Anticoagulant therapy: basic principles, classic approaches and recent developments

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The standard multipotent anticoagulants (unfractionated and low molecular weight heparins, antagonists of vitamin K) are commonly used for treatment and/or prophylaxis of different thrombotic complications, such as deep vein thrombosis, thrombophilia, pulmonary embolism, myocardial infarction, stroke and so on. Advantages and shortcomings of these anticoagulants are considered. The modern tendencies to use small selective direct inhibitors of thrombin or factor Xa are surveyed. The search of the new targets in coagulation cascade for development of new promising anticoagulants and improvement in antithrombotic therapy is discussed. *Blood Coagul Fibrinolysis* 23:000–000 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Blood coagulation abnormalities develop in a very broad range of disorders. These abnormalities frequently result in death following injuries, sepsis, surgical interventions, oncological and cardiovascular diseases, atherosclerosis and so on. One of the most threatening complications is thrombosis, that is, formation of clots (thrombi) where they should not form. These clots obstruct some of the blood vessels and can block the supply of blood to the body's vital organs and tissues. Thromboembolic diseases, such as myocardial infarction, stroke, deep vein thrombosis (DVT), pulmonary embolism and so on, are the main causes of death worldwide. As thrombosis results from increased blood clotting, anticoagulant therapy is employed to counteract thrombosis.

To better understand the principles of target choice in antithrombotic therapy, we will view very briefly the hemostatic system of the blood. The current perception is that the cessation of bleeding is realized jointly by the vascular wall, blood cells, predominantly platelets (vascular – platelet hemostasis), and enzymes (serine proteases) formed in the plasma following coagulation activation from inactive plasma precursors (plasma hemostasis). Coagulation always involves the both systems, but either of them can be predominant depending on the clotting conditions (e.g., depending on the blood flow rate).

The vascular wall is very important in the process of hemostatic reactions. When its integrity is impaired, endothelial cells in the blood vessels produce and / or express on their surface various biologically active substances that modulate blood clot formation. Causes of vessel injury are very diverse and include both exogenous factors (mechanical impacts, radiation, hyper- and hypothermia, toxic influences, including medications, etc.) and endogenous factors. The latter factors include biologically active substances (thrombin, cyclic nucleotides, a number of cytokines, etc.) that become membrane-aggressive under certain conditions. This mechanism of vessel wall damage is characteristic of many conditions accompanied by increased propensity to thrombosis.

The first step in the body's hemostatic response to vessel wall damage is platelet activation and formation of a platelet aggregate at the site of injury. Although the platelet and the plasma parts of hemostasis are activated simultaneously, plasma coagulation works somewhat more slowly. Within several minutes, however, a network of fibrin fibers is gradually formed inside the platelet aggregate, which makes the initial clot much more firm.

Plasma coagulation is a complex sequence of enzymatic reactions with extensive positive and negative feedbacks; it involves consecutively activated serine proteases called coagulation factors. These factors are denominated with Roman numerals (their activated forms are indexed with letter 'a').

The process of plasma hemostasis can be conditionally divided into three phases.

The first phase is a multistage process, which results in formation of trace amounts of thrombin, activation of positive feedbacks by thrombin (through activation of

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cofactors V and VIII) and formation of a prothrombinase complex, which significantly accelerates the conversion of prothrombin to thrombin. This phase lasts approximately 5–7 min. It involves virtually all factors of coagulation system (VII, X, V, VIII, IX, XI, XII).

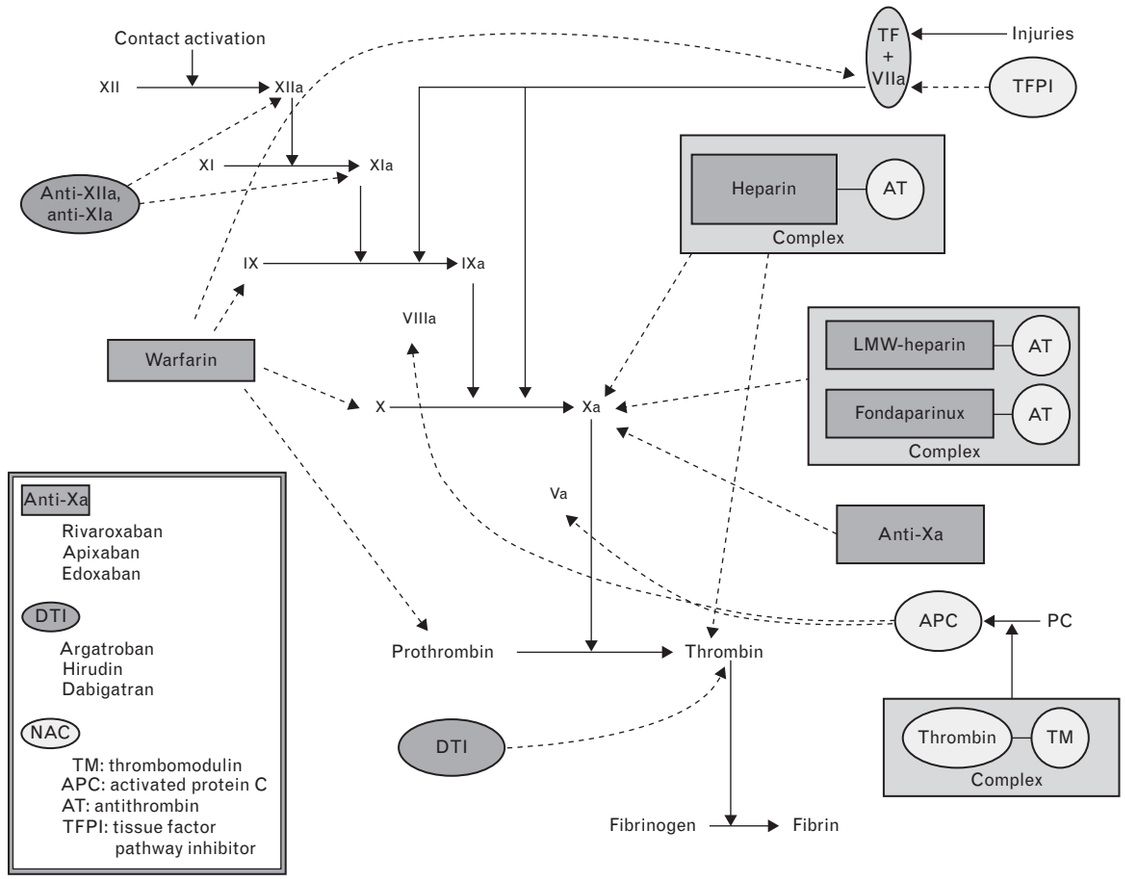
The second phase is accelerated production of thrombin. During this phase, the prothrombinase produced converts the inactive factor II (prothrombin) to the active factor IIa, thrombin.

The third phase is production of fibrin. Thrombin from the previous phase splits two by two peptides, A and B, off the soluble plasma fibrinogen molecule, thus converting the latter to fibrin monomer, which polymerizes nonenzymatically and turns into a slowly, partially soluble polymeric form (fibrin), a component of the blood clot.

To prevent excessive coagulation leading to thrombosis, one can target different elements of the hemostatic systems. The possible targets of anticoagulant therapy are presented in Fig. 1.

In today's clinical practice, anticoagulant therapy is most frequently based on indirect and direct inhibitors of coagulation cascade enzymes, first of all thrombin and factor Xa [unfractionated heparin or low molecular weight heparins (LMWHs), hirudin or its recombinant analogs, argatroban, fondaparinux, etc.); antiplatelet agents that decrease platelet aggregation and, thus, counteract with further activation of coagulation; and vitamin K antagonists that inhibit the production of coagulation factor precursors in the liver (the most important representative of this class, warfarin, is an oral coumarin anticoagulant) [1]. Although these agents are rather efficient in many clinical conditions, each of them has its own therapeutic limitations and undesirable adverse effects, which prompts investigators to continue the search for not only new 'perfect' anticoagulants possessing the same or higher therapeutic efficacy but

Fig. 1



Points of application of standard and new anticoagulants in the blood coagulation cascade. Red dotted lines show the points of application of standard (warfarin, unfractionated heparin, low molecular weight heparins and fondaparinux) and natural (activated protein C, TFPI, etc.) clinically used anticoagulants, whereas green dotted lines represent the points of application of new anticoagulants (clinically important direct inhibitors of thrombin and factor Xa, or new inhibitors only in development for factors XIa or XIIa). Anti-Xa, inactivated factor Xai and direct factor Xa inhibitors; DTI, direct thrombin inhibitors; LMWH, low molecular weight heparins; NAC, natural anticoagulants; TFPI, ???.

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also free of the disadvantages typical for the existing medicines.

The major achievements in this field have until now been related to the introduction into clinical practice of small-sized synthetic direct inhibitors of individual blood coagulation factors, primarily thrombin and factor Xa.

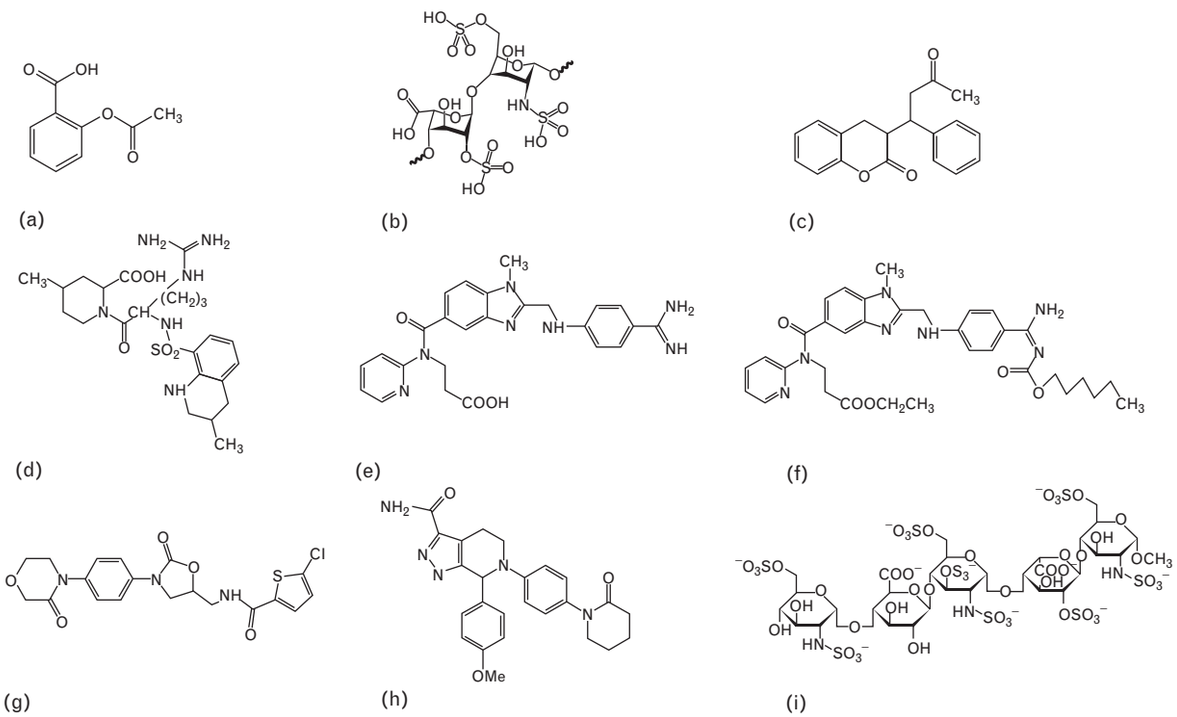
The improved understanding of the fundamentals of the blood coagulation regulation has produced a number of hypotheses proposed lately concerning the possible advantages of inhibitors of factors XIIa and XIa for antithrombotic therapy [2]. The research in this area has not been finished yet, but these studies have been opening new interesting approaches to the fight against potential thrombosis without impairing normal hemostatic function of this system.

Standard anticoagulants

Antiplatelet therapy

A number of drugs affecting platelet hemostasis are available at present. They either block platelet aggregation or inhibit platelet activation, eventually leading to retarded coagulation. The best known antiplatelet agent is aspirin (cyclooxygenase inhibitor) (Fig. 2a). Antiplatelet drugs include also substances that block certain platelet aggregation or activation receptors [glycoprotein IIb/IIa (GPIIb/IIIa), purinoreceptor of the P2Y12 type (P2Y12), protease-activated receptor (PAR)1, PAR4, and so on) [3]. Some examples of these medications are as follows: abciximab (reopro), an antibody-based antagonist of GPIIb-IIIa; tirofiban, a low molecular weight inhibitor of GPIIb/IIIa receptors found on active platelets; and plavix (clopidogrel), which binds irreversibly to platelet P2Y12 ADP receptors and, thus, selectively inhibits the subsequent ADP-induced activation. These antagonists are usually used either to prevent arterial thrombosis in which the role of platelets is believed to be the predominant one, or in a combined therapy, concomitantly with an anticoagulant of another type. Like other antithrombotic agents, they have various adverse effects (first of all, increased risk of bleedings) and disadvantages (first of all, insensitivity in a significant portion of the human population). The enormous body of existing material does not allow a detailed description of all available antithrombotic classes in one survey; therefore, we will not here go into problems on antiplatelet drugs any further.

