INTRODUCTION

Oral anticoagulation with the vitamin K antagonist "Marevan" reduces the rate of thromboembolic events in patients with deep venous thrombosis "DVT" ***(Ansell et al., 2005)***. However, Marevan therapy is challenging because there is wide variation among patients in response and therefore in dose requirement. To achieve and maintain an optimal Marevan dose, the prothrombin time and international normalized ratio "INR" are monitored, and doses are adjusted to maintain each patient's INR within a narrow therapeutic range. An INR of less than 2 is associated with an increased risk of thromboembolism ***(Kearon et al., 2003)*** and an INR of 4 or more is associated with an increased risk of bleeding ***(Levine et al., 2001)***.

Polymorphisms in the gene encoding the cytochrome P-450 2C9 enzyme (CYP2C9) are known to contribute to variability in sensitivity to Marevan. CYP2C9 is the enzyme primarily responsible for the metabolic clearance of s-enantiomer of Marevan ***(Takahashi et al., 2004)***.

Patients with certain common genetic variants of CYP2C9 require a lower dose of marevan and a longer

time to reach a stable dose. They are also at higher risk for over-anticoagulation and serious bleeding ***(Veenstra et al., 2005)***.

Vitamin K epoxide reductase (VKORC1) recycles the vitamin K epoxide to the reduced form of vitamin K, an essential co-factor in the formation of the active clotting factors II, VII, IX and X through γ glutamyl carboxylation ***(Cain et al., 1997)***.

The VKORC1 is the target of Coumarin anticoagulants, and its common genetic variants result in altered sensitivity to Marevan. VKORC1 polymorphisms are associated with a need for lower doses of Marevan during long-term therapy ***(Reider et al., 2005)*** and in some studies, were found to contribute to the variation in dose requirement more than CYP2C9 variants ***(Li et al., 2006)***.

On the basis of these observations, the Food and Drug Administration (FDA) approved a labeling change for Marevan that describes the reported effects of VKORC1 and CYP2C9 on dose requirements, the package insert as of August 2007 stated that "Lower initiation doses should be considered for patients with certain genetic Variations in CYP2C9 and VKORC1 enzymes". The FDA also approved chemical tests for these genetic

variations ***(Higashi et al., 2002)***. However, there is little information about the relative contribution of VKORC1 and CYP2C9 to the anticoagulation response in patients during the initiation of Marevan therapy ***(Beyth et al., 2000)***.

The first months of anticoagulant treatment are particularly problematic, since the safe and effective dose for an individual patient is not known and is determined emperically. Consequently, the risk of over anticoagulation, with the potential for haemorrhagic complications is higher during this time than subsequently. Therefore, genotyping in order to individualize the Marevan dose is likely to have the greatest effect during the initiation of treatment ***(Beyth et al., 2000)*.**

AIM OF THE WORK

To assess the allelic frequencies and to investigate the relationship between "CYP2C9" and "VKORC1" genotype and vitamin K antagonist anticoagulation.

HEMOSTASIS

Hemostasis is a complex process which changes blood from a fluid to a solid state. Intact blood vessels are central to moderating blood's tendency to clot. The endothelial cells of intact vessels prevent thrombus formation by secreting tissue plasminogen activator (t-PA) and by inactivating thrombin and adenosine diphosphate (ADP). Injury to vessels overwhelms these protective mechanisms and hemostasis ensues. Hemostasis proceeds in two phases: primary and secondary hemostasis ***(Furie, 2005).***

Primary Hemostasis: is characterized by vascular contraction, platelet adhesion and formation of a soft aggregate plug. It begins immediately after endothelial disruption. Injury causes temporary local contraction of vascular smooth muscle. Vasoconstriction slows blood flow, enhancing platelet adhesion and activation ***(Ogedegbe and Hill, 2003).***

Secondary Hemostasis:is responsible for stabilizing the soft clot and maintaining vasoconstriction. Vasoconstriction is maintained by platelet secretion of serotonin, prostaglandin and thromboxane. The soft plug is solidified

through a complex interaction between platelet membrane, enzymes, and coagulation factors. Coagulation factors are produced by the liver and circulate in an inactive form until the coagulation cascade is initiated. The cascade occurs in steps. The completion of each step activates another coagulation factor in a chain reaction which leads to the conversion of fibrinogen to fibrin ***(Ogedegbe and Hill, 2003)*.**

