Antithrombotic therapy and thrombolysis: the present and the future

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Abstract
This literature review discusses different aspects of thrombolytic and antithrombotic therapy. These aspects will be reviewed in a logical order, starting with the current guidelines that are used in the Netherlands for clinical treatment of several diseases related to thrombosis. A more in-depth report of the drugs used in these diseases is then presented. Next, pharmaceuticals in the current pipeline or in clinical trials are analyzed for potential use as a new treatment method. Lastly, the most recent research in the field of thrombolytic or antithrombotic therapy is discussed for their possibilities.

List of abbreviations
ACS = acute coronary syndrome, ADP = adenosine diphosphate, AMI = acute myocardial infarction, APT = 4-amino-2-pyrimidinethiol, cAMP = cyclic 3',5'-adenosine monophosphate, cGMP = cyclic 3',5'-guanosine monophosphate, CRUSADE = Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines, CT = computed tomography, DAPT = dual antiplatelet therapy, DAPTh = 4,6-diamino-2-pyrimidinethiol, DHTP = 4,6-dihydroxyl-2-pyrimidinethiol, DVT = deep venous thrombosis, ECG = electrocardiogram, GRACE = Global Registry of Acute Coronary Events, INR = International Normalized Ratio, LMWH = low molecular weight heparin, MDCT = multi detector computed tomography, MRI = magnetic resonance imaging, MRP-14 = myeloid-related protein 14, NSTE-ACS = non-ST-elevation acute coronary syndrome, PAI = plasminogen activator inhibitor, PAR-1 = protease-activated receptor 1, PCI = percutaneous coronary intervention, PTS = post-thrombotic syndrome, rt-PA = recombinant tissue plasminogen activator, scFV-scuPA = antibody-bound single chain urokinase plasminogen activator, scuPA = single chain urokinase plasminogen activator, STEMI = ST-elevation myocardial infarction, VKA = vitamin K antagonist, VTE = venous thromboembolism

Introduction

The focus of this literature review is thrombolytic therapy. Thrombolytic therapy is used to remove obstructive thrombi that occlude the vasculature. This is accomplished through the breakdown or removal of the blood clot. Several topics of thrombosis and thrombolysis will be discussed in this review. First, the main cardiovascular diseases related to thrombosis will be reported, according to their current guidelines. Next, the drugs used for the treatment of these conditions will be described. Alternative (surgical) treatment is also reviewed. After all the current treatment options are reported, novel drugs on the market and drugs in development are examined. Finally, current research that could lead to new thrombolytic therapy is discussed.

Conditions that are eligible for thrombolytic and/or antithrombotic therapy

To identify the thrombolytics and antithrombotics that are the most used, the most common diseases related to thrombosis will be described, which are deep venous thrombosis (DVT), pulmonary embolism, stroke, acute myocardial infarction (AMI) and acute coronary syndrome (ACS). A description of these diseases will be given, together with the diagnosis and treatment of the illness. The diagnosis and treatment plans presented are taken from the guidelines used in the Netherlands.

Deep venous thrombosis (DVT)

DVT is the forming of a thrombus in a vein deep inside the body (veins next to an artery). The majority of these type of thrombi occur in the leg[1]. These blood clots can break off and move through the body. The embolism can lead to pulmonary embolism[2], which will be covered later in this review. In a paper by Cushman et al., the mortality rate after diagnosis of DVT was 9%[3]. DVT is pre-diagnosed through a combination of a clinical model developed by Wells et al.[4] and a D-dimer assay. The Wells rules for DVT and pulmonary embolism are shown in table 1. Depending on the clinical model, the sensitivity of D-dimer assay should be determined. Note that this combination is mainly used to exclude DVT diagnosis. The negative diagnostic value of normal D-dimer levels is too low to exclude DVT by itself, but combined with medium or low risk after assessment of the clinical model DVT can be excluded safely. The actual diagnosis of DVT is then determined using compression ultrasound, but venography and plethysmography are other good options[5]. Therapy of DVT consists of vitamin K antagonists (VKA) in combination with low molecular weight heparin (LMWH) or unfractionated heparin. LMWH is preferred over unfractionated heparin and is administered subcutaneously. Heparin needs to be administered at least for 5 days and can be stopped when the International Normalized Ratio (INR) is stabile and above 2.0 during two days[6]. Only in severe venous thrombosis with direct danger for the limb fibrinolytics (streptokinase, urokinase or tissue plasminogen activator, rt-PA) should be used, due to the increased incidence of bleeding complications[5]. To prevent expansion or recurrence of venous thromboembolism (VTE), prophylactic treatment with VKA for 3 to 6 months is required[7]. In the case of recurring VTE, treatment with VKA can be prolonged even up to lifelong treatment, depending on the severity of recurrence[5]. During long term treatment, the INR has to be between 2.0 and 3.0. In patients with VTE and cancer, prophylaxis with LMWH for 3 to 6 months is the standard[8].

