DETERMINATION OF PROTEIN C ACTIVITY IN BILHARZIAL HEPATIC FIBROSIS

THESIS

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BY

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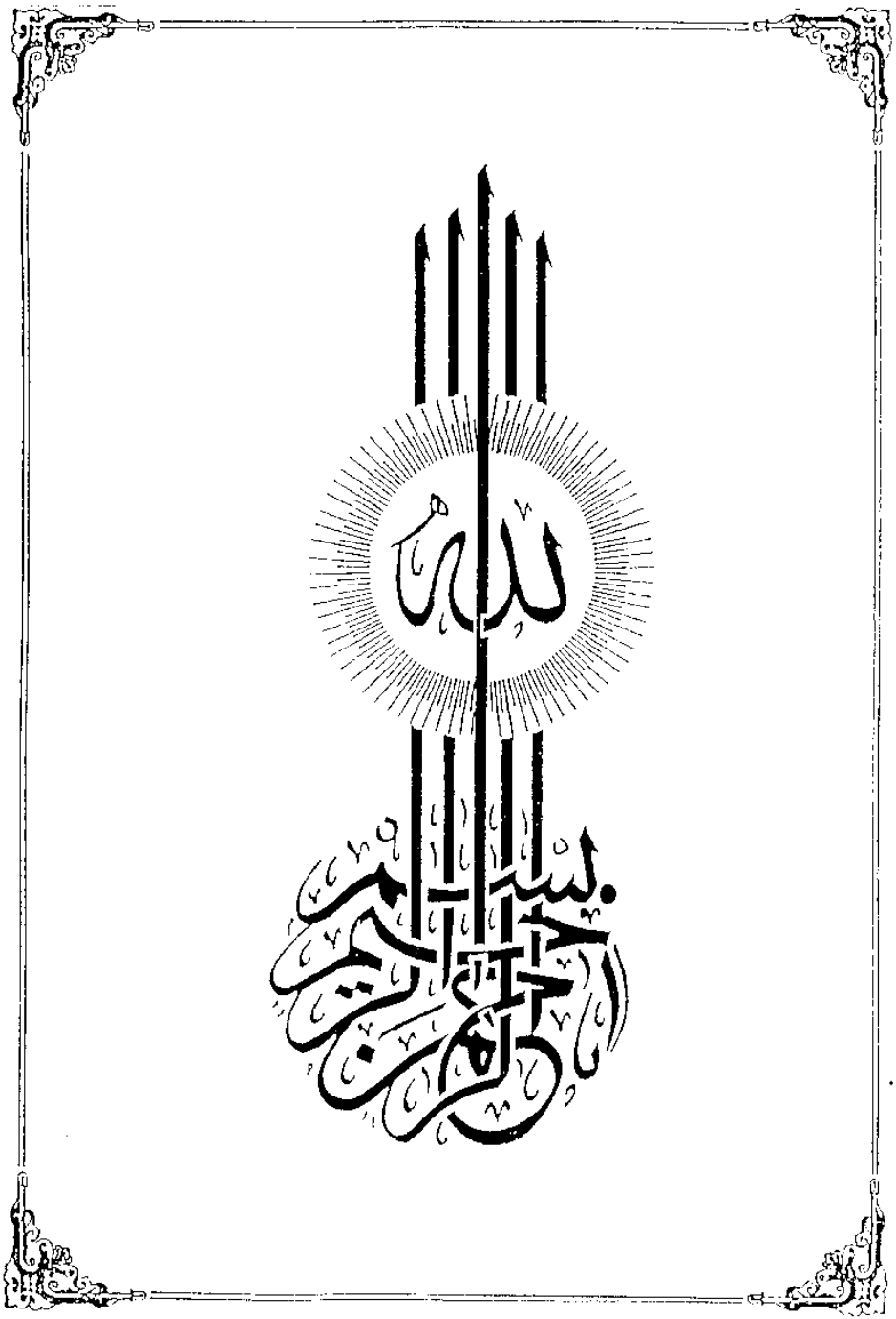

CONTENTS

LIST OF ABBREVIATIONS

A.A.	amino acid
ADP	adenosine dinucleotide phosphate
Ag	antigen
AHF (F-VIII-C)	antihaemophilic factor
α_2 AP	α_2 antiplasmin (α_2 plasmin inhibitor)
APC	activated protein C
APCI	activated protein C inhibitor
aPTT	activated partial thromboplastin time
α_2AT III,AT III	antithrombin III
BLF	bilharzial liver fibrosis
Ca^{++}	calcium ions
CHO	carbohydrate
DBA - buffer	dibarbitone acetate buffer
DIC	disseminated intravascular coagulation
DFP	diisopropylfluorophosphate
DVT	deep venous thrombosis
ELISA	enzyme linked immunosorbent assay
FDPs	fibrin degradation products
GLu	glutamic acid
HC	heavy chain
HMWK	high molecular weight kininogen
HRG	histidine rich glycoprotein
H_2O_2	hydrogen peroxide
IgG	immunoglobulin G
LC	light chain
M.Wt	molecular weight

