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Evolution of Anticoagulant Therapy: A Focus on Newer Anticoagulants

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Abstract

Anticoagulant therapy has evolved from the use of traditional anticoagulants such as unfractionated heparin and low molecular weight heparins (LMWHs) to the use of direct thrombin and factor Xa inhibitors. A limitation in the use of acetyl salicylic acid (Aspirin) has led to the development of drugs that target the Platelet $P2Y_{12}$ ADP receptor. Orally administered newer anticoagulants have an advantage over traditional anticoagulants that have to be administered parenterally. Unlike Warfarin the newer direct Factor Xa inhibitors do not require laboratory monitoring of INR (International normalized ratio) by the measurement of Prothrombin time (PT). Pharmacogenomic variability associated with some of these drugs affect patient management. The coagulation laboratory is challenged to come up with newer assays to assess the efficacy of therapy.

Introduction

Before a discussion of anticoagulants let us discuss some basic concepts of coagulation.

The first step in the formation of a coagulum is the adhesion of platelets to the exposed endothelial surface of the broken blood vessel. The platelets through glycoprotein Ib-IX-V receptor complex (or CD 42) adhere to von Willebrand (vW) factor, a multimeric protein on the exposed endothelial surface. In addition to vW factor other ligands such as, collagen are recognized by specific platelet membrane receptors during the process of platelet adhesion. The adhesion of platelets to ligands on the subendothelial matrix activates platelet membrane lipases resulting in the release of arachidonic acid from the platelet membrane. Arachidonic acid is further converted by cyclooxygenase-1 enzyme to prostaglandin cyclic endoperoxides (PGG_2 and PGH_2). The widely used drug Aspirin (acetyl salicylic acid) inhibits the enzyme cyclooxygenase-1 thus maintaining the fluidity of blood in an intact blood vessel. The cyclic endoperoxides, in turn are converted by thromboxane synthetase enzyme to thromboxane A_2. The latter triggers the release from the platelet dense granules of adenosine diphosphate (ADP) which promotes the aggregation of platelets through the platelet ADP $P2Y_1$ and $P2Y_{12}$ receptors. The activation of platelets initiates a series of intracellular signaling events leading to a conformational change in the platelet glycoprotein IIb/IIIa receptor that allows the receptor to bind fibrinogen readily leading ultimately to the formation of a platelet plug and the initiation of coagulation. As platelets are aggregated by strong agonists such as thrombin and collagen, the platelet membrane phospholipid, phosphatidyl serine is translocated to the outer surface of the platelet membrane upon which two major coagulation factor complexes (the tenase and prothrombinase complexes) are assembled. The tenase complex leads to the generation of activated factor X (Xa) upon binding of activated factors IXa and VIIIa in presence of calcium. The prothrombinase complex is formed upon binding of activated factor V (Va) in presence of calcium to Xa and prothrombin, which ultimately cleaves prothrombin to form thrombin. Thrombin generated on the surface of the platelet plug converts fibrinogen to fibrin and stabilizes it by activating factor XIII (XIIIa) to form a cross-linked fibrin clot [1,2]. Other aspects of coagulation mechanism such as the tissue factor pathway involving factor VII (VIIa), the regulation of coagulation by the fibrinolytic pathway and the role of prostacyclin (PGI_2) in modulating platelet activation, all of which are outside the scope of this review.

Therapeutic approaches to anticoagulation

Numerous new anticoagulant drugs have been evaluated in clinical trials. I have chosen to discuss just a select few of these in this review. These anticoagulants can be classified as "Indirect" or "Direct inhibitors".

Indirect inhibitors

Unfractionated heparin (UFH) by binding to antithrombin (AT) and activating it has long served as an indirect inhibitor of coagulation. UFH with more than 18 pentasaccharide chains can inhibit both thrombin and factor Xa. However, the heparin-AT complex is unable to inhibit thrombin and factor Xa sequestered in the fibrin clot. Other drawbacks associated with UFH isolated from animal sources such as pigs' intestines is the potential for contamination as was seen in 2008 when batches of UFH produced in China had to be recalled since they were contaminated with chrondroitin sulfate. There is also a risk of heparin-dependent antibodies directed to platelet factor 4 binding to platelets and causing heparin-induced thrombocytopenia (HIT).

Low-molecular weight heparins (LMWHs) prepared from chemical or enzymatic treatment of UFH since they have less than 18 pentasachharide chains can by binding to antithrombin inhibit only factor Xa. LMWHs have greater bioavailability and longer half-life making it amenable for once-or twice a-day dosing and do not unlike UFH require laboratory monitoring. They also have less interaction with platelets with a lesser risk for HIT. Synthetic LMWH preparations containing the AT-binding pentasaccharide region designed to inhibit factor Xa are of well defined purity in contrast to LMWHs prepared from UFH. They too have less interaction with platelets and can be administered once a day without the need for laboratory monitoring. LMWHs do not inhibit clot-bound factor Xa and like UFH must be administered parenterally [1,3]. A synthetic hexadeca-saccharide has been reported to inhibit both factor

Xa and thrombin. It does not bind to platelet factor 4 (PF4) or fibrin and hence can also inhibit clot-bound thrombin [3]. It should be noted that unfractionated heparin and low molecular weight heparins are still the major anticoagulants used in cardiac and orthopedic surgery frequently followed by the switch to direct inhibitors of Factor Xa or thrombin.

Direct inhibitors

In contrast to both UFH and LMWHs these inhibitors directed specifically to either thrombin or factor Xa can inhibit both free and clot-bound thrombin and factor Xa respectively.

Direct Thrombin inhibitors: The most potent thrombin inhibitor is hirudin_originally isolated from the salivary glands of the leech *Hirudo medicinalis*. It binds to thrombin very tightly with an inhibition constant (K_i) of 10^{-15} M (femtomolar). Unlike heparin which dissociates from antithrombin upon binding of the complex to thrombin and is reutilized, hirudin binding to thrombin is mole per mole and is irreversible. While excess heparin can be neutralized with protamine sulfate no such antidote is available to neutralize either hirudin or recombinant hirudin. The latter designed for therapy was accompanied with bleeding episodes thus requiring careful dosing and laboratory monitoring by the Ecarin clotting time. The test is based on the fact that Ecarin, an enzyme isolated from venom of snake *Echis carinatus* can convert prothrombin to meizothrombin. Since hirudin inhibits meizothrombin as soon as it is formed, only after all the hirudin has complexed with meizothrombin can the additional meizothrombin generated convert fibrinogen to fibrin and clot the sample [1]. Modifications of hirudin such as hirugen, hirulog and bivalirudin have been introduced.

Bivalirudin whose binding to thrombin is reversible has been found suitable for use in percutaneous coronary intervention (PCI) procedures. It also performed better than UFH plus abciximab (ReoPro), antibody toGPIIb-IIIa in patients with ST elevation in myocardial infarction [4]. A small molecule called **argatroban** (M. wt. 532 Da) belonging to a class of thrombin inhibitors called peptidomimetics is a reversible inhibitor of thrombin (K_i 19 nM) and has been used to treat patients with HIT. While these direct thrombin inhibitors have the advantage in terms of lack of reactivity with platelets they have to be administered intravenously.

A promising specific and reversible thrombin inhibitor is Dabigatran etexilate (M.wt. 627.7 Da), a benzamidine-based molecule, has the added advantage in that it can be administered orally. It is a pro-drug which is converted rapidly in the liver to the active Dabigatran with maximum plasma concentrations in plasma reached within 2 hours after intake. The drug has undergone clinical trials for the prevention of venous thromboembolism (VTE) in patients undergoing total hip and knee replacement surgery and for the prevention of stroke in patients with atrial fibrillation [5,6]. These studies demonstrated that a fixed dose of Dabigatran etexilate was just as effective as warfarin with a similar safety profile and unlike warfarin does not require laboratory monitoring. Indeed the Food and Drug Administration (FDA) in the USA approved Dabigtran in October 2010 for the prevention of stroke in patients with atrial fibrillation. Dabigatran, however, has gastrointestinal side effects since it stimulates the production of excess stomach acid. It may also be unsuitable for patients with renal disease since 80% of the drug is excreted by the kidney.

Direct factor Xa inhibitors

Rivaroxaban, Apixaban and Edoxaban are examples of a few of the direct factor Xa inhibitors that have undergone extensive clinical studies. I will limit my discussion to Rivaroxaban and Apixaban in this review. These inhibitors are small molecules which are highly specific reversible inhibitors of factor Xa and can be administered in fixed doses without the need for routine laboratory monitoring. They inhibit both free and clot-bound factor Xa and prothrombinase activity. As an inhibitory target factor Xa is very attractive since it blocks the thrombin burst considering that one molecule of factor Xa can generate 1000 molecules of thrombin. They have relatively short half-lives when compared to warfarin and have demonstrated their potential in the prevention and treatment of thromboembolic disease (deep vein thrombosis, pulmonary embolism). They are metabolized by cytochrome P-450 3A4 (CYP3A4) isoform and are substrates for P-glycoprotein. Hence drugs or herbs that either induce or inhibit either of these 2 pathways would have a bearing on the pharmacokinetics of these direct factor Xa inhibitors and would require adjustment of dose.

Rivaroxaban: The results of 2 major studies comparing Rivaroxaban, an oxazolidinone derivative (M.wt. 435.9 Da) with a low molecular weight heparin (enoxaparin) followed by a vitamin K antagonist (warfarin or acenocoumarol) one in patients with acute deep-vein thrombosis (DVT) and the other on patients with acute pulmonary embolism have been published [7]. Rivaroxaban at an initial oral dose of 15 mg twice a day for 3 weeks followed by a 20 mg dose once daily when compared with enoxaparin followed by warfarin or acenocoumarol proved to be safe and effective in the treatment of venous thrombosis. Rivaroxaban has a rapid onset of action with a half-life ranging from 7 to 12 hours compared to 20 to 60 hours for warfarin. The rapid onset of action while obviating the need for the administration of heparin also requires strict patient compliance given the short half-life of the drug.

Apixaban: A study has confirmed that this factor Xa inhibitor (M.wt. 459.5 Da) at an oral dose of 2.5 mg twice a day was more effective in patients undergoing total hip replacement when compared to enoxaparin (40 mg/day) [8]. Treatment with apixaban while having a similar bleeding profile as enoxaparin was, however, associated with fewer thromboembolic events. The half-life of apixaban is 12 hours and like rivaraoxaban requires strict patient compliance.

Drugs that target the platelet ADP $P2Y_{12}$ receptor

Limitations in the use of aspirin (acetyl salicylic acid) which by inhibiting cyclooxygenase-1 enzyme prevents the conversion of arachidonic acid to prostaglandin cyclic endoperoxides (PGG_2 and PGH_2) and the subsequent generation of thromboxane A_2 thus keeping the blood from clotting, has led to the development of drugs that target the platelet ADP $P2Y_{12}$ receptor. Limitations of aspirin apart from its gastrointestinal side effects include the finding that some patients are resistant to aspirin. These patients can be managed with oral drugs that inhibit the binding of ADP to the platelet $P2Y_{12}$ receptor thus preventing the platelets from aggregating. The interaction of two platelet receptors $P2Y_1$ and $P2Y_{12}$ are required for the transduction of ADP signal. $P2Y_1$ activation leads to a change in platelet shape and a weak phase of platelet aggregation. However, it is the $P2Y_{12}$ activation that in turn leads to GPIIb-IIIa receptor activation and ultimately to the formation of a stable platelet aggregate. Thienopyridines are a class of molecules that irreversibly inhibit the ADP $P2Y_{12}$ receptor. The widely used drug in this class is clopidogrel: It is a pro-drug which is converted by cytochrome P450 (CYP2C19) isoform in the liver to its active form that inhibits ADP from binding to platelet $P2Y_{12}$ receptor thus preventing platelets from aggregating. It is slow in achieving maximum platelet inhibition taking as long as 4 to 5 days at the standard 75 mg dose, which can however, be reduced to 3 to 5 hours by giving a 300 to 600 mg loading dose. The inhibition is irreversible and persists throughout the lifetime of the platelet which is problematic for patients requiring coronary artery bypass grafting (CABG) procedure who would then be subject to increased risk of bleeding [9]. The widespread use of this drug commercially called Plavix has uncovered that subjects with mutations in the alleles *2 to *5 of CYP2C19 are poor metabolizers of clopidogrel and present a risk of thrombosis compared to wild type *1 allele who are normal metabolizers. In contrast, persons with mutation in