Fig. 2



Chemical structures of major anticoagulants used in clinical practice. Standard anticoagulants: (a) aspirin (an anti-aggregant); (b) structural units of heparins (unfractionated heparin and low molecular weight heparin); (c) warfarin (a vitamin K antagonist). Direct thrombin inhibitors; (d) the parenteral inhibitor argatroban; (e) the inhibitor dabigatran (may be administered parenterally and has low bioavailability in oral administration); (f) dabigatran etexilate (the prodrug form of dabigatran that may be administered orally). Highly specific factor Xa inhibitors: (g) direct inhibitor, rivaroxaban; (h) direct inhibitor, apixaban; (i) indirect inhibitor, fondaparinux.

Unfractionated heparin, low molecular weight heparins and heparinoids

Unfractionated heparin is a natural anionic polysaccharide consisting of chains of different lengths composed of repeating residues of uronic acid and D-glucosamine [4–6] (Fig. 2b). The molecular weight of unfractionated heparin varies from 3000–5000 to 30 000–40 000 Da [7,8] peaking at 12 000–15 000 Da (Fig. 3) [9].

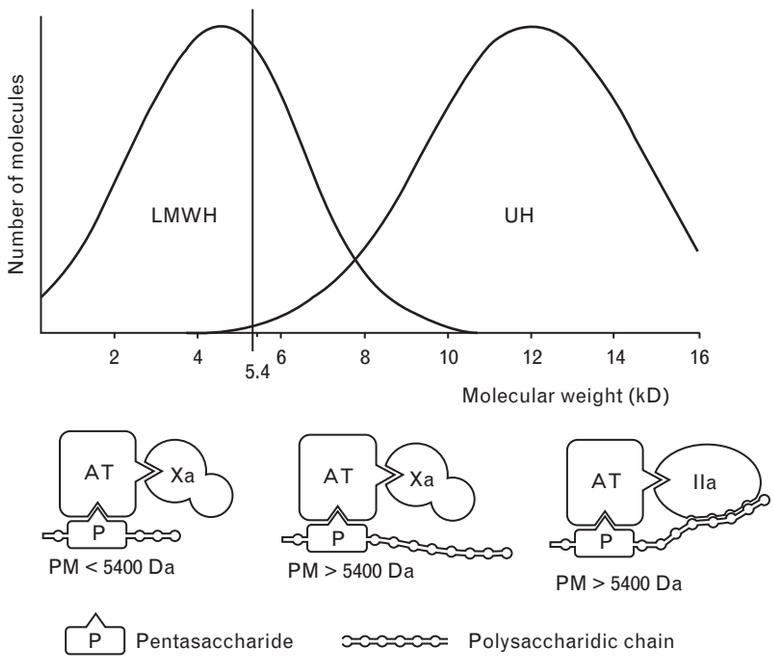
Unfractionated heparin may be divided by chemical or enzymatic fragmentation into fractions with lower molecular weights (from 2000–4000 to 6000–10 000 Da on the average) that possess different biological properties depending on their average molecular weight (Fig. 3) [5,10].

Sulfated mucopolysaccharide analogs of heparin called heparinoids have been identified in animal tissues. They have the same principal structure and a similar polysaccharide composition.