Norm	normal
PC	protein C
PCI	protein C inhibitor
PF-3	platelet factor 3
PK	prekallikrein
P.NA	p - nitroaniline
PS	protein S
PT	prothrombin time
PTT	partial thromboplastin time
rpm	revolution per minute
SD	standard deviation
TF	tissue factor
T.L.C.	total leucocytic count
t-PA	tissue type plasminogen activator
u-PA	urokinase type plasminogen activator
F-VIII-VW	Von Willibrand factor
$\bar{X}$	mean value

INTRODUCTION
AND
AIM OF THE WORK

INTRODUCTION :

Protein C, a recently studied plasma factor by Stenflo (1976) who gave the first report discribing its molecular basis. This protein which is synthesized by the liver parenchyma with dependence on vitamin K excerts a controlling action on the coagulation mechanism.

It has an inhibitory action on coagulation by verture of interaction with factor V (Kisiel et al., 1977) and factor VIII (Vehar and Davie, 1980).

Many reports are available relating the acquired or hereditary changes of protein C with conditions known to have hypercoagulability or haemorrhagic tendency.

AIM OF THE WORK :

Protein C is a vitamin K - dependent plasma factor which is synthesized by liver cells.

Patients with bilharzial liver disease are known to have great liability to bleed, the aim of this work is to determine the plasma protein C activity in these patients to find out if it has a relationship to the bleeding tendency in bilharzial patients or not.

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REVIEW OF THE LITERATURE

CHAPTER I

NORMAL HAEMOSTASIS

Haemostasis means to prevent blood loss from intact vessels and to arrest bleeding from severed vessels. This involves three processes namely: Vascular response, platelet adhesion & aggregation and activation of blood coagulation mechanisms.

1. Role of blood vessels :

Vasoconstriction occur following injury, thus assisting plug formation by reducing blood flow. The mechanism appears to be humoral & it is probably due to the release of (thromboxane A_2), a vasoconstrictor substance, from aggregated platelets (Hamberg et al., 1975).

The stimulus of hemostatic plug formation is the damage of the vascular endothelium. Endothelial cells synthesize and secrete three substances involved in plug formation and localization. These are Von Willibrand factor (F. VIII VW), prostacylin (PGI_2) & plasminogen activator (Davidson, 1977).

2. Platelet - Vessel wall interaction :

The primary hemostatic plug is composed of a mass of aggregated platelets held together by fibrin. Physiological stimulus of aggregation is exposure of platelets to collagen fibrils in subendothelial tissue or to ADP liberated from adherent platelets. Collagen, ADP, thrombin, adrenaline & serotonin, all can induce platelet aggregation by activating the enzyme "phospholipase A_2" in platelet membrane causing formation of arachidonic acid which after a series of reactions, is converted to thromoboxane A_2, a powerful inducer

of platelet aggregation & releases ADP from the dense granules of platelets, with the result of further platelet aggregation.

3. Blood coagulation :

According to the coagulation cascade or waterfall hypothesis (Mac Farlane, 1964; Davie & Ratnoff, 1964) coagulation is initiated by two fundamental different mechanisms: The process of contact activation & the action of tissue factor, which proceed via two separate (intrinsic & extrinsic) pathways with the end result of factor X activation, then they converge by activating a third common pathway leading to fibrin formation.

(A) Intrinsic pathway :

This involves activation of factors XII, XI and IX in addition to kinin system. It ends by the formation of intrinsic F X activator complex by the interaction of F IXa, PF-3, Ca^{++} and F VIII.

* Factor XII activation :

This can be achieved by two ways : either contact activation or proteolytic cleavage & fragmentation (Wintrobe, 1981).

Contact activation involves the adsorption of four "contact sensitive" factors: XII, XI, Prekallikrein and High molecular weight kininogen on any active surface resulting in a change in the conformation of F XII, thus exposing an enzyme active site (Vroman, 1964).

Contact activation of F XII requires the presence of other coagulation factors, but may occur without alteration of any covalent bonds (Revak, 1977). Biologically active surfaces include: unbroken skin, vascular basement membrane, human cartilage, bacterial lipopolysaccharides, sebum, uric acid and long

chain fatty acids. The role of collagen in contact activation is contradictory (Wintrobe, 1981).

