PROTEIN - C IN HEPATIC CIRRHOSIS

THESIS

SUBMITTED IN PARTIAL FULFILMENT OF MASTER DEGREE IN INTERNAL MEDICINE

By

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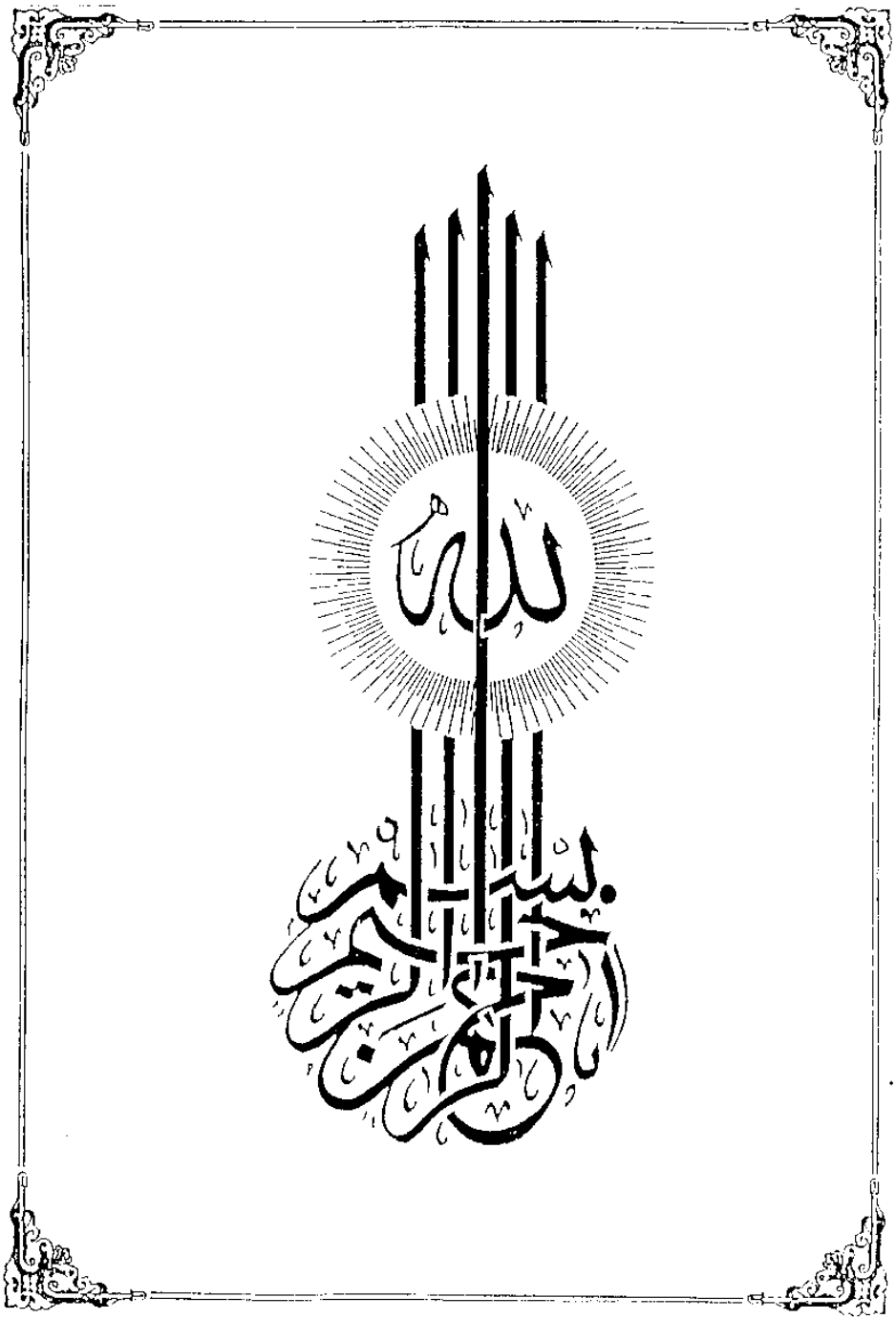