Pulmonary embolism

Pulmonary embolism (PE) is defined as the blockage of the main artery of the lungs or one of the branches of this artery by an embolism (a blood clot which broke off somewhere else in the body). This is usually caused by DVT, but is not necessarily the case. Pulmonary embolism is much more fatal than DVT, as shown in a trial by Barritt and Jordan in 1960, the untreated cohort had a 26% mortality rate[9]. In pulmonary embolism, exclusion diagnostics for PE are performed with Wells rules. However, the clinical model of Wells in pulmonary embolism is different than the one used in DVT[10]. If the clinical model of Wells has a negative result, a D-dimer assay is performed to ensure that no pulmonary embolism is present. The Wells rules for DVT and PE are shown in table 1. If the Wells test is positive, a multi detector computed tomography (MDCT) is performed, because it is a non-invasive procedure. However,pulmonary angiography is still one of the best diagnostic tools and can be used

as an alternative. In an electrocardiogram (ECG) it can be shown that the pressure on the right ventricle is increased during a pulmonary embolism. However, not all the pulmonary embolism lead to increased pressure on the right ventricle and thus ECG cannot be used as a diagnostic tool[5]. The treatment for pulmonary embolism is the same as it is for DVT. The treatment is started with VKA and LMWH. The LMWH has to be administered for at least 5 days until the INR is stabile and above 2.0 during two days[5,6]. The only difference is that in patients with massive pulmonary embolism and hemodynamic instability (systolic blood pressure <90 mm Hg) 100 mg of alteplase should be administered intravenously during 2 hours[6,11].Long term treatment is the same as in DVT as well, prophylactic treatment with VKA for 3 to 6 months[6,7]. In the case of recurring VTE events, up to lifelong treatment with VKA can be prescribed[5].

Table 1: Wells rules for DVT and PE[5].

Wells rules for DVT			
Risk factor			*Score*
Active cancer (treatment ongoing or within previous 6 months or palliative)			1.0
Paralysis, paresis, or recent plaster immobilization of the lower extremities			1.0
Recent bedridden > 3 days and/or major surgery in the past 4 weeks			1.0
Thigh and calf swollen			1.0
Calf swelling > 3 cm on the symptomless side			1.0
Pitting oedema in the symptomatic leg			1.0
Dilated superficial veins in symptomatic leg only			1.0
Pain in course of deep veins			1.0
Alternative diagnosis			-2.0
Wells rules for PE			
Clinical signs of DVT			3.0
Probability of PE higher than alternative diagnosis			3.0
Heart frequency higher than 100 beats per minute			1.5
Paralysis, paresis, recent plaster immobilization or operation in the past 4 weeks			1.5
History of DVT or PE			1.5
Hemoptysis			1.0
Active cancer (treatment ongoing or within previous 6 months or palliative)			1.0
Risk assessment DVT		**Risk assessment PE**	
High risk	≥ 3 points	High risk	≥ 6 points
Moderate risk	1-2 points	Moderate risk	3-5 points
Low risk	≤ 0 points	Low risk	≤ 2 points
Probable	≥ 2 points	Probable	> 4 points
Not probable	< 2 points	Not probable	≤ 4 points

Stroke

There are two subtypes of stroke: ischemic stroke and hemorrhagic stroke. Ischemic strokes account for approximately 87% of the stroke cases[12] and happens when a blood vessel is vasoconstricted or a blood clot (thrombotic or embolic) is obstructing the artery. Hemorrhagic stroke occurs when a blood vessel in the brain ruptures[13]. Diagnosis of ischemic or hemorrhagic stroke can be accoplished with computed tomography (CT) or magnetic resonance imaging (MRI). In both types of stroke the preferred tool is CT, since it is faster and more readily available in most hospitals[14]. Since the two different types of stroke have different causes, the treatment of the two types also deviates. In acute ischemic stroke, if the patient can be treated within 3 hours of the onset of the symptoms, alteplase (tissue plasminogen activator, rt-PA) should be administered intravenously. Symptoms of stroke are numbness or weakness in the face, arm or leg on one side, confusion/trouble understanding people,

difficulty speaking, struggling while walking or staying balanced, dizziness, severe headache with sudden onset and trouble seeing[15]. Intravenous administration of alteplase within 3-4.5 hours after the start of the symptoms could be beneficial and should be considered by the doctor. After 4.5 hours no fibrinolytic agents should be given to the patient, because it is associated with higher mortality[6,14]. The dose of alteplase should be 0.9 mg/kg administered over one hour, with 10% of the total dose as a bolus in the first minute of administration[11]. In severe cases of ischemic stroke, intra-arterial treatment with rt-PA in between 3-6 hours after the onset of the symptoms is performed[6,14]. In all cases of ischemic stroke, chronic treatment with aspirin should be started as soon as possible, sometimes in combination with dipyridamole[14]. In the case of acute primary hemorrhagic stroke, no fibrinolytic drugs are administered, because hemorrhagic stroke itself is a bleeding of the brain and fibrinolytic drugs would only increase the bleeding time. However, after a few days LWMH should be administered to prevent VTE attacks[6].

Acute myocardial infarction (AMI)

Acute myocardial infarction (AMI) is the death of heart muscle due to lack of oxygen caused by an ischemic event in one of the coronary arteries. AMI could cause cardiac arrest and is therefore life threatening. Diagnosis of AMI is performed with a combination of patient symptoms (chest pain lasting for 20 minutes or more, not responding to nitroglycerin), ECG and angiography[16]. AMI is also known as ST-elevation myocardial infarction (STEMI) and always needs to be treated immediately. The treatment consists of primary percutaneous coronary intervention (PCI) with a periprocedural treatment of aspirin, adenosine diphosphate (ADP) receptor blocker and a parenteral anticoagulant (preferably unfractionated heparin). The preferred ADP-receptor blockers are prasugrel or ticagrelor[16], but if those are not available for the patient, clopidogrel is used. In cases where primary PCI cannot be offered within the time limits to STEMI patients, fibrinolytic therapy with alteplase (rt-PA) is recommended. The recommended dose is 100 mg alteplase over 3 hours, of which 60 mg should be administered in the first hour and 40 mg over the next two hours[11]. Next to that, administration of aspirin and clopidogrel should be started[16]. After STEMI, aspirin should be taken indefinitely as a long term treatment.