All heparins are not direct inhibitors of thrombin or other serine proteases in the coagulation cascade. These agents exert their antithrombotic activity working as cofactors of the natural inhibitors of these proteases, antithrombin III (ATIII) and cofactor II heparin [8,11]. ATIII neutralizes (to a different extent) virtually all activated blood coagulation factors (except for factor VIIa) and is responsible for more than half the complete plasma inhibitory activity [12]. When thrombin (as well as factors IXa and XIa) is inhibited in the presence of heparin, a ternary complex is formed in which heparin binds to thrombin nonspecifically and simultaneously binds a molecule of ATIII [13]. To make this possible, the polysaccharide chain has to be long enough, not less than 18–20 sugar residues, which approximately corresponds to a molecular weight of 5400 Da. The binding of heparin and antithrombin results in alteration of the ATIII molecule conformation, which increases the rate of its reaction with factor Xa and, to a lesser extent, with thrombin [14]. It should be mentioned that unfractionated heparin, similarly to its analogs, exhibits heterogeneous affinity to ATIII. Just one-third of the chains contain a pentasaccharide that has high affinity to ATIII. This pentasaccharide has been artificially synthesized [15].

The ATIII-mediated neutralization of factor Xa (and factor XIIa) requires heparin binding to ATIII only (Fig. 3). Hence, the main difference in the biological activities of unfractionated heparin and LMWHs. Being different in the polysaccharide chain length only, they have significantly differing antithrombin and antifactor Xa activities. Unfractionated heparin, which has long polysaccharide chains, is similarly effective in both thrombin and factor Xa inhibition (the anti-Xa/antithrombin activity ratio for this heparin is 1). However, LMWHs, which have shorter chains, bind thrombin generally much weaker, which translates into their higher anti-Xa/antithrombin activity ratio (Fig. 3). These agents

Fig. 3



Distribution of unfractionated heparin (UH) and low molecular weight heparin (LMWH) preparations by molecular weight (top panel). The mechanisms by which heparins of different chain lengths bind to antithrombin III (ATIII) and factors Xa or thrombin [9] (bottom panel).

exert their antithrombotic action predominantly by inhibiting factor Xa activity [9,16]. The various LMWH manufacturing methods produce drugs with somewhat different molecular weight distributions; therefore, different LMWH preparations have different anti-Xa/antithrombin activity ratios (from 1.5 to 6.1) [16].

The abundant available literature (beginning from the 1980s) makes it clear that, although standard antithrombotic therapy with unfractionated heparin is now the most common option that has been rather successful in treatment of many diseases, such as unstable angina pectoris and myocardial infarction, as well as in the treatment of postoperative and chronic DVT and so on [17–20], use of unfractionated heparin is associated with a number of limitations and disadvantages.

(1) Heparin exerts its antithrombotic effect indirectly. This mediated effect requires the presence of ATIII in the system. Therefore, the anticoagulant efficacy of heparin is decreased in patients with ATIII deficiency (such as those with severe septic shock), as well as in long-term heparin therapy associated with reduced ATIII concentrations.
(2) Unfractionated heparin has a very short plasma life time (30–60 min); therefore, its effects disappear very quickly after discontinuation of therapy. As a result, treatment with unfractionated heparin is unable to decrease the risk of recurrent thrombotic events [21,22].
(3) The same heparin dose can produce unpredictable anticoagulant responses in different patients, which is due to a whole range of causes, including the plasma ATIII concentration, individual drug elimination rates and binding and neutralization of heparin by various plasma proteins and activated platelets (platelet factor 4, heparinase, etc. [23–25]). Besides, heparin has nonlinear pharmacodynamics, because it is eliminated from the body through different routes: by saturable cellular mechanisms involving endothelial and reticulo-endothelial cells and through the kidneys. The half-life of heparin depends on the administered drug dose: low doses are very quickly eliminated, whereas at higher doses the drug is eliminated significantly more slowly [26]. This determines the need for frequent monitoring of the coagulation system status [using the activated partial thromboplastin time (APTT) test in most cases] and the risk of hemorrhagic complications.
(4) Heparin is active only against circulating thrombin, but it exerts virtually no inhibition of thrombin adsorbed on the clot [4,27,28]. Therefore, heparin is unable to prevent recurrent thrombosis after discontinuation of heparin therapy, when blood clots that were formed earlier are completely lysed by the fibrinolytic system and thrombin released from them enters the plasma again.
(5) Heparin therapy may be associated with immune-mediated heparin-induced thrombocytopenia (HIT) accompanied by a quantitative reduction but strong activation of the platelets and by the risk of thrombosis [29].
(6) Long-term heparin therapy (longer than 6 months), as well as very high heparin doses (>15 000 units), may induce another complication, osteoporosis [5,30].
(7) The bioavailability of unfractionated heparin is low, being approximately 20–30%. Heparin may be administered only intravenously, in in-hospital settings.
(8) Unfractionated heparin therapy requires monitoring, which is routinely done with APTT assay, although various thrombin generation assay and thrombelastography protocols for this also exist.