allele *17 of CYP2C19 are ultra rapid metabolizers in whom a smaller dose of the drug is required. This heighted awareness of the fact that clopidogrel therapy needs to be tailored to a person's genotype has led to the use of an automated assay to detect CYP2C19 mutations in alleles *2,*3 and *17. In a meta-analysis of 9 studies of patients who had coronary artery stents and were on clopidogrel therapy, carriers with just one reduced- function CYP2C19 allele had a 167% increased risk for stent thrombosis compared to those who had wild type allele. The risk increases even more dramatically in carriers of 2 reduced-function alleles [10]. Doubling the standard dose of clopidogrel in non-responders appeared to have little effect as was gleaned from the results of the GRAVITAS (Gauging responsiveness with a Verify Now Assay-Impact on Thrombosis and Safety). (Verify Now Assay is a platelet function testing assay that measures inhibition of the $P2Y_{12}$ receptor). The GRAVITAS, a multi-center placebo controlled study was designed to ascertain whether a high maintenance dose of clopidogrel therapy established on the basis of results obtained with the Verify Now assay reduces ischemic events post-percutaneous coronary intervention (PCI). In addition to CYP2C19 polymorphism the ABCB1 gene involved in drug transport may also have a bearing on patients' responsiveness to clopidogrel. This brings us to the use of alternative drugs to achieve platelet inhibition in patients who are non-responders to clopidogrel.

Prasugrel: This drug also belongs to the family of thienopyridines. It, like clopidogrel, is a pro-drug. However, unlike clopidogrel, it achieves faster and more pronounced platelet inhibition at a relatively lower dose (60 mg loading dose and 10 mg maintenance dose for prasugrel, compared to 300 to 600 mg loading dose and 75 to 150 mg maintenance dose with clopidogrel). Like clopidogrel platelet inhibition is irreversible during the life time of the platelet. Prasugrel is converted by esterases to an intermediate metabolite which in turn is converted to an active metabolite by any one of the four different CYP isoforms. As such it is less affected by reduced function alleles of CYP2C19 as clopidogrel is. Prasugrel (trade name Effient) has been approved for use on patients with either reduced function alleles of CYP2C19 or those with high platelet reactivity. However, there was increased bleeding in elderly patients and in those with a history of transient ischemic attack and stroke when compared to clopidogrel [11].

While both Prasugrel and clopidogrel are pro-drugs and are irreversible platelet inhibitors other direct acting and reversible platelet inhibitors are on the scene. One such drug is Ticagrelor.

Ticagrelor: This drug can also be administered orally. It is an ATP analogue that inhibits the platelet ADP $P2Y_{12}$ receptor reversibly. Ticagrelor has been studied extensively including a trial that compared it favorably with clopidogrel on 18,624 hospital patients admitted with an acute coronary artery syndrome [12].

Pharmacogenomic Variability: I addressed previously the effect of mutations in some of the alleles of CYP2C19 that influences the pharmacokinetics of clopidogrel. Warfarin therapy is influenced by variations in genes involved in its metabolism. Warfarin exists in two enantiomeric forms (R- and S- warfarin). R-warfarin is metabolized by CYP1A2 and CYP3A4 isoforms. S-warfarin which is two to five times more potent than the R-enantiomer is metabolized by the hepatic microsomal CYP2C9 isoform to the inactive S-7-hydroxywarfarin. Carriers of CYP2C9*2 and CYP2C9*3 variant alleles had a 30% and 80% decrease in enzymatic activity respectively subjecting them to an increased risk for overanticoagulation and bleeding unless the warfarin dose was reduced [13]. Variations in vitamin K epoxide reductase complex subunit 1 (VKORC1) gene also affect the efficacy of warfarin. The efficacy of warfarin is dependent on its inhibiting vitamin K epoxide reductase enzyme. This enzyme is involved in the pathway of the production of the active form of vitamin K which is required to add gamma carboxyl groups to vitamin K dependent clotting factors II, VII, IX and X and thus facilitate the process of clotting [1]. Variations in the VKORC1 gene dictated the warfarin dose required to maintain stable anticoagulation. Compared to wild type, the two variants of the VKORC1 gene (the CT and TT genotypes) required 27% and 47% reduction in warfarin dosage respectively to maintain stable anticoagulation [14]. This inter-individual genetic variability makes it imperative for warfarin dosage to be determined by montoring the patient's INR (international normalized ratio) derived from prothrombin time measurements. Ultimately determination of the patient's genotype is the best way to establish the stable warfarin dosage required to maintain anicoagulation without the risk of encountering overanticoagulation and bleeding or insufficient anticoagulation and clotting.