Acute coronary syndrome (ACS)

Acute coronary syndrome (ACS) is a collection of diseases that are related to the blockage of coronary arteries. ACS can lead to stable or unstable angina pectoris (unstable and improper contraction of the heart muscle), myocardial infarction or heart failure[17]. Although ACS can lead to STEMI, treatment of STEMI is described in different guidelines and is therefore reviewed in the AMI section. Furthermore, ACS causes non-ST-elevation myocardial infarction (NSTEMI) or unstable angina as well. Both fall under the definition of non-ST-elevation ACS (NSTE-ACS). Diagnosis of NSTE-ACS consists of physical examination, ECG and measurement of biomarks (cardiac troponin levels). If additional diagnostic tools are necessary, echocardiography or coronary angiography are performed[17]. Treatment of NSTE-ACS should start with dual anti platelet therapy (DAPT) as soon as possible, consisting of aspirin and a $P2Y_{12}$ receptor inhibitor (clopidogrel, prasugrel or ticagrelor). The DAPT should lasts for at least 12 months. In high risk patients undergoing invasive procedures (such as PCI), GP IIb/IIIa receptor inhibitors should be given next to DAPT if the risk of bleeding is low[17]. Anticoagulation treatment is recommended next to the antiplatelet therapy. Fondaparinux (factor Xa inhibitor) is highly recommended, but LMWH is used in cases where fondaparinux is not available. After an invasive procedure, the anticoagulation therapy should be discontinued. The duration of the anticoagulant treatment is until the patient is discharged from the hospital[17].

Current standard thrombolytic and antithrombotic agents

The thrombolytics currently in use, as described in the guidelines, will be discussed in detail here. There are three classes of thrombolytics or antithrombotics: antiplatelet agents, anticoagulants and fibrinolytics. Based on the disease section, the treatment cases will be summarized for each drug, as well as the mechanism of action, the pharmacokinetics and side effects.

Antiplatelet agents

Platelet aggregation is one of the most important aspects in the formation of a thrombus. Therefore, antiplatelet drugs are crucial in secondary stroke prevention[18]. Aspirin, dipyridamole (combined with aspirin), $P2Y_{12}$ receptor inhibitors and glycoprotein IIb/IIIa receptor inhibitors fall in this category.

Aspirin. Aspirin is commonly used in ischemic stroke, sometimes in combination with dipyridamole. Next to that, the drug is also used in STEMI and NSTE-ACS. It's widely available, low cost and is proven to be beneficial in the prevention of secondary ischemic events[19]. Production of thromboxane A2 is inhibited through the irreversible inhibition of cyclo-oxygenase I. The decrease in thromboxane A2 leads to reduced ability for platelet aggregation[20]. Due to the irreversible binding of aspirin, the effect lasts as long as the life span of the platelet, which is 7 to 10 days[21]. However, some patients can develop aspirin resistance due to non compliance with the treatment, incorrect dosing (too low), other drugs interacting with the mechanism of aspirin and genetic polymorphisms[22]. Aspirin is administered orally and rapidly absorbed in the stomach and the upper intestine. 30 to 40 minutes after the ingestion of aspirin is when peak plasma levels occur. Peak plasma levels can take up to 3 to 4 hours after the ingestion of enteric-coated aspirin. The bioavailability of aspirin is 40 to 50%[23]. The side-effects of treatment with aspirin include ulcers in the upper gastrointestinal tract, thrombocytopenia and pancytopenia[18].

Dipyridamole. Dipyradimole is only given to patients in combination with aspirin. It is used in patients with ischemic stroke. The mechanism of action of this drug consists of two parts. First, the cyclic 3',5'-adenosine monophosphate (cAMP) concentrations is increased by inhibition of adenosine uptake by the platelet. This results in inhibited platelet aggregation by collagen, platelet activating factor and ADP. Second, dipyridamole inhibits cyclic 3',5'-guanosine monophosphate (cGMP) as well, which leads to decreased platelet aggregation[20]. Dipyridamole also has a vasodilation effect through inhibiting the nucleoside transport, resulting in increased levels of endogenous adenosine[24]. Adenosine then induces vasodilation through interaction with the A_2 subtype adenosine receptor and a intracellular site[25]. Dipyridamole is administered in an extended release formulation with aspirin, due to the short half-life of dipyridamole[18]. The aspirin and dipyridamole combined treatment has several side-effects: headaches, gastro-intestinal disturbances (such as diarrhea) and increased bleeding risk[26].

$P2Y_{12}$ receptor inhibitors (clopidogrel, prasugrel, ticagrelor). $P2Y_{12}$ receptor inhibitors are used in the treatment of STEMI in combination with aspirin, whether percutaneous catheter intervention (PCI) is performed or not. Treatment of NTSE-ACS also consists of dual anti platelet therapy with aspirin and $P2Y_{12}$ receptor inhibitors. Clopidogrel and prasugrel have a slightly different mechanism of action than ticagrelor. Clopidogrel and prasugrel irreversibly bind to the $P2Y_{12}$ receptor, inhibiting ADP signaling of platelet aggregation[18]. On the contrary, ticagrelor binds reversibly to the $P2Y_{12}$ receptor. The reversible binding of ticagrelor is also noncompetitive with ADP, since increasing concentrations of ADP do not stop the inhibition of the receptor[27]. Clopidogrel requires a loading dose of at least 300 mg and several daily doses of 75 mg to reach a steady state which leads to drug activity[28]. The main difference between clopidogrel and prasugrel is that prasugrel has increased potency and a quicker peak effectiveness[29]. Ticagrelor peak plasma levels occur 1.3 to 2 hours after oral intake. Steady state is accomplished by doses of 100 mg twice daily[27]. The safety profiles of clopidogrel and prasugrel are only slightly different. Both drugs can induce bleeding, but bleeding events induced by prasugrel are usually more severe and more frequent[30]. Side effects of ticagrelor include bleeding, dyspnea and ventricular pauses. The incidence of bleeding is the same as in clopidogrel[27].

