PHARMACOGENETICS OF THE DRUGS THAT ACT ON HEMOSTASIS

Hemostasis is a dynamic process that maintains the liquid state of blood, restores damage to blood vessels and limits blood loss, and at the same time prevents occlusion of blood vessels (thrombosis), insufficient blood supply to organs. Drugs affecting hemostasis can be divided into two groups:

Drugs for thrombosis treatment	Drugs for bleeding treatment
Anticoagulants	(Pro)coagulants
Antiaggregants	(Pro)aggregants
Fibrinolytics	Inhibitors of fibrinolysis

Coumarin derivatives play an important role among anticoagulants (also known as indirect/oral anticoagulants). The clinical use of the coumarin anticoagulants began with the discovery of an anticoagulant substance formed in spoiled sweet clover silage, which caused hemorrhagic disease in cattle. At the behest of local farmers, a chemist at the University of Wisconsin identified the toxic agent as bishydroxycoumarin. Dicumarol, a synthesized derivative, and its congeners, most notably warfarin (Wisconsin Alumni Research Foundation, with "-arin" from coumarin added), were initially used as rodenticides. In the 1950s, warfarin was introduced as an antithrombotic agent in humans.

Coumarin anticoagulants block the γ-carboxylation of several glutamate residues in prothrombin and factors VII, IX, and X as well as the endogenous anticoagulant proteins C and S. The blockade results in incomplete coagulation factor molecules that are biologically inactive. The protein carboxylation reaction is coupled to the oxidation of vitamin K. The vitamin must then be reduced to reactivate it. Warfarin prevents reductive metabolism of the inactive vitamin K epoxide back to its active hydroquinone form. Mutational change of the gene for the responsible enzyme, vitamin K epoxide reductase (VKORC1), can give rise to genetic resistance to warfarin in humans and rodents.

The resulting inhibition of coagulation is dependent on their degradation half-lives in the circulation. These half-lives are 6, 24, 40, and 60 hours for factors VII, IX, X, and II, respectively. Importantly, protein C has a short half-life similar to factor VIIa. Thus the immediate effect of warfarin is to deplete the procoagulant factor VII and anticoagulant protein C, which can paradoxically create a transient hypercoagulable state due to residual activity of the longer half-life procoagulants in the face of protein C depletion. For this reason in patients with active hypercoagulable states, such as acute DVT or PE, UFH or LMW heparin is always used to achieve immediate anticoagulation until adequate

warfarin-induced depletion of the procoagulant clotting factors is achieved. The duration of this overlapping therapy is generally 5–7 days. Cutaneous necrosis with reduced activity of protein C sometimes occurs during the first weeks of therapy in patients who have inherited deficiency of protein C. In such patients, the drug should be started without a loading dose of warfarin, even if the patient is administered heparin.

Warfarin is metabolized by CYP2C9, which is polymorphic and for which about 50 alleles are known (www.cypalleles.ki.se/cyp2c9.htm). At the same time, most of the variability is associated with two CYP2C9∗2 and ∗3 alleles. CYP2C9∗2 and *3 alleles are accompanied by amino acid substitutions, which slow down the metabolism of warfarin by 30-40% and 80-90%, respectively. Both alleles are more common in Europeans than in Africans or Asians (7–13% vs < 5%, correspondently).

Vitamin K epoxide reductase complex subunit 1 (VKORC1), encoded by the *VKORC1* gene, is the target of anticoagulant warfarin and a key enzyme in the vitamin K recycling process. Activated vitamin K is an essential cofactor for activation of blood clotting factors II, VII, IX, and X, as well as endogenous anticoagulant proteins C and S. Rare genetic variants in the coding region of VKORC1 may lead to bleeding disorders, eg, multiple coagulation factor deficiency type 2A, or warfarin resistance. A polymorphism common across all major ethnicities results in reduced expression of VKORC1 in the liver. The most important consequences of the VKORC1 polymorphism are increased sensitivity to warfarin. The VKORC1-1639G>A polymorphism occurs most frequently in Asian populations (~90%) and least often in Africans (~10%), which explains, in part, the difference in dosing requirements among major ethnic groups.

Treatment with warfarin should be initiated with standard doses of 5–10 mg. The initial adjustment of the prothrombin time takes about 1 week, which usually results in a maintenance dosage of 5–7 mg/d. The prothrombin time (PT) should be increased to a level representing a reduction of prothrombin activity to 25% of normal and maintained there for long-term therapy. When the activity is less than 20%, the warfarin dosage should be reduced or omitted until the activity rises above 20%. Inherited polymorphisms in 2CYP2C9 and VKORC1 have significant effects on warfarin dosing; however, algorithms incorporating genomic information to predict initial warfarin dosing were no better than standard clinical algorithms in two of three large randomized trials examining this issue.

When using coumarin derivatives (warfarin, acenocoumarol, phenprocoumon), it is recommended to regularly check the international normalized ratio (INR) in order to prevent the development of side effects of coumarins - bleeding. The recommended INR for prophylaxis and treatment of thrombotic disease is 2–3. Dosing of coumarin derivatives can be based on genetic testing of patients at the start of treatment.

In the table 1 one can see an example warfarin doses adjustment according CYP2C9 and VKORC1 genotype. An isolated polymorphism of the CYP2C9 and VKORC1 genes can explain the variability of the warfarin dose in 18 and 37% of abnormal cases, respectively, and the simultaneous determination of the polymorphism of both genes allows to explain up to 50% of the cases of warfarin dose changes. The presence of variant alleles of the CYP2C9 gene reduces the recommended dose of the drug by 3–10 times (from 5–7 to 0.5–2 mg/kg); the presence of mutant alleles of the VKORC1 gene reduces the dose by 2-6 times.

Table 1. DOSE ADJUSTMENT OF WARFARIN (mg/kg)

Genotype *VKORC1*	***CYP2C9* genotype**					
	***1/*1**	***1/*2**	***1/*3**	***2/*2**	***2/*3**	***3/*3**
GG	**5-7**	**5-7**	**3-4**	**3-4**	**3-4**	**0,5-2**
GA	**5-7**	**3-4**	**3-4**	**3-4**	**0,5-2**	**0,5-2**
AA	**3-4**	**3-4**	**0,5-2**	**0,5-2**	**0,5-2**	**0,5-2**

Changes in the concentration of coumarin derivatives may be associated with drug interaction and the patient's condition. The interaction may refer to pharmacokinetic and pharmacodynamic interactions. Pharmacokinetic mechanisms usually involve induction/inhibition of CYP2C9 and decreased plasma protein binding. Pharmacodynamic mechanisms include synergism (disturbance of hemostasis, reduced synthesis of blood coagulation factors, for example in liver disease), competitive antagonism (vitamin K), disruption of the vitamin K cycle (congenital resistance to oral indirect anticoagulants).

The most dangerous is the interaction with warfarin, which increases the anticoagulant effect and bleeding, for example, pharmacokinetic interaction with pyrozolone derivatives (phenylbutazone or butadione), salicylic acid (aspirin). The latter reduce platelet aggregation, suppress warfarin metabolism in the liver, and displace warfarin from binding to plasma proteins. Antimicrobial agents (metronidazole, fluconazole and co-trimoxazole), amiodarone, disulfiram and cimetidine inhibit the metabolism of warfarin in the liver. Third-generation cephalosporins eliminate bacteria from the gastrointestinal tract that produce vitamin K, and inhibit the cycle of vitamin K in the liver.

Barbiturates and rifampicin can reduce the anticoagulant effect of warfarin by inducing liver enzymes; cholestyramine - by reducing absorption in the gastrointestinal tract. Some diuretics (spironolactone, chlorthalidone) increase the content of blood clotting factors and thus weaken the anticoagulant effect.

Among the unwanted effects, warfarin easily passes through the BBB and can cause bleeding. Moreover, warfarin suppresses the synthesis of proteins in the bones of the fetus, so it can disrupt the formation of bones in the fetus. Tissue necrosis and peripheral thrombosis may also occur.

ANTIAGGREGANTS (ANTIPLATELETS)

Platelet function is regulated by three categories of substances. The first group consists of agents generated outside the platelet that interact with platelet membrane receptors, eg, catecholamines, collagen, thrombin, and prostacyclin. The second category contains agents generated within the platelet that interact with membrane receptors, eg, ADP, prostaglandin D2, prostaglandin E2, and serotonin. A third group comprises agents generated within the platelet that act within the platelet, eg, prostaglandin endoperoxides and thromboxane A2, the cyclic nucleotides cAMP and cGMP, and calcium ion. From this list of agents, several targets for platelet inhibitory drugs have been identified:

- inhibition of prostaglandin synthesis (aspirin);
- inhibition of ADPinduced platelet aggregation (clopidogrel, prasugrel, ticlopidine);
- blockade of glycoprotein IIb/IIIa (GP IIb/IIIa) receptors on platelets (abciximab, tirofiban, and eptifibatide);
- others - dipyridamole and cilostazol.

THIENOPYRIDINES: Ticlopidine, clopidogrel, and prasugrel reduce platelet aggregation by inhibiting the ADP pathway of platelets. These drugs irreversibly block the ADP P2Y12 receptor on platelets. Unlike aspirin, these drugs have no effect on prostaglandin metabolism. Use of ticlopidine, clopidogrel, or prasugrel to prevent thrombosis is now considered standard practice in patients undergoing placement of a coronary stent.

Ticlopidine is approved for prevention of stroke in patients with a history of a transient ischemic attack (TIA) or thrombotic stroke, and in combination with aspirin for prevention of coronary stent thrombosis. Adverse effects of ticlopidine include nausea, dyspepsia, and diarrhea in up to 20% of patients, hemorrhage in 5%, and, most seriously, leukopenia in 1%. Development of thrombotic thrombocytopenic purpura has also been associated with the ingestion of ticlopidine. The dosage of ticlopidine is 250 mg twice daily orally. Because of the significant side effect profile, the use of ticlopidine for stroke prevention should be restricted to those who are intolerant of or have failed aspirin therapy. Dosages of ticlopidine less than500 mg/d may be efficacious with fewer adverse effects.

Clopidogrel is approved for patients with unstable angina or non-ST-elevation/ ST-elevation acute myocardial infarction (NSTEMI/STEMI) in combination with aspirin; for patients with myocardial infarction; or stroke, or established peripheral arterial disease. For NSTEMI, the dosage is a 300-mg loading dose orally followed by 75 mg daily of

clopidogrel, with a daily aspirin dosage of 75–325 mg. For patients with STEMI, the dosage is 75 mg daily of clopidogrel orally, in association with aspirin as above; and for recent myocardial infarction, stroke, or peripheral vascular disease, the dosage is 75 mg/d.

Clopidogrel has fewer adverse effects than ticlopidine and is rarely associated with neutropenia. Thrombotic thrombocytopenic purpura has been reported. Because of its superior adverse effect profile and dosing requirements, clopidogrel is frequently preferred over ticlopidine. The antithrombotic effects of clopidogrel are dosedependent; within 5 hours after an oral loading dose of 300 mg, 80% of platelet activity will be inhibited. The maintenance dosage of clopidogrel is 75 mg/d, which achieves maximum platelet inhibition. The duration of the antiplatelet effect is 7–10 days. Clopidogrel is a prodrug that requires activation via the cytochrome P450 enzyme isoform CYP2C19. Depending on the single nucleotide polymorphism (SNP) inheritance pattern in CYP2C19, individuals may be poor metabolizers of clopidogrel, and these patients may be at increased risk of cardiovascular events due to inadequate drug effect. The FDA has recommended CYP2C19 genotyping to identify such patients and advises prescribers to consider alternative therapies in poor metabolizers. However, more recent studies have questioned the impact of CYP2C19 metabolizer status on outcomes. Drugs that impair CYP2C19 function, such as omeprazole, should be used with caution.

In EM and UM the dosage remains standard (*CYP2C19*1/*1*); in IM (*CYP2C19*1/*2; *1/*3*) and in SM (*CYP2C19*2/*2; *2/*3; *3/*3*) the effectiveness will be diminished.

Prasugrel, similar to clopidogrel, is approved for patients with acute coronary syndromes. The drug is given orally as a 60-mg loading dose and then 10 mg/d in combination with aspirin as outlined for clopidogrel. Prasugrel showed greater efficacy in reducing mortality during and after coronary angioplasty compared with clopidogrel, but showed a higher risk of bleeding. At the same time, CYP-450 polymorphism in patients does not affect the pharmacology of prasugrel.