C O N T E N T S

LIST OF ABBREVIATIONS

Ag	:	Antigen.
APC, PCa	:	Activated protein C.
APTT	:	Activated partial thromboplastin time.
ATIII	:	Antithrombin III.
AVH	:	Acute viral hepatitis.
Ca^{++}, Ca^{2+}	:	Calcium ions.
C4BP	:	C4b - binding protein.
DIC	:	Disseminated intravascular coagulation.
ELISA	:	Enzyme linked immunosorbent assay.
F	:	Factor.
FDPS	:	Fibrinogen, fibrin degradation products.
Gla	:	Glutamic acid.
HC	:	Heavy chain.
HMWK	:	High molecular weight Kininogen.
HRG	:	Histidine rich glycogen.
Mol. Wt., M.W.:	:	Molecular weight.
OC	:	Oral contraceptives.
OV	:	Oesophageal varices
PAF	:	Platelet activating factor.
PC	:	Protein C.
PCI	:	Protein C inhibitor.
PSBP	:	Protein S. binding protein.
PT	:	Prothrombin time.
TF	:	Tissue Factor.
t-PA	:	tissue - type plasminogen activator.
u-PA	:	urokinase - type plasminogen activator.

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Protein C is the zymogen of a serine protease involved in blood coagulation that has been isolated from both bovine and human plasma (Stenflo , 1976; Kisiel, 1979). It is a vitamin-K-dependent glycoprotein (Griffin et al., 1982) Protein C received its present name because it was purified from a protein fraction (Pool C) obtained after gradient elution of a prothrombin complex concentrate on a DEAE-Sephadex column (Stenflo, 1976). Activated protein C destroys the activity of activated factors V and VIIIC (Kisiel et al.,1977; Marlar et al., 1982) and stimulates fibrinolysis by inducing a rise in plasma plasminogen activator activity (Zolten & Seegers, 1973; Comp & Esmon 1981). This rise in plasma plasminogen activator activity is due to a neutralizing effect of activated protein C on a circulating inhibitor of t-PA (Van Hinsberg et al., 1985).

The soluble components of protein C pathway (Protein C, protein C – inhibitor and protein S) are produced by the liver parenchymal cells (Fair and Marlar, 1986). In marked liver dysfunction, severe depression of protein C levels would occur (Clouse and Comp, 1986).

Aim of the work:

Estimation of protein C in hepatic cirrhosis as it might have some general implications for the pathophysiology of haemostasis in liver disease.

BLOOD COAGULATION CASCADE

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(Figure I)

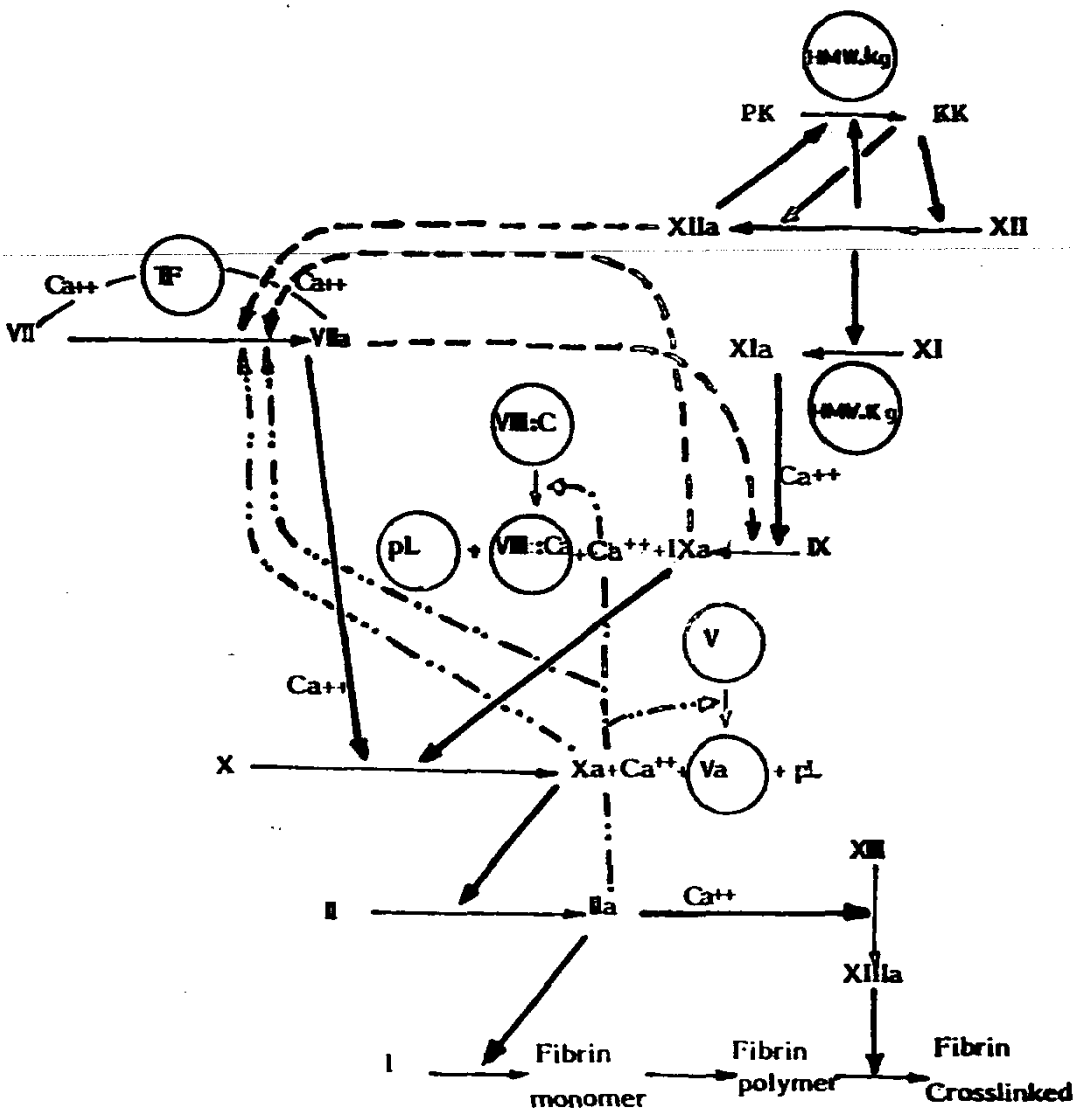
The coagulation mechanism is composed of a series of reactions which functions as a biological amplifier. This view was enunciated independently by Macfarlane (1964) and Davie & Ratnoff (1964) and termed the cascade hypothesis.