Glycoprotein IIb/IIIa receptor inhibitors. These drugs are given in high risk NSTE-ACS patients who undergo PCI (while having a low bleeding risk), next to DAPT. The risk assessment for patients is performed using the Global Registry of Acute Coronary Events (GRACE) risk score[31]. The glycoprotein

IIb/IIIa receptor is blocked by binding of the inhibitors. This leads to decreased binding of platelet aggregation agonists collagen, thrombin and thromboxane A_2[18]. The major side effects are bleeding and thrombocytopenia. The frequency of severe bleedings reported in patients treated with glycoprotein IIb/IIIa receptor inhibitors was high, which is why this treatment is available for patients with lower risk of bleeding[18]. The bleeding risk can be determined through the use of the bleeding risk score assessment developed in the Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA guidelines (CRUSADE)[32].

Anticoagulants

The formation of blood clots is highly dependent on the coagulation factors. Anticoagulants are therefore used for the treatment of different thrombosis related diseases. The different anticoagulants are vitamin K antagonists (VKA), low molecular weight heparin (LMWH), unfractionated heparin and fondaparinux (a factor Xa inhibitor).

Vitamin K antagonist (VKA, warfarin). Vitamin K antagonists are used as the first in line treatment of deep venous thrombosis and pulmonary embolism, combined with LMWH or unfractionated heparin. The mechanism of action of VKA is the inhibition of vitamin K epoxide reductase, which normally reduces vitamin K epoxide to vitamin K. Vitamin K is necessary for the γ-carboxylation of factor II, VII, IX and X. This step is necessary for the binding of the GLA domains of these coagulation factors to membranes, and thus indirectly for the procoagulant activity. Due to the VKA, depletion of activated factor II, VII, IX and X occurs[33,34]. Warfarin is the most commonly used VKA and is rapidly absorbed from the gastrointestinal tract and reaches peak plasma concentrations 90 minutes after oral administration[35]. A reduction in the amount of prothrombin is the antithrombotic effect of warfarin. The half-life of prothrombin is 60 to 72 hours, which means that the anticoagulation effects induced by warfarin are at their peak after 4 days[33]. The side effects of warfarin are bleeding, skin necrosis and limb gangrene. Protein C and factor VII are inhibited stronger by warfarin due to shorter half-lives than the other vitamin K-dependant coagulation factors, causing a hypercoagulable state in the patients. This results in thrombosis of the venules and capillaries in the subcutaneous fat, causing skin necrosis in warfarin treatment[36]. Limb gangrene in warfarin treatment is caused by the blockage of outflow of the venous system of the limb[33].

Unfractionated heparin. Unfractionated heparin is used in DVT, pulmonary embolism and STEMI. In DVT and pulmonary embolism unfractionated heparin is a combined treatment with VKA. Heparin binds to antithrombin III, which undergoes a conformational change to inhibit factor Xa[37]. Thrombin is inhibited through an interaction with heparin, resulting in the bridged inhibition of thrombin by antithrombin III[38]. However, heparin has a short-lived effect, because only free thrombin is inhibited by the complex[18]. Next to that, heparin is not taken up when administered orally. The preferred route of administration is thus continuous intravenous infusion or subcutaneous injection[39]. Unfractionated heparin has several adverse effects. The main adverse event is bleeding, but some patients develop heparin-induced thrombocytopenia and osteoporosis[39]. Reversal of the anticoagulation effect of unfractionated heparin can be achieved by intravenous administration of protamine sulfate[39].

Low molecular weight heparin (LMWH). Low molecular weight heparins are used in similar cases as unfractionated heparin in DVT and pulmonary embolism, but are preferred over unfractionated heparin due to having a better benefit-to-risk ratio[40]. LMWHs are derived from unfractionated heparin and thus have a similar mechanism of action. However, LMWHs cannot bind to thrombin and antithrombin III at the same time, which means that LMWHs only inhibit factor Xa[41]. LMWHs have a higher bioavailability than unfractionated heparin after subcutaneous injection. The inhibition of factor Xa peaks after 3 to 5 hours after dosing of LMWH[39]. The side effects of LMWHs are the same as described in unfractionated heparin, but the heparin-induced thrombocytopenia and osteoporosis events are less frequent[39]. Contrary to unfractionated heparin, reversal of anticoagulant effect of

LMWHs with intravenous administration of protamine sulfate is not complete and variable in patients[39].