Influence of herbs on therapy

Herbs that induce or inhibit cytochrome P450 (CYP) isoforms affect anticoagulation therapy. The effects of herbs on warfarin therapy can range from loss of efficacy and clotting to life threatening complications such as bleeding as a result of overdosage.

St. John's Wort, the widely used herb to treat depression, by inducing CYP2C9, CYP1A2 and CYP3A4 isoforms affects the bioavailability of both R- and S-warfarin necessitating the adjustment of dose upward. The decrease in INR by as much as 50% can occur due to consumption of ginseng for two weeks with the INR normalizing after discontinuation of the herb. Decreases of INR have also been reported with the consumption of soy milk for four weeks. The Chinese herbs Dong quai, Quilinggao, Danshen and Go-qi-zi have been reported to increase INR. Some of the other examples of herbs or herb-based preparations that increase INR include chamomile tea and Royal Jelly [15]. Both the clinician and the laboratory should be alert to such herb-anticoagulant drug interactions in order to optimize therapy.

Newer laboratory tests to assess therapeutic effectiveness

I have already addressed the use of molecular assays to genotype patients to identify polymorphisms in the CYP2C19 allele to be able to tailor dosage of clopidogrel to a patient's genotype. I also mentioned the use of genotyping to identify polymorphisms in the CYP2C9 and VKORC1 genes in order to effectively optimize warfarin dosage. While these tests are still not in the realm of the routine coagulation laboratory they do allow clinicians to optimize doses of drugs such as clopidogrel and warfarin and avoid life threatening situations of either bleeding or thrombosis. Furthermore, molecular testing needs to be performed just once to obtain a patient's genotypic profile to guide all subsequent treatments.

Cartridge-based microbead agglutination technology using turbidimetric-based optical detection has been used to determine resistance to aspirin and platelet ADP $P2Y_{12}$ receptor inhibitors such as clopidogrel [16]. The automated system called Verify Now, designed for point-of-care testing, consists of an analyzer and disposable assay cartridges consisting of fibrinogen-coated beads, platelet activators and buffer. Separate cartridges with specific agonists are available to measure aspirin or platelet ADP $P2Y_{12}$ receptor inhibitor-drug resistance. As whole blood is added the platelet agglutination process results in an increase in light transmittance which is measured. Inhibition of platelet aggregation will result in decrease in light transmittance. Results are expressed either in "aspirin reaction units" (ARUs) for aspirin resistance or $P2Y_{12}$ reaction units (PRUs) for clopidogrel resistance. The assay, since it is based on the agglutination of fibrinogen-coated beads by activated platelets cannot be used for patients who may be taking GPIIb-IIIa receptor inhibitors. Aspirin inhibition can also be followed by measuring urinary 11-dehydro thromboxane B_2 levels.

The inhibition of platelet ADP $P2Y_{12}$ receptors by clopidogrel and other thienopyridine class of drugs can also be followed by flow cytometry measurement in whole blood of intracellular platelet vasodilator-

stimulated phosphoprotein (VASP) phosphorylation [17]. The rationale for this testing lies in the fact that the phosphorylation of VASP which is an intraplatelet actin regulator protein is dependent on the level of activation of the platelet ADP $P2Y_{12}$ receptor which is inhibited by thienopyridine class of drugs.

The insensitivity of activated partial thromboplastin time (APTT) to monitor heparin therapy has led to the increasing use of anti-factor Xa chromogenic assay to more accurately assess heparin levels. APTT is also inadequate to monitor direct thrombin inhibitors such as hirudin and has given way to the Ecarin clotting time which I discussed earlier. The many variables that affect the INR estimated by measurement of prothrombin time (PT), would hopefully be of historical interest if newer orally administered anticoagulants replace warfarin.

Conclusions

As new anticoagulant drugs are introduced we are learning that one dose doesn't fit all. Therapy has to be individualized based on a patient's genotype. Lifestyle such as diet, medications and herb-based supplements can interfere with enzyme isoforms involved in the metabolism of anticoagulant drugs and both the clinician and the laboratory should be alert to such interferences. As new assays are introduced the laboratory has the challenge of validating such assays and delineating its performance characteristics including its limitations. The scope of coagulation practice is expanding with the progresses made in the discovery and use of newer anticoagulants.

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