, platelets have 2 major mechanisms to recruit additional platelets to the growing hemostatic plug. They release proaggregatory materials by the release reaction, and they synthesize thromboxane A2 from arachidonic acid. Thus, the release reaction and prostaglandin synthesis act to consolidate the initial hemostatic plug by promoting the participation of other platelets in the growing hemostatic plug. In addition, when platelets are activated, negatively charged phospholipids move from the inner to the outer leaflet of the membrane bilayer. This negative surface provides binding sites for enzymes and cofactors of the coagulation system, resulting in the formation of a clot (secondary hemostasis) (*Maguire &Fitzgerald, 2003*)

Beyond an eminent role in hemostasis and thrombosis, platelets are characterized by expert functions in assisting and modulating inflammatory reactions and immune responses. This is achieved by the regulated expression of adhesive and immune receptors on the platelet surface and by the release of a multitude of secretory products including inflammatory mediators and cytokines, which can mediate the interaction with leukocytes and enhance their recruitment. Among those factors are cytokines and chemokines, which are key molecules in that they bridge innate and adaptative immunity; Their activity extends on T cells, B cells, monocytes and macrophages, dendritic cells and endothelial cells lining the blood vessels. This means that when a platelet concentrate is transfused to a recipient, a huge amount of cytokines and chemokines is also infused *(Cognasse et al., 2007)*

In addition, platelets are characterized by an enormous surface area and open canalicular system, which in concert with specialized recognition receptors may

contribute to the engulfment of serum components, antigens, and pathogens. Platelet-dependent increases in leukocyte adhesion may not only account for an exacerbation of atherosclerosis, for arterial repair processes, but also for lymphocyte trafficking during adaptive immunity and host defense (*Von handelshausen & Weber, 2007*).

Platelet granule secretion or exocytosis is required for normal platelet function and plays an important role in the pathogenesis of cardiovascular diseases. Platelets secrete molecules that amplify thrombosis, induce vascular remodeling, recruit and activate cells. The platelet secretory process begins in megakaryocytes where molecules are targeted to developing granules through specific vesicle trafficking and transporter mechanisms. Secretory granules may continue to mature in the circulation after the platelet has been released from the megakaryocyte. The platelet secretory process culminates when ligands interact with specific platelet receptors to trigger exocytosis. A convergence of new insights from several different organisms has begun to illuminate the molecular mechanisms responsible for the platelet secretory process, from granule development through membrane fusion and exocytosis (*Reed, 2004*)

Extensive studies enabled the discovery of over than 300 proteins in thrombin activated platelet secretome (*Cagney & Emilia, 2002*)

Defects that impair function can affect platelet receptors, secretory responses, or intracellular signaling pathways. The treatment of platelet disorders is primarily with platelet concentrates. However, in patients with abnormalities of their platelet surface receptors, platelet transfusion may provoke an immune response (*White,2006*).

Aim of the Work:

This essay aims to shed some light on the role of platelet secretory functions in hemostasis as well as modulating cellular parameters of immunity both in health & disease.

References:

- **Cagney G; Emili A. (2002);** *De novo peptide sequencing and quantitative profiling of complex protein mixtures using mass coded abundance tagging.* Nat Biotechnol; 20:163-70
- **Cognasse F; Osselaer JC; Garraud O.(2007);** *Platelets cytokines and their effects on platelet transfusion*. Transfus Clin Biol. 14(1):69-78
- **Fukami H, Holmsen H, Kowalska MA, Niewiarowski S (2000);***Platelet secretion* in: Haemostasis & thrombosis by Colman, Hirsh, Marder, Salzeman(eds.) 4th edn. Philadelphia 561-73
- **Maguire PB; Fitzgerald DJ. (2003);** *Platelet proteomics*. J thromb haemost 1: 1593-1601
- **Reed GL. (2004)** *Platelet secretory mechanisms*. Semin Thromb Hemost. 30(4):441-50
- **Von Hundelshausen P; Weber C.(2007) ;***Platelets as immune cells: bridging inflammation and cardiovascular disease*. Circ Res. 100(1):27-40
- **White GC. (2006);** *Congenital and acquired platelet disorders: current dilemmas and treatment strategies.* Semin Hematol. 43(1 Supp1 1):537-41

الوظائف الافرازية للصفائح الدموية
فى الصحة و المرض

رسالة

مقدمة توطئة للحصول على درجة الماجستير

فى امراض الباطنة العامة

مقدمة من

الطبيبة/ سالي محمود اسماعيل موسى

تحت اشراف

الاستاذ الدكتور / عبد الرحمن عبد الحميد سليمان
استاذ امراض الباطنة العامة و امراض الدم
كلية الطب – جامعة عين شمس