Fondaparinux (factor Xa inhibitor). Fondaparinux is used next to antiplatelet therapy in the case of NSTE-ACS. Fondaparinux is a synthetic, heparin-derived pentasaccharide with high affinity for antithrombin. Again, the mechanism of fondaparinux is the same as unfractionated heparin (inhibiting factor Xa through binding to antithrombin), but fondaparinux does not bind thrombin[42]. Administered through a once-daily subcutaneous injection of 2.5 mg, the steady state is reached after the third or fourth injection. No laboratory monitoring is needed for fondaparinux treatment, because there is no variability in the anticoagulant response of fonaparinux. In cases where laboratory monitoring is necessary due to side effects, fondaparinux activity should be measured using an anti-Xa assay[39]. The only side effect of fondaparinux is bleeding, no heparin-induced thrombocytopenia and osteoporosis events have been reported[39].

Fibrinolytics

Fibrin is one of the most important components of a blood clot. The polymer is necessary for joining platelets together to form the thrombus. The most commonly used fibrinolytic is alteplase, but to be complete, urokinase and streptokinase will be discussed as well. In order to understand the position these drugs have in the fibrinolytic system, this system will first be reviewed. The breakdown of fibrin polymers happens through cleavage by plasmin. Plasmin itself is a product of the activation of plasminogen. Plasminogen can be activated by several enzymes: rt-PA, urokinase or streptokinase. Streptokinase can activate plasminogen directly, but urokinase and rt-PA activate plasminogen through binding to other proteins. Urokinase binds to the urokinase receptor (uPAR, expressed on monocytes, macrophages, fibroblasts and endothelial cells) together with plasminogen to form a complex that releases plasmin. rt-PA associates to fibrin to activate plasminogen to plasmin. The activation of plasminogen to form plasmin results in fibrinolysis[43]. Plasminogen activator inhibitor (PAI) inhibits the activation of plasminogen by urokinase or rt-PA. Plasmin itself is inhibited by α2-plasmin inhibitor. The net results of these interactions is the inhibition of fibrinolysis[43].

Alteplase (recombinant tissue plasminogen activator, rt-PA). Severe cases of DVT and pulmonary embolism warrant the use of alteplase. However, alteplase treatment is mainly used in ischemic stroke. Alteplase is a tissue plasminogen activator, a serine protease which catalyzes plasminogen to plasmin. Plasmin can then cleave the fibrin polymers in a blood clot[18]. Alteplase is administered intravenously, but in the case of acute ischemic stroke, intra-arterial administration adjacent or into the thrombus is warranted[44]. Dosage of alteplase has been discussed in the disease section, but is repeated here. In PE, 100 mg of alteplase should be administered intravenously during 2 hours[6,11]. Stroke patients should receive a dose of 0.9 mg/kg alteplase administered over one hour, with 10% of the total dose as a bolus in the first minute of administration[11]. Ischemic stroke patients should not receive fibrinolytics after 4.5 hours, since it is associated with higher mortality[6,14]. The recommended dose of alteplase for AMI patients is 100 mg alteplase over 3 hours, of which 60 mg should be administered in the first hour and 40 mg over the next two hours[11]. Bleeding is the main side effect of rt-PAs, but in some cases immune hypersensitivity or tongue angioedema can develop[18,44]. It has also been shown that rt-Pas can cause neurotoxicity[45].

Prourokinase, urokinase and streptokinase. Prourokinase is the proenzyme of urokinase. After conversion, it can activate plasminogen into plasmin. Plasmin will then degrade fibrin in the thrombus. However, prourokinase is no longer available. Even though fibrin-specific agents (rt-PA) are recommended over urokinase in AMI[16], urokinase is still used in the treatment of PE[14]. In 1988, a German study showed that urokinase and rt-PA treatment in AMI were non-inferior to one another[46], but three years later a Japanese study showed superiority of rt-PA in their randomized trial with Japanese patients[47]. The administration of urokinase in PE consists of a loading dose of 4,400 IU/kg intravenous injection over 10 minutes. Then a continuous infusion of 4,400 IU/kg per hour for 12

hours should be applied[48]. Three clinical trials on streptokinase treatment in ischemic stroke were started, but ended prematurely due to safety precautions (a significantly higher amount of patients developed intracranial bleedings)[14]. Streptokinase can be used in AMI and PE, but rt-PA is preferred over streptokinase. If streptokinase is used, a dose of 1.5 million IU over 30 to 60 minutes intravenously is used in AMI. In PE, a loading dose of 250,000 IU followed by 100,000 IU per hour for 24 hours is used.

Alternative treatment methods

Although medical treatment is often the preferred option for thrombotic diseases, alternative methods are available. The current guidelines used in the Netherlands for DVT, pulmonary embolism, stroke, acute myocardial infarction and ACS also recommend other (surgical) treatments options for these diseases. This section discusses the alternatives to medical treatment, or sometimes in combination with medical treatment.

Thrombectomy and embolectomy

Thrombectomy is the removal of the thrombus that is blocking the vein through a surgical procedure. The difference with embolectomy is that in embolectomy an embolus is removed. An embolus is a thrombus that has been detached from its place of origin and then becomes stuck somewhere else. This procedure is usually not performed in DVT or pulmonary embolism, unless the patient cannot receive thrombolytic therapy or the patient is at immediate risk of losing an organ, limb or his/her life. However, these procedures have a higher mortality rate than medical intervention and therefore thrombolytics are preferred as the first treatment in DVT and pulmonary embolism[5].

Percutaneous coronary intervention (PCI) and catheter extraction

In acute myocardial infarction, percutaneous coronary intervention is the golden standard of treatment, as opposed to medical treatment[16]. PCI is a non-surgical procedure that leads a catheter to the site of blockage and then inflates a balloon to widen the vein. At that position in the vein a so-called 'stent' is placed (a spring) to keep the vein widened. Due to the severity and mortality rate of AMI, PCI is preferred over medical intervention. PCI is also indicated for high-risk NSTE-ACS patients. Another non-surgical procedure is catheter extraction, in which a catheter is moved towards the blood clot. The blockage is then fragmented by physiological salt solution under high speed flow[5]. Catheter extraction can be performed in patients with pulmonary embolism who either cannot undergo thrombolytic therapy or who are in a critical condition[5].