Substances required for the proper functioning of the coagulation cascade include:

- Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade ***(Ogedegbe and Hill, 2003)*.**

- Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding

the gamma-carboxyl group to glutamate residues on the immature clotting factors, Vitamin K is itself oxidized. *Vitamin K epoxide reductase*, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target for anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Other deficiencies of vitamin K (e.g., in malabsorption), or disease (hepatocellular carcinoma) impairs the function of the enzyme and leads to the formation of PIVKAs (proteins formed in vitamin K absence); this causes partial or non-gamma carboxylation, and affects the coagulation factors' ability to bind to expressed phospholipid ***(Giangrande, 2003)*.**

STEPS OF COAGULATION PROCESS

Hemostasis, the stoppage of bleeding, is accomplished through three steps:

Vascular Role in Hemostasis

Vascular Spasm, a constriction of the damaged blood vessel, occurs at the site of injury. Vasoconstriction is initiated by the smooth muscle of

the blood vessel in response to the injury and by nerve signals from pain receptors ***(Furie, 2005).***

The normal vascular endothelium maintains blood fluidity by inhibiting blood coagulation and platelet aggregation, and promoting fibrinolyisis ***(Feinstein et al., 2001).***

The endothelial cells are activated when exposed to endotoxin, the cytokines interleukine-1 (IL-1) and tumor necrosis factor (TNF), thrombin, low oxygen tension and increased shear stress ***(Feinstein et al., 2001).*** As the endothelial cells are negatively charged, they repel the negatively charged platelets. This anionic surface, as well as, other antithrombogenic properties of endothelium could be important in limiting the intravascular extension of the hemostatic reaction induced by vessel injury ***(Feinstein et al., 2001).***

When the vessel is injured, it constricts, thereby diverting blood from the site of injury, and the shed blood is exposed to these subendothelial structures that stimulate hemostatic plug formation by promoting platelet adhesion and aggregation, and by activating blood coagulation ***(Middeldrop et al., 2001).***

Role of Platelets in Coagulation Process:

Platelet plug consisting of a mass of linked platelets, fills the hole in the damaged blood vessel. Platelet plug formation follows these steps:

- Platelet adhesion: Platelets adhere to the exposed collagen fibers in the damaged blood vessel wall.
- Platelet release: Platelets release ADP (which attracts other platelets to the injury), serotonin (which stimulates vasoconstriction), and thromboxane A_2 (which attracts platelets and stimulates vasoconstriction). Cellular extensions from the platelets interconnect and form a loose mesh.
- *Platelet aggregation*: Additional platelets arrive at the site of the injury in response to the released ADP and expand the accumulation of platelets.

(Furie, 2005)

Platelet activation

Damage to blood vessel walls exposes subendothelium proteins, most notably von Willebrand factor (vWF), present under the endothelium. vWF is a protein secreted by healthy endothelium, forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated,

underlying vWF is exposed to blood and recruits Factor VIII, collagen, and other clotting factors. Circulating platelets bind to collagen with surface collagen-specific glycoprotein Ia/IIa receptors. This adhesion is strengthened further by additional circulating proteins vWF, which forms additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrils. These adhesions activate the platelets ***(Thiagarajan, 2004).***

Platelet release reaction

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A_2 (TXA_2), which, in turn, activate additional platelets. The granules' contents activate a protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase A_2 (PLA_2). PLA_2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets ***(Thiagarajan, 2004).***

Coagulation (Blood Clotting):

Is a complex series of reactions that transform liquid blood into a gel (clot) that provides a secure patch to the injured blood vessel. Thirteen coagulation factors (numbered I through XIII in order of their discovery) are involved (Table 1). Most of these factors are proteins released into the blood by the liver. Factor III is Ca^{2+}. Vitamin K is required for the synthesis of some of these factors ***(Feinstein et al., 2001)*.**

Most of the traditional diagrammatic representations of haemostasis represent the process as consisting of three separate systems, the intrinsic, extrinsic and common pathways. Most modern evidence would suggest this is not a good reflection of the in vivo process in healthy individuals. It is now known that both tissue factor and thrombin play a much more important role than was previously thought and the role of factor XII in the activation of coagulation needs to be reassessed as more physiological mechanisms for the activation of factor XI have been described. It must also be remembered that the traditional coagulation diagrams are only a partial picture as the role of the cellular component and the fibrinolytic process are not usually included. The new coagulation jet series of diagrams have been developed in an attempt to better represent the whole physiological system of

normal haemostasis in a schematic form ***(Jenny and Mann, 2002).***

The Traditional View of Coagulation:

Histologically the coagulation mechanism was separated in three separate sections.