Knowledge of the individual components and reactions has greatly expanded during the succeeding years and additional factors were identified (Prekallikrein and high molecular weight Kininogen).

Contact activation system (intrinsic pathway):

The contact system is composed of factor XII (Hageman factor), prekallikrein, high molecular weight kininogen (HMWK) and factor XI, the interaction of which results in the conversion of factor XII to factor XIIa which in turn activates factor XI to XIa. The latter then proteolytically activates factor IX to factor IXa, which in the presence of factor VIIIa, Ca^{++} and phospholipid vesicles or membranes activates factor X and leads to the formation of prothrombinase complex which consists of factors Va (a cofactor), Xa (an enzyme), Ca^{++} and membranes. This complex converts prothrombin to thrombin, which leads to clot formation (Nemerson Y., 1988).

COAGULATION PATHWAYS, INITIATION AND REGULATION



Transformations of precursors into active species.

"Forward" reactions of classical extrinsic, intrinsic and common pathways.

Positive feed back reactions.

Reactions between components of classical extrinsic and classical intrinsic systems.

Fig. (1): Clot promoting reactions of the extrinsic and intrinsic systems, positive feedback reactions and linkages between both coagulation systems. (Lämmle et al., 1985).

So factors IX and X are precursors of active enzymes and must be activated by limited proteolysis to express coagulant activity. Factors VIII and V are precursor forms of cofactors for factors IXa and Xa respectively. In their native state, they have little or no activity and must also be proteolytically cleaved to promote clot formation (Nemerson Y., 1988).

Factor XII (Hageman factor or glass factor):

The liver is the principal site of synthesis of factor XII (Saito et al., 1983).

Activation of factor XII requires prekallikrein,HMWK and an activating surface. Factor XIIa activates plasma prekallikrein and leads to the formation of kallikrein which is capable of reciprocally activating further factor XII. Both prekallikrein and factor XI circulate in plasma in a complex with HMWK. The HMWK functions as a non-enzymatic cofactor localising the prekallikrein and factor XI to the surface so that reciprocal interaction with surface bound factor XII can take place. The surface bound factor XIIa proceeds to activate factor XI to factor XIa by limited proteolysis (Derek & Bruce,1985).

Factor XI (plasma thromboplastin antecedent, PTA):

It consists of two identical polypeptide chains held together by one or more disulphide bonds (Bouma & Griffin, 1977). During the activation of factor XI by factor XIIa an

internal peptide bond in each of the two chains is cleaved giving rise to a pair of disulphide_linked heavy and light chains. The light chain region of factor XIa contains the entire enzymatic active site while the heavy chain contains the high affinity binding site for HMWK (Van der Graaf et al., 1983).

Factor IX (Christmas factor):

Activation of factor IX is achieved by a calcium-dependent two step mechanism. Initially an internal arginyl - valine bond in factor IX is cleaved, giving rise to a two - chain disulphide - linked inactive intermediate which is then converted to factor IXa by a second cleavage caused by the action of factor XIa and resulting in the release of an activation peptide (Fujikawa et al, 1974; Osterud et al., 1978). The activation of factor IX in the intrinsic pathway by factor XIa requires calcium ions (Amphlett et al., 1981).