Coronary artery bypass graft

Coronary artery bypass graft is a surgical procedure in which a vein from another part of the body is grafted to the coronary arteries to bypass the blockage in the artery. This procedure is performed in patients with AMI who cannot undergo PCI[16]. Furthermore, it is advised in patients who suffer from NSTE-ACS who have had to be stabilized medically. This can happen when initial PCI has not delivered the necessary effect and additional coronary artery disease (CAD) is diagnosed[17].

Vena cava filter

The vena cava filter is a filter that is placed into the vena cava inferior, which happens percutaneously through the vena femoralis or vena jugularis. This filter lower the mortality of pulmonary embolism by catching emboli caused by DVT. The vena cava filter is applied during the treatment for DVT. The protection this serves is only needed temporarily and therefore retrievable vena cava filters are usually used. This is an alternative treatment method specific for pulmonary embolism and is used in patients with a contra-indication for anticoagulants[5].

Compression therapy

Compression therapy is wearing an elastic stocking over the lower leg. It has been shown that wearing an elastic stocking with 34-46 mm Hg of pressure for two years decreases the chance of

developing post-thrombotic syndrome (PTS)[49], which is a long term complication of DVT. The symptoms of PTS are skin induration, swelling, venous ectasia, pain and edema of the affected limb. In severe cases, venous leg ulcers can develop[50]. This therapy is started next to the normal medical treatment and is only indicated in DVT[5].

Novel drugs

After discussing all current treatment options mentioned in the guidelines, this section reports on the novel thrombolytic drugs that are currently on the market and still in development. First, the new agents which are already in use, but not yet part of the guidelines, are reviewed. Then the thrombolytics currently in development are discussed.

Anticoagulants

Idraparinux. Idraparinux is a pentasaccharide that inhibits factor Xa, similar to fondaparinux. After investigation of the drug in a phase III trial, it was shown that the drug had safety problems (major bleedings). These were probably caused by the long half-life of the drug (120 hours) and thus the drug is not used[51].

Dabigatran. Dabigatran is an orally active drug that directly inhibits thrombin without associating to antithrombin III. Dabigatran peak plasma concentration occur within 2 hours and the drug has a terminal half-life of 14 to 17 hours after multiple dose administration[52]. Again, effects are dose-dependent which means dabigatran does not need laboratory monitoring. Dabigatran was compared to warfarin treatment in VTE for 6 months, after initial treatment with unfractionated heparin or LMWHs was finished. This study showed that dabigatran was non-inferior to warfarin in efficacy, while the safety of dabigatran was better[53]. Another study for the extended treatment of VTE with dabigatran showed the same results[54].

Ximelagatran. Ximelagatran is an oral direct thrombin inhibitor as well. Efficacy results were promising when compared with warfarin, since the drug was non-inferior to the vitamin K antagonist in VTE treatment. Safety was also the same, but ximelagatran did not need laboratory monitoring[55]. However, it was shown by Schulman et al. that ximelagatran was hepatotoxic[56].

Bivalirudin. Bivalirudin is a direct thrombin inhibitor which has been on the market for some time, but the guidelines do not use it as a first-in-line drug. Bivalirudin is a hirudin-analogue (a natural peptide occurring in medicinal leeches, *Hirudo medicinalis*), that shows lower renal excretion and immunogenicity[57]. Bivalirudin has been shown to bind to clot-bound thrombin, whereas unfractionated heparin and LMWHs cannot bind to fibrin-bound thrombin[58]. The dose-dependacy of the effects is therefore more clear in bivalirudin and it has been shown that bivalirudin as a monotherapy in PCI is just as effective as heparin product combined with glycoprotein IIb/IIIa receptor inhibitors. Next to that, bivalirudin causes less bleeding complications[59].

Rivaroxaban. Rivaroxaban is an orally active drug that inhibits factor Xa directly. This means that binding to antithrombin III is not necessary for factor Xa inhibition. Pharmacodynamic effects of rivaroxaban are dose-dependent, meaning that laboratory monitoring is not mandatory[60]. Rivaroxaban can be used as a monotherapy in DVT, meaning that the dual therapy of VKA with unfractionated heparin or LMWHs can be replaced[61]. In the form of tablets, peak plasma concentrations are reached after 2 hours. The bioavailability of the drug is 80%[60]. The ability to replace two currently used drugs in combination with dose-dependent responses which require no laboratory monitoring makes this treatment attractive for use. Bleeding is also the side-effect of rivaroxaban, but the safety of the agent is tolerated well with respect to the efficacy of the drug[61].

Apixaban. Apixaban is an oral direct factor Xa inhibitor. Apixaban is rapidly abosorbed, has a half-life of 12 hours and is excreted through the liver and kidneys. The bioavailability of apixaban is 43-46%[62].

Currently, apixaban is used for stroke prevention in atrial fibrillation instead of warfarin (VKA) treatment. Apixaban does not require laboratory monitoring and has a lower risk profile with less bleeding events compared to warfarin treatment[63]. In 2013, a study has shown that apixaban can be used in the extended treatment of VTE (in which patients have already received 6 to 12 months of anticoagulants and need to continue thrombolytic therapy) to prevent recurrence of venous thromboembolism[64].