F XII may also be activated by proteolysis & fragmentation in the absence of an active surface, resulting in the cleavage of F XII into fragments (XII_f) smaller in size. These are: α XIIa fragment which is formed of a heavy chain & a light chain connected by disulphide bond (Fujikawa, 1977) and β XIIa fragment equivalent to the light chain of F XII or prekallikrein activator (PKA) which is released into solution (Cochrane et al., 1979) leaving a large fragment which contains the sites for F XII binding to contact surface (kaplan, 1978). Proteolytic activation of F XII can be achieved by the action of Kallikrein (Fujikawa et al., 1980), plasmin (Mc Millin, 1974) and F XIa (Revak, 1976) but Kallikrein is the most important (Kaplan, 1978). Factor XIIa and all XII_f can activate prekallikrein, but only F XIIa can activate F XI (Wintrobe, 1981).

* Activation of Kinin system:

It was found that optimum activation of the intrinsic pathway of coagulation requires the participation of the Kinin system (Weiss, 1974), however, the exact mechanism is unclear.

Prekallikrein activation is achieved by proteolytic action of F XIIa, all XII_f fragments, plasmin & other proteins (Bagdasarian, 1973). Kallikrein accelerates, several coagulation reactions in vitro (Kaplan et al., 1975) including the conversion of F XII to F XIIa & F XII fragmentation thus amplifying the activation of F XII (Weiss, 1974). It may directly activate F IX (Osterud, 1975) and plasminogen (Mandle & Kaplan, 1977).

HMW kininogen forms complexes in the plasma with F XI or prekallikrein which function cooperatively causing enhancement of several reactions (Thompson, 1977, 1979) :

- Activation of PK by F XIIa & XIIf (Thompson, 1978).
- Action of kallikrein on F XII activation & fragmentation (Revak, 1977).
- Activation of F XI by F XIIa (Saito, 1977).

Thus HMWK acts mainly to enhance F XIIa action on its natural substrates (Meier, 1977).

Factor XI activation into F XIa occurs by the enzymatic action of F XIIa (Ratnoff et al., 1964) and the serine protease F XIa subsequently activates F IX into F IXa in a calcium dependent reaction (Davie et al., 1975). Other activators of F IX may include tissue factor - F VII complex (Osterud et al., 1977), kallikrein (Osterud, 1975) and F Xa which activates F IX in vitro (Kalousek, 1975).

Formation of inrinsic F X activator :

It is achieved through the interaction of F IXa, VIII, PF-3 in a calcium dependent reaction which needs PF-3 traces or platelet substitute (Schiffman, 1966). The reaction is accelerated by the action of traces of thrombin on F VIII. Platelet factor 3 on the platelet membrane acts as a surface on which the components of the complex (IXa, VIII, PF-3 &Ca^{++}) are adsorbed & oriented leading to enhanced enzymatic action of the complex (Henker, 1970).

Factor IXa is the active enzyme acting on F X while F VIII is a cofactor.

(B) Extrinsic pathway :

This involves the interaction of tissue factor and F VII in the presence of Ca^{++} ions to form a complex which behaves as "enzyme" activating F X (Williams et al., 1966), but neither of the two factors can activate F X alone (Jesty et al., 1974).

Tissue factor has calcium binding sites & may act in the same way as F V & F VIII behave (Wijngaards et al., 1977). The proteolytic activity derived from the intrinsic "Contact phase" increases F VII activation (Shanberge et al., 1966) by the action of kallikrein, plasmin, XIIa, XIIf & IXa leading to the formation of F VIIa, a protease with two chains (Radcliff et al., 1976).

(C) Common pathway :

This starts with F X activation in a calcium dependent reaction, to yield F Xa (Discipio, 1977) which interacts with F V, Ca^{++} & PF-3 to form "Prothrombinase complex" (Papahadjopoulos et al., 1964). Factor Xa is the active enzyme, while F V forms the receptor site for F Xa binding to PF-3 by Ca^{++} bridges (Miletech, 1978) and it must be activated by thrombin before F Xa binding is optimal (Kane et al., 1980). It may have accessory binding sites to prothrombin (Esmon, 1973).

The rate of formation of prothrombinase complex is accelerated by the action of thrombin & F Xa on F V (Smith et al., 1976) prothrombinase then activates prothrombin in a calcium dependent reaction to yield thrombin, which itself enhances prothrombin activation (Aronson, 1977).

Thrombin - Fibrinogen reaction proceeds in three steps :