الدكتورة / امل مصطفى العفيفى
استاذ مساعد امراض الباطنة العامة و امراض الدم
كلية الطب – جامعة عين شمس

الدكتور / هانى محمد عبد الله حجاب
مدرس امراض الباطنة العامة و امراض الدم
كلية الطب – جامعة عين شمس

كلية الطب – جامعة عين شمس
٢٠٠٨

المقدمة

تنشأ الصفائح الدموية من التشرف الهيولى للنواء فى النخاع العظمى و تدور فى الدم على شكل جسيمات قرصية عديمة النواة و تحتوى الصفائح على عدد كبير من حبيبات لتخزين مواد فعالة مختلفة شكليا الالفا و المكثفة و اليحلول.
حبيبات الالفا تحتوى على بروتينات متخصصة للصفائح ، برتنات كتايونية ، عناصر مخثرة و بروتينات سكرية.
الحبيبات المكثفة تحتوى على عناصر سابقة لتكدس الصفيحات الدموية مثل ثانى فوسفات الادينوزين ، الكالسيوم و السيروتونين.
الحبيبات اليحلولية و هى تتشابه مع الحبيبات الالفا من حيث الشكل و تحتوى على عدد من احماض الهيدرولاز و هى تماثل اليحلول الموجود فى الخلايا الاخرى و تشارك فى عملية نكوص البروتينات و الدهون.
فى حالات التنشيط تتمركز الحبيبات فى الوسط و تفرز محتوياتها داخل جهاز قينوى يوصلها الى خارج الخلية (عملية الافراز) و يتبع التفعيل اليتان كبيرتان للصفائح الدموية لتوظيف عدد اكبر من الصفائح ال سدادة التخثر حيث تفرز مواد سابقة لتكدس الصفائح عن طريق عملية الافراز كما تصنع الثرومبوكسان أ٢ من الحمض الاراكيدونى اى ان عملية تصنيع البروستاجلاندين تتشارك مع عملية الافراز فى تصلد سدادة التخثر النامية بالاضافة الى ان الصفائح الفعالة و الشحميات الفوسفورية المشحونة سالبا تتحرك من الوريقة الداخلية الى الوريقة الخارجية من الغشاء المزدوج حيث يقدم السطح السالب اماكن ربط للانزيمات و العناصر المساعدة للجهاز التخثرى مما ينتج عنه صناعة الجلطة الدموية (المرقىء الثانوى)
بالاضافة للدور الهام للصفائح الدموية فى الارقاء و التخثر فانها تلعب دورا هاما فى التفاعل الالتهابى و الاستجابة المناعية و هو ما يتحقق عن طريق التمثيل المنظم للمستقبلات المناعية و اللاصقة على صطح الصفائح و عبر افراز عدد من المواد التى تساعد على التفاعل مع كرات الدم البيضاء و تسهل توظيفها من بينها السيتوكينات و الكيموكينات التى تعتبر اساسية حيث انها تعد المعبر بين المناعة البدائية و المناعة المكتسبة كما ان نشاط الصفائح يمتد الى الخلايا الليمفاوية ب و ت والخلية الوحيدة و البلعم و الخلايا التغصنية و الخلايا المبطنة للاوعية الدموية .
كما ان الصفائح الدموية تتميز بسطح هائل و نظام قنوى مفتوح بالاضافة الى نظام استقبال متخصص يسهم فى حصار و هضم مكونات المصل، المستضدات و الجراثيم المرضية ويعتبر افراز الحبيبات من الصفائح و تسرب محتوياتها اساسى لوظائف الصفائح الطبيعية. كما تفرز الصفائح مواد مضخمة للتخثر و مواد تحرض على اعادة تشكيل الاوعية الدموية.
ان عملية افراز حبيبات الصفائح تبدأ فى النواء حيث توجه الجزيئات لتصنيع الحبيبات و نقلها فى طرق متخصصة و تكمل نموها فى الدورة الدموية بعد افراز الصفائح من النواء و تتصاعد عملية افراز الحبيبات من الصفائح عندما تتحد الربيطة مع المستقبلات و تحفز عملية الافراز و قد اظهرت الدراسات اكتشاف اكثر من ٣٠٠ نوع من البروتينات التى تفرزها الصفائح.

الهدف من العمل

تهدف هذه الرسالة الى تسليط الضوء على الدور الافرازى للصفائح الدموية فى الصحه و المرض

INTRODUCTION

The platelets arise from the cytoplasmic fragmentation of megakaryocytes in the bone marrow and circulate in blood as disc-shaped anucleate particles. Platelets are known to contain a number of pre-formed morphologically distinguishable storage granules, the alpha granules, the dense granules, and lysosomes. The alpha granules contain platelet specific proteins, cationic proteins, coagulation factors and glycoproteins. The dense granules contain proaggregatory factors such as adenosine-diphosphate (ADP), calcium, and 5-hydroxytryptamine (serotonin). Lysosomes are morphologically similar to alpha granules and contain a number of acid hydrolases, they are analogous to lysosomes in other cells and are involved in protein and lipid degradation (***Fukami et al., 2000).***

During activation, the granules are centralized and their contents are discharged into the lumen of the open canalicular system, from which they are then released to the exterior (the release reaction) (***Maguire and Fitzgerald, 2003***).