Edoxaban. Edoxaban is also a factor Xa inhibiting drug that is administered orally. The bioavailability of edoxaban is at least 50% and peak plasma concentrations are achieved after 1 to 2 hours. The half-life of the drug is 8 to 10 hours[65]. Edoxaban has been used for the stroke prevention in atrial fibrillation and was found to be non-inferior in efficacy to warfarin, but edoxaban had a better safety profile with less bleeding events. These bleeding events were also dose-dependent and more predictable[66]. A study using edoxaban treatment in patients with venous tromboembolism showed that edoxaban treatment is non-inferior to heparin with regards to efficacy, but edoxaban has superior safety[67].

Fibrinolytics

Tenecteplase. Tenecteplase is a modified form of alteplase, produced by recombinant DNA technology through several point mutations. Tenecteplase has 14-fold more specificity for fibrin and a half-life of approximately 18 minutes. It has 80-fold greater resistance to plasminogen activator inhibitor 1[68]. These characteristics of tenecteplase were observed in a trial where patients with acute ischemic stroke were treated with tenecteplase or alteplase. Tenecteplase was found to be superior to alteplase in reperfusion and clinical improvement after 24 hours. Clinical benefit on a longer term was also shown in the tenecteplase group. The side effect of tenecteplase is bleeding, but fewer major complications developed in the high dose tenecteplase treatment group, which shows that tenecteplase has a predictable dose-response relationship[69].

Desmoteplase. Desmoteplase is a recombinant form of the plasminogen activator which was isolated from the saliva of the vampire bat *Desmodus rotundus*. Desmoteplase is highly specific for fibrin and exerts almost no plasminogen conversion when fibrin is not present. This new generation tissue plasminogen activator is thus very specific for the conversion of fibrin-bound plasminogen[70]. Desmoteplase is currently evaluated in clinical trials with high anticipations for its safety and efficacy, due to succes in the DIAS-2 clinical trial[71,72].

Antiplatelet agents

Vorapaxar. Vorapaxar is the first drug that is classified as a protease-activated receptor 1 (PAR-1) antagonist. PAR-1 is normally present on human platelets and mediates platelet activation through the association of thrombin to PAR-1[73]. Vorapaxar is orally available and shows rapid absorption after oral intake. Peak plasma concentration is observed in 0.5 to 2 hours. The half-life of vorapaxar is long and steady state is attained after 21 days with once-daily administration[74]. In a large clinical trial in which standard treatment was combined with vorapaxar treatment, a decrease in thrombotic events was noticed, although vorapaxar did increase bleedings. In patient without a history of stroke, vorapaxar had such increased benefits that the drug was approved for use in myocardial infarction and peripheral arterial disease[75,76].

Novel research for new treatment methods

This section of the review discusses novel research that could lead to new methods for thrombolytic treatment. The thrombolytic or antithrombotic aspect of these papers will be highlighted and the results will be summarized.

Antibody targeted plasminogen activator for thrombolysis[77]

Current fibrinolytic treatment options with alteplase show side-effects such as bleeding, but research has also shown that neurotoxicity is an issue in these agents[45]. Next to that, the current generation fibrinolytics are rapidly neutralized and have bad clot penetration[78]. To combat these characteristics of current generation fibrinolytics, single chain urokinase plasminogen activator (scuPA) is attached to a recombinant antibody that is specific for activated GP IIb/IIIa receptors. *In vitro* tests of the binding of the antibody-bound scuPA (scFV-scuPA) showed that scFV-scuPA targeted activated platelets while inhibition fibrinogen binding on these activated platelets. High concentrations of scFV-scuPA inhibit ADP-induced platelet aggregation and the urokinase plasminogen activator showed conversion of plasminogen to plasmin, which degraded fibrin around platelet aggregates in another *in vitro* assay[77]. *In vivo*, the scFV-scuPA showed prophylactic properties when the material was used beforehand in a ferric chloride-induced thrombosis model in mice. In this mouse model, the Doppler flow velocity of the blood was determined 20 minutes after inducing thrombi with ferric chloride. The Doppler flow velocities of the mice treated with scFV-scuPA were significantly higher than the different controls. Next to that, in another thrombosis model, the size of the thrombus was decreased by scFV-scuPA. Low doses of scFV-scuPA (doses that had antithrombotic effect) did not show increased bleeding in mice models. From these results, it seems like an antibody targeted approach for tissue plasminogen activators could have a double effect, namely reduction in platelet aggregation and fibrinolysis[77]. This approach could be the next step towards thrombolytic agents without bleeding side-effects. However, further safety of scFV-scuPA should be determined, as well as possible generation of resistance against this type of drug.