The activation of bovine factor IX by surface - bound factor XIa was found to be accelerated by cephalin, indicating that phospholipid participates in this reaction as well as in subsequent stages of the coagulation sequence (Mannhalter, et al., 1984).

The interaction between factors VIIIa, IXa and X:

The activation of factor X through the intrinsic pathway requires factor IXa, factor VIIIa (thrombin-modified form), negatively charged phospholipid and calcium ions. All these agents form a complex in which the enzymatic activity of the factor IXa is responsible for the activation of factor X (Derek & Bruce, 1985).

Factor X (stuart Prower factor): In its zymogen form is a two-chain molecule (light & heavy) held together by one or more disulphide bonds. The activation of factor X involves the cleavage of a specific arginyl-isoleucine bond with release of a peptide from the amino-terminal end of the heavy chain. (Derek & Bruce, 1985).

Factor VIIIc is a plasma glycoprotein that acts as a cofactor to factor IXa in the activation of factor X. Modification of the factor VIII (antihaemophilic globulin, AHG) molecule is achieved by thrombin (Hultin & Nemerson, 1978). Factor Xa has also been shown to be capable of activating factor VIII (Vehar & Davie, 1980).

Hultin (1982) reported that the maximum velocity (V max) of factor X activation reaction was increased 56 fold by human factor VIIIa.

Extrinsic coagulation mechanism:

This requires a tissue factor which gains access to the blood only when tissue is damaged. It is initiated by the formation of a complex of the tissue factor with factor VII. Factor VIIa in the presence of tissue factor and calcium is capable of activating factor X and in addition can activate factor IX (Zur & Nemerson, 1980). So in tissue factor (TF) initiated coagulation, factor Xa is generated directly by the action of the TF: VIIa complex and indirectly by the activation of factor IX; the instantaneous concentration of factor Xa is determined by the summation of the two pathways (Nemerson, 1988).

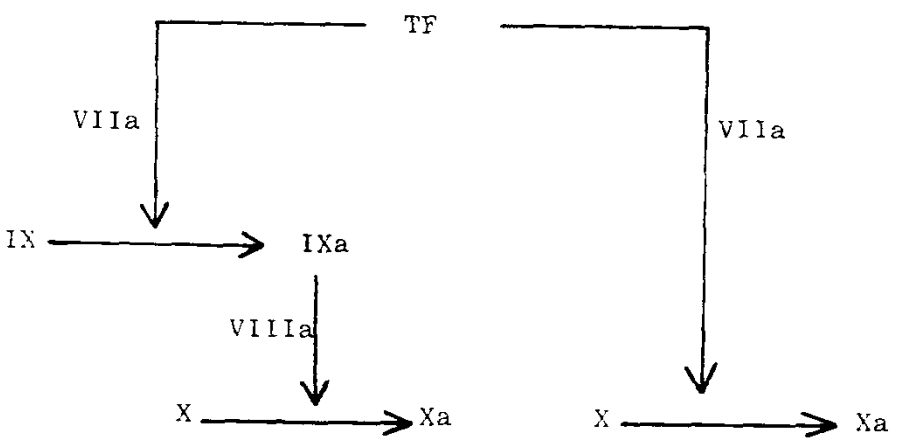


Figure (2): Tissue factor-initiated coagulation
(Nemerson, 1988: Tissue factor and haemostasis)

The mature TF protein consists of 263 residues and has a derived molecular weight of 29, 593. The complete cDNA and protein structure has been deduced from a single 2, 147 - base pair cDNA insert derived from a placental library and cloned in a bacteriophage (Spicer E., 1987), also it has been deduced from overlapping clones (Scarpati et al., 1987).

By using somatic cell hybridization techniques, the TF gene has been localized to chromosome 1 (Carson et al., 1985).

TF resides on the surface of many cells that are not normally in contact with the blood. Subjecting intact cells to a variety of agonists such as interleukin I, tumour necrosis factor and endotoxin results in the induction of TF synthesis (Bevilacqua et al., 1985). Because TF induction is a relatively slow process, it seems unlikely that it could be involved in haemostasis; Induction, however may be important in the pathogenesis of thrombosis. (Nemerson Y., 1988).

The structure of factor VII is very similar to the other vitamin K- dependent clotting factors. Its amino acid sequence, has been deduced from clones of cDNA. The circulating protein consists of 406 amino acid. Conversion to factor VIIa is accomplished by cleavage of an arginine-