Beyond an eminent role in hemostasis and thrombosis, platelets are characterized by expert functions in assisting and modulating inflammatory reactions and immune responses. This is achieved by the regulated

expression of adhesive and immune receptors on the platelet surface and by the release of a multitude of secretory products including inflammatory mediators and cytokines, which can mediate the interaction with leukocytes and enhance their recruitment. Among those factors are cytokines and chemokines, which are key molecules in that they bridge innate and adaptative immunity; Their activity extends on T cells, B cells, monocytes and macrophages, dendritic cells and endothelial cells lining the blood vessels ***(Cognasse et al., 2007)***.

In addition, platelets are characterized by an enormous surface area and open canalicular system, which in concert with specialized recognition receptors may contribute to the engulfment of serum components, antigens, and pathogens. Platelet-dependent increases in leukocyte adhesion may not only account for an exacerbation of atherosclerosis, for arterial repair processes, but also for lymphocyte trafficking during adaptive immunity and host defense (***Von Handelshausen and Weber, 2007***).

Platelets secrete molecules that amplify thrombosis, induce vascular remodeling, recruit and activate cells. The platelet secretory process begins in megakaryocytes where molecules are targeted to developing granules through

specific vesicle trafficking and transporter mechanisms. Secretory granules may continue to mature in the circulation after the platelet has been released from the megakaryocyte. The platelet secretory process culminates when ligands interact with specific platelet receptors to trigger exocytosis. A convergence of new insights from several different organisms has begun to illuminate the molecular mechanisms responsible for the platelet secretory process, from granule development through membrane fusion and exocytosis (***Reed, 2004***).

Extensive studies enabled the discovery of over than 300 proteins in thrombin activated platelet secretome (***Cagney and Emilia, 2002***).

Defects that impair function can affect platelet receptors, secretory responses, or intracellular signaling pathways. The treatment of platelet disorders is primarily with platelet concentrates. However, in patients with abnormalities of their platelet surface receptors, platelet transfusion may provoke an immune response (***White, 2006***).

AIM OF THE WORK

This essay aims to shed some light on the role of platelet secretory functions in hemostasis as well as modulating cellular parameters of immunity both in health and disease.

THROMBOPOIESIS

Historical review

As recent as 100 years ago platelets were referred to as "dust of blood" platelets were described by Addison in 1841 as extremely minute granules in blood and were termed platelets by ***Bizzozero*** who also observed their adhesive qualities of increased stickiness when a vascular wall is damaged ***(De Gaetano, 2001)***. The same was identified by microscopic examination of blood smears in the late nineteenth century but the elegant camera lucida studies of (Howell) in 1890 and his coining the term of megakaryocyte that led to its broader appreciation as distinct entities. In (1906) ***James Homer Wright*** suggested that blood platelets are derived from the cytoplasm of megakaryocytes and the basic elements of thrombopoiesis were established. The adult human produces 150-400 x 10^9 platelets daily at a steady state, that level can be increased up to 20 folds or more in times of heightened demand reflecting the importance of megakaryopoiesis and thrombopoiesis in clinical medicine that affects morbidity and mortality from bleeding (***Kaushansky, 2008***).

Platelet Formation

Megakaryocytes are highly specialized haemato-poietic precursor cells for platelets and together with platelets play a crucial role in thrombosis and haemostasis as well as immune functions. Megakaryocytes are the largest haematopoietic cells residing in the bone marrow. Mature megakaryocytes (large=mega, nuclei=karyo, cyte=cell). The size of megakaryocytes varies from 20 to 100 micrometers there are estimated $4x10^7$ megakaryocyte comprising 0.02:0.05 % of the total nucleated cells in the human bone marrow (***Sun et al., 2006***).

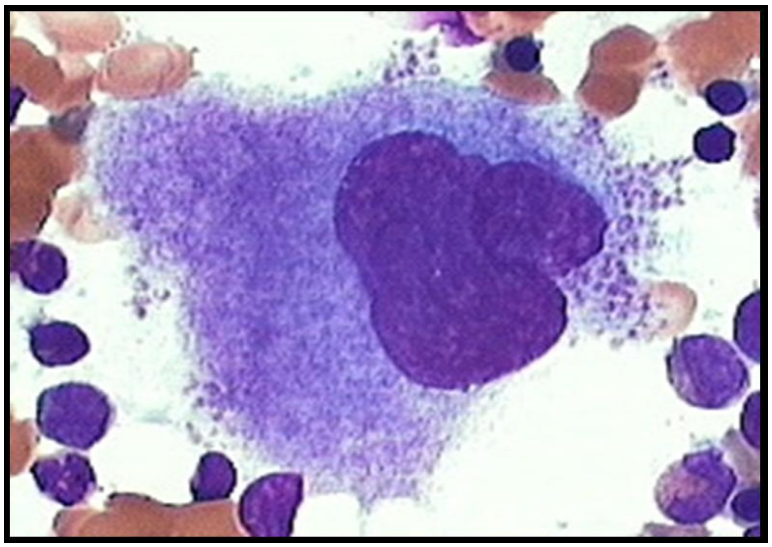

Fig. (1): Megakaryocyte in a bone marrow smear, with a wide cloudy granular cytoplasm as a sign of incipient platelet budding ***(Theml et al 2004).***

A normal megakaryocyte can be easily recognized in bone marrow smears by its characteristic morphology of large size and abundant cytoplasm associated with large polymorphic nuclei and cytoplasmic fragments (platelets) within the cytoplasm or being shed off from the cell surface (***Zucker–Franklin and Philipp, 2000***).

In the late 1970's using several semisolid media 2 colony morphologies that contain exclusively megakaryocytes have been described:

- The Colony Forming Unit – Megakaryocyte CFU-MK which develops into a simple colony containing from 3 to 5 mature megakaryocytes.
- The Burst Forming Unit – Megakaryocyte BFU-MK which is larger more complex colonies that include satellite collections of megakaryocytes and contain up to several hundred cells.

Because of the difference in their proliferative potential BFU-MK and CFU-MK are thought to represent the primitive and mature progenitors restricted to the lineage respectively ***(Kaushansky, 2008)***.

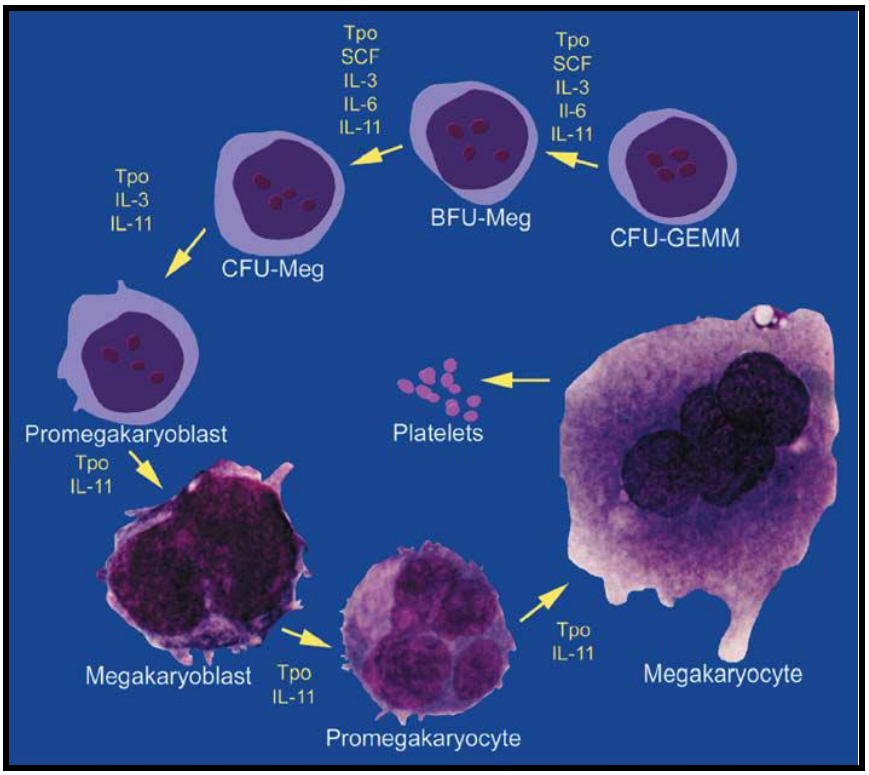

Fig. (2): The cytokine requirements for CFU-MK (***Michelson, 2003).***

TPO: Thrombopoietin. ***SCF:*** Stem cell factor ***IL:*** Interleukin

Although megakaryocytes are mainly found in the bone marrow they can also be found in other organs such as the lungs, liver and spleen where the lungs are even thought to be the major source of platelet release by some investigators (***Zucker-Franklin and Philipp, 2000***).

However the megakaryocytes from liquid cultures are significantly smaller with lower DNA ploidy than those naturally existing megakaryocytes in the bone marrow;