Coated gold nanoparticles[79]

In a recently published paper, the researchers measure the effect of coated nanoparticles on the blood coagulation in different animals. Previously, gold nanoparticles coated with thrombin-binding aptamers have shown anticoagulant activity, but no research was done on potential side effects. Pyrimidine derivatives have also shown anticoagulant activity[80], as well as antibacterial activity[81,82]. In this paper, 3.3 nm gold nanoparticles were coated with pyrimidine derivatives. The inactive molecule 4,6-diamino-2-pyrimidinethiol was chosen to be investigated, because it already showed antibacterial effects when present on golden nanoparticles in previous research. In this research, the golden nanoparticles were coated with 4,6-diamino-2-pyrimidinethiol (DAPTh), while other nanoparticles were coated with 4-amino-2-pyrimidinethiol (APT) and 4,6-dihydroxyl-2-pyrimidinethiol (DHPT) since their chemical structures are similar (see figure 1 and 2), which could lead to other properties, perhaps better than DAPTh. Lastly, gold nanoparticles were also coated with citrate as a negative control[79]. Using these different nanoparticles, the researchers performed *in vivo* assays to show that Au_DAPTh nanoparticles increased APTT and PT times, inhibit tail thrombosis in a mice model and decreased mortality in a mice model for pulmonary embolism. Next to that, Au_DAPTh nanoparticles did not increase bleeding times *in vivo* shown through a tail-transection model, but further studies should be performed to ensure that these nanoparticles do not cause bleeding side effects. *In vitro* assays suggested that Au_DAPTh nanoparticles influence platelet aggregation, thrombin generation and also the polymerization of fibrin[79]. However, to be able to convert this into treatment, biochemical mechanisms for these observations should be investigated.

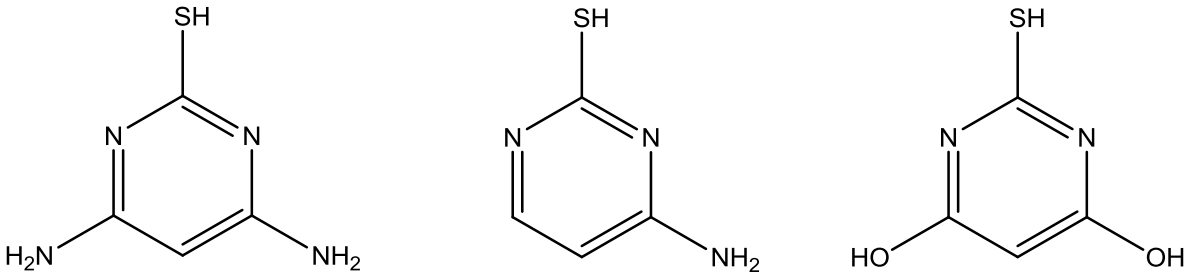

Figure 1: The different coating molecules that were attached to golden nanoparticles.

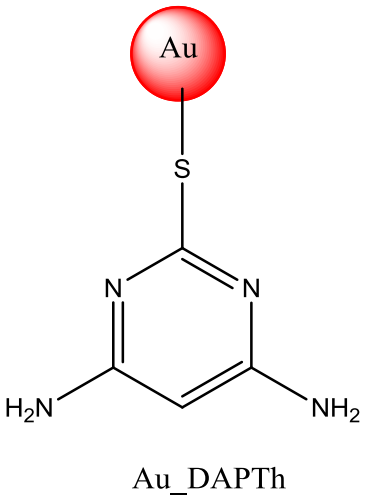

Figure 2: Example of coat attached to golden nanoparticle.

Thrombosis reduction by FXII inhibition[83]

Before the publication of the paper by Matafonov et al., it was shown that factor XII deficient mice have defective thrombus formation and are this resistant to arterial and venous thrombosis[84]. To test this further, antibodies against human factor XII were developed. These antibodies were verified by factor XII activation assays i*n vitro.* The antibodies were then tested for thrombus-forming inhibition in a flow test, a mouse model and finally the antibodies were used in a baboon thrombosis model. The results were promising, in all models the antibodies decreased the rate of thrombus formation[83]. Because factor XII is not part of the intrinsic pathway, it is an interesting target for inhibition of pathological thrombus formation while retaining haemostatic function. However, this approach should be monitored intensively due to the fact that factor XII is part of the contact activation system.

Myeloid-related protein 14 is a regulator of thrombosis[85]

After the discovery that expression of the gene encoding myeloid-related protein 14 (MRP-14) is increased in platelets from acute myocardial infarction patients compared to patients presenting with stable coronary artery disease[86], research on myeloid-related protein 14 was initiated. MRP-14 is more abundant than CD40L (an agonist that binds to GP IIb/IIIa receptors, present in the α-granules of platelets)[85]. Mice with MRP-14 deficiency were bred and it was shown that these mice have a significantly prolonged thrombotic occlusion time in multiple models of vascular injury. In *MRP14*$^{-/-}$ mice, wildtype platelets and addition of purified recombinant MRP-14 decreased the thrombin occlusion time after vascular injury. This indicates that MRP-14 derived from platelets regulates thrombosis. The researchers then identified the target of MRP-14, which is CD36, a protein

that was already described to have a role in thrombosis[87]. The binding of MRP-14 to CD36 was shown through a plate binding assay. Infusion of MRP-14 in double deficient mice (*MRP14$^{-/-}$ CD36$^{-/-}$*), did not decrease the prolonged thrombotic occlusion time. These results combined suggest that platelet MRP-14 and CD36 are involved in a molecular pathway of thrombosis and could be a target for future novel drugs or used as a biomarker[85].

Discussion

After reviewing current disease treatment for DVT, pulmonary embolism, stroke, AMI, ACS and their medical and alternative treatment options, it seems like the current guidelines should be updated with the new generation drugs, since they are already used in certain cohorts of patients with good results. Next to that, the new generation of antithrombotic agents have a higher specificity for their targets, making their dose-response relationship more predictable. This reduces the need for laboratory monitoring in treatment. The novel therapeutics also show better availability in higher risk patients and induce less severe side-effects. Current research in thrombosis shows room for improvement, either in the formulation of drugs or through the identification of new pathways. Once more future drugs make use of new pathways, decision on thrombolytic therapy for patients can be made on an individual level using (new) biomarkers as a basis.

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