The intrinsic pathway: This pathway was considered to be the more physiologically relevant route as it could be used to explain the bleeding disorders found in haemophiliacs. All the components of this pathway are found circulating in the blood of healthy individuals ***(Morrissey, 1995).***

The extrinsic pathway: As the name implies, this pathway involves a key component, tissue factor, not found circulating in its active form in the blood of healthy individuals. Tissue factor is a transmembrane protein found mainly on the endothelium, but has also been described as a constituent of the monocyte cell membrane. This is the key protein required for initiation of the extrinsic pathway. Historically this was considered to be the less important pathway ***(Morrissey, 1995).***

The common pathway: This covers the final steps of the coagulation process which result in the conversion of soluble fibrinogen into the insoluble fibrin clot by the action of

thrombin and factor XIII. This pathway is common to both the intrinsic and extrinsic pathway (hence its name) ***(Morrissey, 1995).***

The traditional representation of the coagulation cascade, first described in the mid-1960s, fits well with the commonly used routine laboratory testing parameters, namely Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT). These commonly used routine laboratory tests were developed before the coagulation cascade was first described and they were used to help scientists to develop the three traditional, theoretical pathways. Although these tests five good and clinically relevant information, they are artificial and non-physiological. Reagents for the APTT assay use artificial negatively charged materials to initiate clotting, while the reagents used for PT assays contain non-physiological concentrations of tissue factor, brain or other tissue extracts. It is therefore not surprising that the resulting pathway is not a completely true reflection of the normal haemostasis process ***(Jenny and Mann, 2002).***

The Modern View of Haemostasis:

Since the mid-1960's, a number of other key components of the coagulation system (e.g., Antithrombin, Protein C pathway, TFPI), the fibrinolytic system (e.g., PAI, tPA, TAFI) and other haemostasis related factors (e.g., EPCR,

Protein Z, ZPI) have been discovered. These components need to be fitted into any new diagram representing the haemostasis system. Historically the intrinsic pathway in the coagulation cascade was considered to be the most important pathway. One major weakness was always that this pathway could not correctly explain the minimal or even non-existent role for high molecular weight kininogen, prekallikrein and factor XII (Hageman factor) in the activation of coagulation. Their involvement had always been questionable because deficiencies did not resulting a bleeding disorder. Mr. Hageman, in whom the deficiency was first described, did not have a haemorrhagic disorder ***(Jenny and Mann, 2002).***

In the last years, it has been shown that the traditional extrinsic pathway (tissue factor and factor VIIa) is the major pathway for activation of coagulation and since the 1960's thrombin has been found to play a far more important role, acting as both an activator and an inhibitor in the haemostasis process. Tissue factor in complex with factor VIIa has been shown to activate factor IX as well as factor X, helping to further break down the artificial barriers of the extrinsic and intrinsic pathway theory. Thrombin, the key enzyme for the conversion of soluble fibrinogen into fibrin, has also been shown to be required for the activation of the protein C system which down regulates haemostasis, and also for the activation

of TAFI which is involved in the down regulation of the fibrinolytic process. Activation of FXI of FXIa by thrombin has also been demonstrated and this is considered to be the normal physiological mechanism. Thrombin is therefore not only involved in fibrin formation, but is also involved in controlling its own production and inhibition ***(Jenny and Mann, 2002).***

The sum of all these discoveries has changed the modern view of coagulation and led to the development of alternative representations of the coagulation pathways that better represent the normal in vivo process. A series of diagrams have been developed that are intended to better reflect the current state of knowledge of the coagulation process **(Morrissey, 1995).**

The Coagulation Jet Diagram:

In this haemostasis diagram, the whole coagulation process is shown as a jet with tissue factor playing its key central role in triggering the activation of coagulation. To fit with modern ideas, factor XII and the higher members of the traditionally intrinsic pathway have been excluded. This updated representation of the coagulation pathway is a single integrated system of intrinsic, extrinsic and common pathways. The pathways shows: