Towards improvement of oral anticoagulant therapy

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CHAPTER 1

General Introduction

General introduction

Thrombosis, or the formation of a blood clot which hampers blood flow in a bloodvessel, is a very serious disease which can potentially be fatal. Thrombosis can occur in arteries (arterial thrombosis) as well as in veins (venous thrombosis). The most commonly known forms of arterial thrombosis are thrombosis of the coronary arteries and the carotid arteries, which may cause myocardial infarction or ischaemic stroke. Venous thrombosis most commonly presents as an isolated thrombus in vessels of the leg, as a pulmonary embolism (which may be a clot formed in the legs, which has embolised to the pulmonary vasculature), or as a combination of both. An arterial thrombus mainly consists of platelets, whereas in venous thrombosis fibrin is the main component. Treatment and prevention of arterial and venous thrombosis is aimed at inhibiting platelet function or inhibition of coagulation. Three classes of anti-platelet drugs are currently approved for clinical use; cylooxygenase inhibitors (aspirin), P2Y12 inhibitors (such as clopidogrel), and inhibitors of platelet aggregation (such as Reopro). The most commonly used anticoagulant drugs are heparin and heparin derivatives and vitamin K antagonists.

Vitamin K was discovered in 1929 by the observation of bleeding syndromes among chickens that were fed a fat-free diet [1]. This postulated the existence of a nutritional factor that was essential for normal haemostasis and Dam et al named this factor vitamin K. From clinical observations on patients with liver cirrhosis and obstruction from bile ducts it became clear that there was a direct link between liver function, bile secretion, vitamin K and the synthesis of clotting factors [2].

In the early 1920s a veterinarian in North Dakota, USA described a haemorrhagic diathesis in cattle that was caused by the ingestion of spoiled, sweet clover [3]. In 1929 it was demonstrated that this bleeding disorder was caused by a

deficiency of functional prothrombin [4]. It wasn't until 1940 that chemists of the University of Wisconsin discovered that the anticoagulant substance in the moldy sweet clover was the coumarin derivative 4-hydroxycoumarin [5]. In 1948 the first anticoagulant, warfarin (named for the Wisconsin Alumni Research Foundation), was produced [6]. It was first registered for use as a rodent poison. In 1954 warfarin was approved for medical use in humans. The exact mechanism of action remained unknown until it was demonstrated, in 1978, that warfarin inhibited vitamin K epoxide reductase and hence interfered with vitamin K metabolism [7].

Vitamin K is essential for the function of vitamin K dependent coagulation factors. These specific proteins involved in the coagulation cascade are modified after synthesis by a vitamin K dependent process. Specific amino acid residues in the so-called gla-domain are modified from a glutamic acid to a γ-carboxy glutamic acid by a vitamin K dependent carboxylase. In this process the vitamin K is oxidised to vitamin K epoxide. The γ-carboxy glutamic acid residues have a double negative charge at physiological pH, which is in contrast to the single negative charge of the (non modified) glutamic acid. The introduction of extra negative charge in the gla-domain of the vitamin K-dependent proteins is essential for their Ca^{2+}-mediated interaction with negatively charged cell membranes. Coagulation reactions take place on cellular surfaces – typically an activated platelet or endothelial cell. If the gla-domain of a vitamin K-dependent coagulation factor is not modified, the protein loses its capacity to bind to negatively charged cellular structures and thus is no longer able to participate in coagulation reactions.

Vitamin K antagonists exhibit their effect by interfering with the vitamin K cycle. Because the body is not able to store vitamin K, it is recycled through the vitamin K cycle. When vitamin K is oxidised in the carboxylation process described above it is no longer biologically active, and needs to be reduced by

vitamin K epoxide reductase (VKOR). Vitamin K antagonists have structural similarity to vitamin K, and therefore reduce availability of biologically active, reduced vitamin K.

Vitamin K is a necessary factor to produce the vitamin K-dependent clotting factors II, VII, IX and X, and the anticoagulant proteins C and S. Although both pro- and anticoagulant proteins are no longer post-translationally modified to biological active proteins in the presence of warfarin, the net effect of warfarin is an anticoagulant state. However, when warfarin treatment is initiated, it takes time for already circulating active coagulation factors to be replaced by inactive ones. The half-life of vitamin K-dependent coagulation factors varies from 4-6 hours (FVII) to 42-72 hours for prothrombin, so it will take 4-6 days before levels of activated coagulation factors are reduced to such an extent that sufficient anticoagulation is achieved. In some cases, particularly patients with anticoagulant protein deficiencies, this may lead for a short period to a hypercoagulable state with a risk of thrombosis. In order to bridge the period between initiation of anticoagulant treatment and the moment of sufficient vitamin K antagonist-induced anticoagulation, sometimes patients receive heparin for the first few days after initiation of anticoagulation, as heparin inhibits the coagulation system instantly.

Worldwide there are different types of vitamin K antagonists available. The vitamin K antagonists most frequently used are warfarin, acenocoumarol and phenprocoumon. Warfarin is the vitamin K antagonist of choice in the United States of America, the United Kingdom and many other countries around the world; acenocoumarol and phenprocoumon are frequently used in many European countries. These three vitamin K antagonists mainly differ in their half-life. Acenocoumarol has the shortest half-life of 11 hours, followed by warfarin with 36-42 hours and the longest half-life is seen in phenprocoumon with approximately

140 hours [8-11]. The clearance of these vitamin K antagonists is also different. Acenocoumarol is for its elimination completely dependent on hydroxylation by cytochrome p450 (CYP). Warfarin is also dependent on reduction processes [12]. Phenprocoumon can, in addition to elimination as hydroxylated metabolites, be eliminated as parent compound and is thus less dependent on hydroxylation by CYP.

While vitamin K antagonists decrease the risk of a thrombotic event by inhibiting coagulation, through the same mechanism they increase the risk of severe or even fatal haemorrhage. Prescription of vitamin K antagonists should therefore always be preceded by a careful evaluation whether the benefit will outweigh the bleeding risk. Vitamin K antagonists have a narrow therapeutic window, and frequent monitoring with adjustment of anticoagulant dosage is required to maintain patients within the therapeutic window. The response to vitamin K antagonists in a single patient is highly variable and unpredictable. The intensity of anticoagulation is assessed with a simple laboratory test. In this test, plasma is allowed to clot by addition of a reagent containing tissue factor (the physiological initiator of coagulation), phospholipids and calcium. The time to clot formation is a measure of the functionality of the so-called extrinsic pathway of coagulation, which consists of coagulation factors VII, X, V, II, and fibrinogen. This particular coagulation test is referred to as the prothrombin time (PT). Although the PT is sensitive for anticoagulation with vitamin K antagonists, the tests are poorly standardised between different laboratories. Use of different reagents and equipment results in substantially different PT values from a single blood sample. To overcome this standardisation problem, the INR or international normalised ratio has been developed.

The INR is assessed according to the formula:

$$INR = (\text{patient PT}/\text{ mean normal PT})^{isi}$$

In this formula ISI is the International Sensitivity Index, which is the calibration factor to correct for the type of thromboplastin and equipment used.

The introduction of the INR system to reflect anticoagulation led to several studies to determine the optimal level of anticoagulation, i.e. the level at which least complications occur [13-16]. The general recommendation is an INR between 2.0 and 3.0. Sometimes, dependent on the indication, a more intense anticoagulation is needed for which the recommendation is an INR between 3.0 and 4.0. In the Netherlands, the Dutch Federation of Anticoagulation Clinics (Federatie Nederlandse Trombosediensten, FNT) proposes target ranges of 2.5 – 3.5 and 3.0 – 4.0.

Patients who are insufficiently anticoagulated (i.e., an INR below the therapeutic window appropriate for their indication) are at increased risk for (re)thrombosis, whereas over-anticoagulated patients show a sharp increase in bleeding risk [16]. In spite of frequent monitoring, the annual risk for experiencing a serious bleeding complication is 1-2%[17,18]. Several studies investigated potential risk factors for haemorrhagic complications, such as increased age, indication for anticoagulant therapy and the use of interacting medication [19-21]. Besides these acquired factors, also genetic factors are shown to be of influence. Several studies have investigated the association between CYP2C9 genotype and warfarin response. Aithal et al. were the first to demonstrate an association between CYP2C9 genotype and warfarin sensitivity. Carriers of a CYP2C9*2 or CYP2C9*3 allele have lower dosage requirement and showed an increased risk for over-

anticoagulation and major bleeding complications in the initial phase of treatment compared to wild-type patients [22]. The presence of polymorphisms in the VKORC1 gene has also been identified to be associated with warfarin response. Carriers of VKORC1 polymorphisms showed a reduced requirement of warfarin dosage [23]. Most studies that investigate the effect of both the genotypes of CYP2C9 and VKORC1 showed that most variation in dosage is explained by polymorphisms in the VKORC1 gene.

Although the quality of oral anticoagulant treatment is already high, improvement is important. The risk for complications rises sharply with INR values below 2.0 and exponentially with INR values above 5.0 [16]. As a result of the large inter- and intra-patient variability in response to a certain dosage, patients may frequently be under- or overanticoagulated, despite frequent monitoring and adjustment of anticoagulant dose. Approximately 30 to 50% of the time, patients' INR is out of range. Improvement can be targeted at several points. First, dosing of vitamin K antagonists can be improved. If physicians are able to predict a patients' required maintenance dosage better, this would result in spending more time within the therapeutic range, and therefore in less complications. Dosing is classically performed by monitoring sequential INR values and the effect of previous dosing adjustments on the INR. Computer algorithms have been introduced to facilitate dosing and to produce a dosing advice based on mathematical processing of previous INR values and anticoagulant dosages. The use of these computer algorithms to assist physicians with their dosing decisions has been shown to lead to equal or improved quality of control of oral anticoagulant treatment compared to unassisted dosing [24-28]. However, a major disadvantage of these algorithms is that they do not generate a dosage proposal in all cases and they do not account for

the sensitivity of the individual patient for the anticoagulant (which may change over time), the half-life of the drug, and the non-linearity of the dose-INR relation.

Second, it is important to identify those patients who are at increased risk for experiencing either a thrombotic or a bleeding complication. Both patients who are unstably anticoagulated (large differences in sequential INRs) and patients who spend much time outside the therapeutic range are at risk. These patients can be more easily recognised when there is a measure to reflect their instability and if risk factors, either environmental or genetic, are identified. If one can recognise these patients actions such as patient education and more frequent monitoring can be taken.

Outline of this thesis

The studies included in this thesis aim to optimise dosing of vitamin K antagonists and control of oral anticoagulant treatment.

In **chapter 2** we describe the results of a double-blind randomised controlled trial in which we compared two computer algorithms for anticoagulant dosing. A newly developed algorithm which incorporated the sensitivity for vitamin K antagonists (ICAD) was compared to an algorithm frequently used in the Netherlands (TRODIS).

The relationships between maintenance dosages between the three most used vitamin K antagonists acenocoumarol, warfarin and phenprocoumon were studied in **chapter 3**. We calculated transition factors for switching from one vitamin K antagonist to another among participants in a randomised controlled trial who were treated with 2 different vitamin K antagonists.

In **chapter 4** the effects of polymorphisms in the CYP2C9 and VKOR genes were investigated in a cohort of patients starting with oral anticoagulant

treatment with acenocoumarol in Italy. We described the effect of these polymorphisms on the dose requirement and the risk of over-anticoagulation.

Instability is considered a risk factor for developing hemorrhagic and thrombotic complications. In **chapter 5** we studied several methods to reflect instability and investigated which method was best associated with hemorrhagic and thrombotic events in patients with mechanical heart valve prosthesis treated with vitamin K antagonists. Determinants of instability were investigated in **chapter 6**.

Finally, **in chapter 7** we present the study design and general results of a trial of which the primary aim was to compare the quality of an oral anticoagulant treatment with warfarin to the quality of treatment with phenprocoumon.

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