A STUDY OF HEMOSTATC MECHANISMS AND PROTEIN " C " IN NEPHROTIC CHILDRDN

THESIS

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BY

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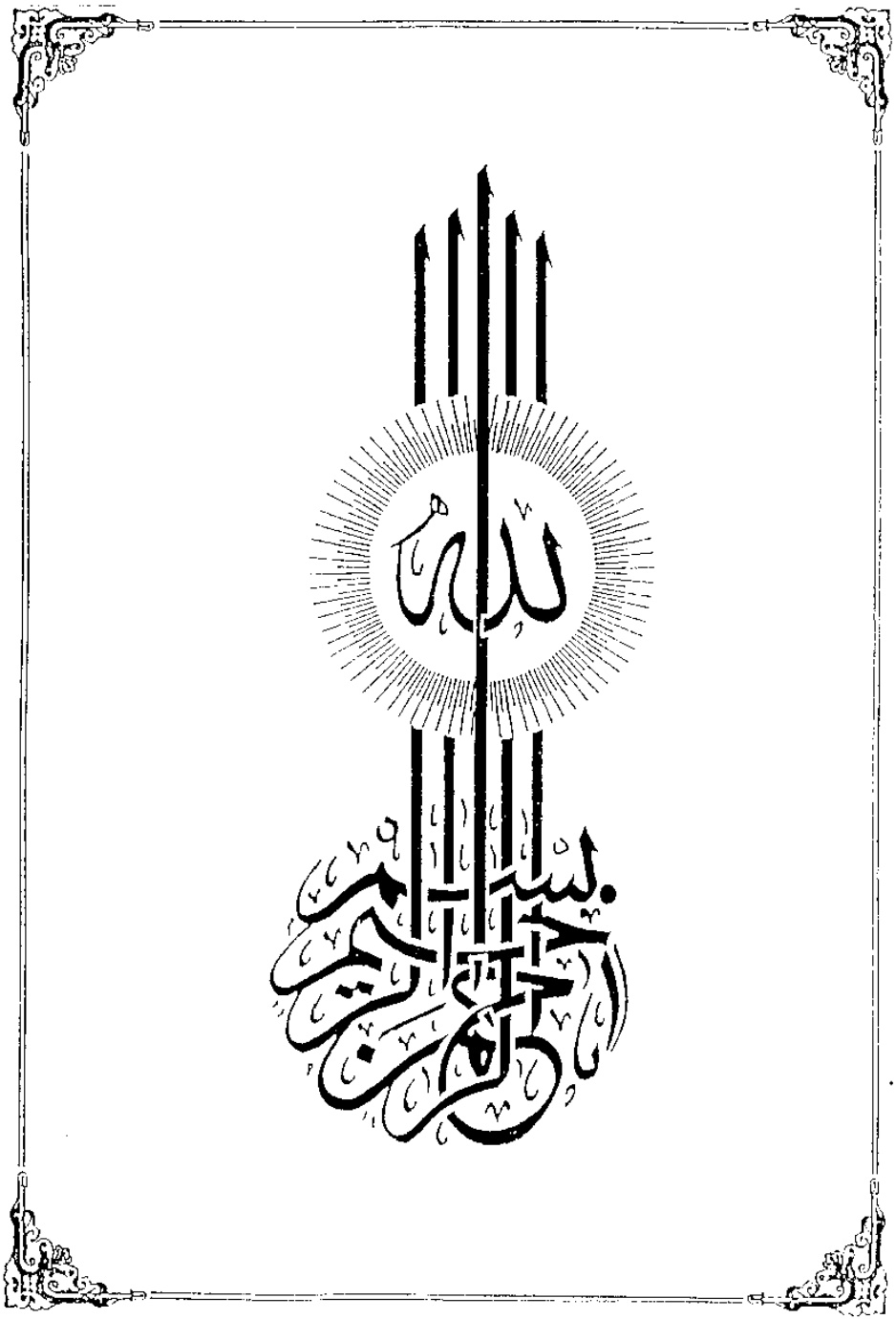

ABBREVIATIONS

α	Alpha
Ag	Antigen
APC	Activated protein C
AT-III	Antithrombin-III
β	Beta
Ca++	Calcium ions
DIC	Disseminated Intravascular Coagulopathy
E.S.R.	Erythrolyte sedimentation rate.
Fig.	Figure
F.V	Factor V
F.Va	Activated factor V
FX	Factor 10
Fxa	Active Factor 10
γ	Gamma
gm	gram
hr	Hour
I^{125}-thrombin	hrombin-labelled with radioactive iodine
I.U.	International unit
<	less than
kg	Kilogram
L.	liter
mg	Milligram
ml	milliliter

ABBREVIATIONS(cont.)

mol.wt.	Molecular weight
>	More than
NS	Nephrotic syndrome
OA	oral anticoagulant
OC	oral contraceptive
PA	plasminogen activator
P.A.I.	plasminogen activator inhibitor
P.C:Ag	Protein C:antigen
P.C	Protein C
P.C.I.	Protein C inhibitor
P.S.	Protein S
P.T.T.	Partial thromboplastin time
S.D.	Standard deviation
SI units	systemic international units
Tab.	Table
t-pA	Tissue type-plasminogen activator
Ug	microgram
uL	microliter

LIST OF FIGURES

CONTENTS

INTRODUCTION

AND

AIM OF THE WORK

INTRODUCTION AND AIM OF THE WORK

Protein C is a vitamin K-dependent plasma protein. It was discovered by Stenflo[1976]. It was named after a protein fraction [pol] C] obtained after gradient elusion of a prothrombin complex concentrate.

Protein C, when activated by thrombin and the endothelial cell cofactor "thrombomodulin", is a major inhibitor of blood coagulation through selective inactivation of factor V[Walker et al .,1979]and factor VIII [Marlar et al.,1981]. Furthermore protein C generates a potent fibrinolytic activity in the plasma [Comp and Esmon, 1981].

The nephrotic syndrome may be complicated by a hypercoagulable state. Hence, it may be complicated by thrombotic episodes of the venous or arterial circulation [Strauss et al., 1987]. Recently association between deficiency of protein C and thrombosis has demonstrated the clinical importance of this protein.

The aim of this study is to find out any association between the hypercoagulable state in nephrotic patients and protein C.

REVIEW OF LITERATURE

PROTEIN C STRUCTURE

Human protein C (PC) is a vitamin K-dependent glycoprotein of a mol.wt.which equals 62.000 that circulates in the plasma as an inactive zymogen at a concentration of 4 ug/ml [Griffin et al.,1982]. When activated its mol.wt. is 54.000 [Walker et al.,1979].

Stenflo,[1976] during his work on pc,found that on reduction of pc,two bands appeared with apparent mol.wt.of 21.000 and 35.000 He termed one as light chain and the other as heavy chain , that are linked together by interchain disulfide bonds.In order to determine which of the two chains contained the Ca++ binding groups,the electrophoretic mobilities of the isolated chains were analysed by agarose gel electrophoresis both with and without Ca ions. The heavy chain produced a smear on electrophoresis,probably due to extensive aggregation,whereas the light chain produced a distant band.

Recently ,a single chain pc was isolated from the plasma. It has the same enzymatic properties and also the same molecular weight of the double chain pc. It presents 10% of the whole plasma pc [Mitchell et al.,1981; Marlar 1985].

Both human and bovine pc are similar structurally and functionally,but each is species specific [Marlar et al.,1982] they differ only in their cabohydrate content and mol.wt.The carbohydrate content of human pc equals 23 % of the total mol.wt. [Kisiel,1979],while it is 18 % of the total mol.wt.of bovine pc [Kisiel and Davie , 1981].The heavy chain of human pc has a mol.wt. of 41.000 and that of the light chain is 21.000 [Kisiel,1979] while the bovine pc heavy chain is of a mol.wt. 43.000 and light chain 23.000 [Kisiel and Davie,1981].

The heavy chain has 260 amino acid residues and has three carbohydrate side chain , at residues aspargine 93,154 and 170 [Stenflo and Frenlund,1982]. When pc is activated by thrombin, an arginine (12) - leucidine (13) bond is cleaved [Kisiel,1979] and a tetradecapeptide of mol.wt.1,400 is released [Kisiel and Davie,1981] which is present in the heavy chain,i.e., the heavy chain contain the active part of the zymogen [Stenflo and Frenlund,1982].

The light chain has 155 amino acid residues and has one carbohydrate side chain at aspargine 97. All the eleven (11) glutamyl residues are carboxylated as γ-carboyglutamic acid residues and are present in the region (1-44) amino acid . This part of the molecule is involved in calcium mediated phospholipid binding . The disulfide interaction bond of the light chain lies at cystein (.122) residue. [Frenlund and Stenflo,1982 ,1983].

PHYSIOLOGICAL ASPECTS OF PC

(A) BIOSYNTHESIS AND SECRETION OF PROTEIN C :

The liver is probably the site of synthesis of pc, as its concentration was very low in patients with chronic liver diseases [Mannucci and Vigano, 1982].

Fair and Marlar (1986) , reported that factor VIII, PC , PC inhibitor and protein S are synthesized by hepatocytes and that each molecule represents its plasma protein counterparts structurally, immunochemically and functionally. The authors noted that PC is synthesized and secreted as a single chain molecule. This is consistent with the recently reported data from recombinant deoxyribonucleic acid cloning , indicating that PC is transcribed as a single contiguous messenger ribonucleic acid [Long et al., 1984] . All the molecules in PC appear to be the products of individual genes [Fair and Marlar, 1986]. The half -life of PC = 6 -8 hours [Widdershoven et al., 1987], while that of activated PC = 10 - 15 minutes [Comp et al., 1982]. Both PC and factor VIII have high secretion rates , relative to factor X , as PC and factor VIII have shorter half-lives.

The secretion of PC and protein S are reduced by warfarin like drugs while PC inhibitor is not affected. This decrease of PC and protein S secretion may be due to a decrease in the synthetic

rate and an increase in the catabolic rate may also occur and need to be investigated . Vitamin K did not significantly increase production of these molecules [Fair and Marlar , 1986].

(B) ACTIVATION OF PROTEIN C :

PC is activated by thrombin : thrombomodulin , a protein present on the endothelial cell surface ,acts as a cofactor in this reaction [Horellou et al.,1984]. Factor Va (active v) and thrombomodulin have been found to accelerate the rate of PC activation [Salem et al.,1984].

* THRUMBOMODULIN GENERAL CHARACTERS :

Thrombomodulin has a molecular weight of 74.000 [Esmon et al.,1982] . Its highest content is in the lung and the placenta but it was also detected in the spleen , the pancreas, the kidney,the liver, the skin, the heart and aorta.It is absent from the brain. The physiological significance of its lack in the white and the gray matter in the brain is uncertain. Perhaps the possibility of intracranial hemorrhage is so threatening to survival that the molecule is not contained in vessels that are within the substance of the brain. However , basilar and internal carotid arteries,pia-arachnoid and choroid plexus which supply the brain contain thrombomodulin [Ishii et al., 1986] . The human plasma and urine contain a soluble form of thrombomodulin and

appear to be smaller than cellular thrombomodulin [Ishii and Majerus , 1985].

* THE ROLE OF THROMBOMODULIN :

THROMBIN – THROMBOMODULIN COMPLEX :

Activated PC must be proteolytically activated to function as an anticoagulant [Marlar et al ., 1982]. Although thrombin can activate PC , the rate of activation is rather slow [Kisiel , 1979] and inhibited further by physiological Ca++ concentration [Esmon et al., 1983]. As a result little PC is activated when blood clots in vitro [Salem et al., 1983 3b. Thrombomodulin forms a 1:1 complex with thrombin and this complex rapidly activate PC [Esmon et al., 1983]. The following model is suggested by Comp et al.,(1982) for PC activation : [Fig. [1].

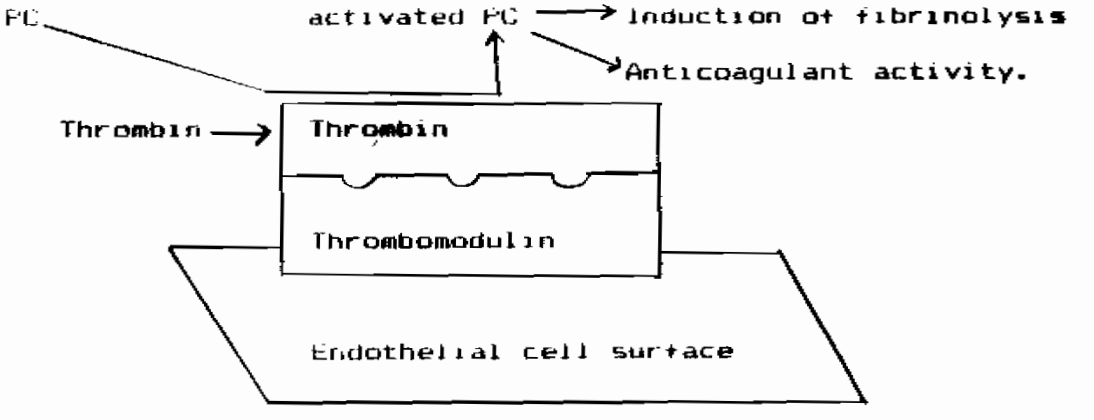

Fig.[1]:A proposed model for in vivo PC activation [Comp et al., 1982].