Some of the disadvantages were in many respects overridden by the introduction of LMWHs into hospital practice. In contrast to unfractionated heparin, LMWHs undergo mainly renal excretion [31–33] and are weakly bound to endothelial cells. As a result, the half-life and elimination rate of the drug remain virtually unchanging over the entire therapy period. At the doses used in clinical practice, the half-life of LMWHs is longer than that of unfractionated heparin, varying from 1.5 to 4.5 h. This allows a switch from regimens employing constant drug infusion to once a day therapeutic regimens. As the nonlinear dependence of the pharmacodynamics on the drug dose is avoided in such regimens, the bioavailability in subcutaneous administration is about 100% at any dose [26]. As a result, a drug dose calculated only on the body weight basis can be used in clinical practice. It allows clinicians to resign the regular monitoring of the coagulation system in the process of treatment. Additionally, this allows many patients to continue necessary antithrombotic prophylaxis over a longer period being at home rather than in the hospital. [34,35].

LMWHs have a comparatively effective and safe activity profile. They appear to be less immunogenic and cause complications (such as hemorrhage, thrombocytopenia and osteoporosis) less frequently.

Vitamin K antagonists

Another class of indirect anticoagulants is composed of vitamin K antagonists. These agents include coumarins, with their best known representative in clinical practice being warfarin (Fig. 2c).

The mechanism of action of vitamin K antagonists with regard to the blood coagulation system is an effective block of the production of vitamin K-dependent coagulation factors in the liver. Posttranslational γ-carboxylation of the N-terminal end of the future coagulation factor is required for a normal factor molecule to be synthesized. This is necessary to make newly synthesized factor molecules able to bind (by means of calcium ions) to

the negatively charged phospholipid surface of activated platelets and exert their function. Vitamin K is an indispensable cofactor to this carboxylation reaction. In the course of the reaction, vitamin K is gradually converted from its hydroxyquinone form, which participates in the carboxylation, to the oxidized epoxide form. The latter form is reduced again by the enzyme vitamin K reductase and can, thus, take part in the carboxylation reaction again. Coumarin group agents block this reaction of chemical reduction [36].

Warfarin has been employed in anticoagulant therapy for more than 50 years now. Its use has significantly expanded in recent years. It was the only agent with anticoagulant activity that could be taken orally in a long therapy course until very recently; therefore, it finds very common use in long-term anticoagulant prophylaxis [36]. However, it possesses a number of limitations and shortcomings as well.

(1) Slow response to therapy. It begins to manifest itself in 1 day, but reaches its peak some days later.
(2) Warfarin has a narrow therapeutic window. Therapeutic anticoagulation [usually at international normalized ratio (INR) values in the range of 2–3] can be attained in patients on warfarin therapy with doses differing between individuals more than 10-fold. The underlying causes of that are numerous, including genetic polymorphism in the activity of warfarin-metabolizing enzymes, such as cytochrome P450 (CYP29) and vitamin K epoxide reductase (VKORC1) [37,38] and the rather strong binding of warfarin to various food components and interactions with many pharmaceutical substances.
(3) This means that warfarin therapy needs certain dietary limitations and systemic monitoring, which is routinely done with prothrombin time assay providing the INR values. Thrombin generation assay is also used.

Warfarin is effective in the prevention of postoperative DVT (although somewhat less effective compared with LMWHs), but it needs long-term therapy [39–41]. Besides, it is administered in the treatment of thromboembolism and in the prevention of recurrent thromboembolic episodes. Treatment usually consists of concomitantly administered unfractionated heparin (intravenously) and warfarin (orally) until a sustained response to warfarin is achieved. Heparin is discontinued afterwards, whereas warfarin therapy should be continued for 3–6 months. The chance of recurrent thromboembolism developing within 1 year after such therapy is 5–10%; however, longer warfarin therapy cannot be recommended because of the risk of bleeding [42,43]. Warfarin is also used as a chronic therapy in the prophylaxis of atrial fibrillation.

Selective thrombin and factor Xa inhibitors

Both heparins and vitamin K antagonists (warfarin) are multipotent, that is, they interact in the coagulation system with several targets at the same time. This fact interferes with predicting how these agents will affect the system on the whole. As a result, the concept of selective inhibition of individual coagulation system factors has emerged in the field of anticoagulant therapy.

Thrombin is the key enzyme in the coagulation cascade. It converts soluble plasma fibrinogen to fibrin gel in the clot and is also involved in the realization of principal positive (activation of cofactors V and VIII, as well as of factor XI and platelets) and negative (activation of protein C) feedbacks in this system. Although the substrates of thrombin are numerous, it exhibits substrate specificity, because a specific binding site exists for each of the substrates (for instance, fibrinogen binds to thrombin through exosite 1). Any thrombotic complication basically results from a thrombin excess, and this excessive thrombin has to be eliminated from the system to avoid these complications. That is why thrombin became the first actual target to be inhibited with the aim to prevent thrombosis.

Another coagulation cascade target suitable for selective inhibition is factor Xa. The search for small-sized inhibitors of this factor has been particularly active in recent years. This is due to the fact that synthetic direct thrombin inhibitors, which have doubtless advantages over heparin, are not perfect either. They tend to increase the probability of hemorrhagic complications, especially in coadministration with thrombolytic agents, such as tissue plasminogen activator [44–46]. Their significant disadvantage is their inability to prevent on-going thrombin production in the therapeutic dose range. A dramatic dose escalation is thus necessary, and this may result in an unacceptably high level of anticoagulation and, as a consequence, lead to hemorrhage. If the prothrombinase complex responsible for rapid thrombin production is inhibited, accelerated thrombin generation is arrested (although a very low level of basal activity may persist and maintain the necessary level of coagulation [47–49]). This perception initiated a search for direct inhibitors able to suppress the active component of the prothrombinase complex, factor Xa [50–52].

Hirudin and its analogs

The first selective thrombin inhibitors that found use in clinical practice were the naturally occurring protein hirudin obtained from the salivary glands of the medicinal leeches (such as *Hirudo medicinalis)* and its analogs.

Hirudin is a very potent and selective bivalent direct inhibitor of thrombin. The dissociation constant value for the complex of thrombin and hirudin is K_d of 10^{-14} mol/l [53,54]. When bound to thrombin, hirudin remains in this complex thanks to simultaneous binding to two

fragments of the thrombin molecule: one of them is near the active center and the other fragment is the fibrinogen-recognizing (anion-binding) exosite of thrombin [55,56].

Recombinant hirudin is manufactured at present (desirudin [57] and lepirudin [58]). The constant for inhibition of thrombin with recombinant hirudin is approximately 230 fmol/l. Because of the double-centered binding to the enzyme molecule, this inhibitor remains one of the most potent and selective of all currently known thrombin inhibitors [59]. It should be mentioned at the same time that it is a weak inhibitor of clot-bound thrombin, just like heparins [60].

The main adverse effect of hirudin therapy is rather frequent hemorrhagic complications [58,61]. Monitoring is common and is usually done either by measuring hirudin level or with APTT, ecarin clotting time or thrombelastography.

A number of peptide analogs of hirudin have been synthesized (hirulogs); their design employs the same bivalent manner of thrombin binding. They also act as potent (dissociation constants in the order of nanomoles per liter) and very selective direct thrombin inhibitors [62,63]. The only representative of this inhibitor class in clinical practice at present is the peptide agent bivalirudin (D-Phe-PRPGGGGDGDFEEIPEEYL). Its inhibition constant K_I is of 1.9 nmol/l, and the half-life ($t_{1/2}$) following intravenous infusion is of 36 min. Clinical studies have demonstrated that in some ischemic diseases, bivalirudin is at least as effective as high-dose heparin therapy; however, treatment with bivalirudin is associated with a significantly decreased risk of bleeding [64].

Low molecular weight synthetic thrombin and factor Xa inhibitors

All conventional antithrombotic agents have limitations in use and a whole range of disadvantages that have been already mentioned. As a result, an intensive search has been under way for many years to find new effective inhibitors free from the shortcomings of the standard drugs. In this respect, the strategy of developing small-sized synthetic inhibitors of thrombin and factor Xa turned out to be very attractive [65]. The investigators take into account the following requirements that the desired inhibitor should conform to, if possible:

(1) High affinity to the target enzyme (i.e. highly effective inhibition).
(2) Considerable selectivity toward the target enzyme compared with other related serine proteases (at present this requirement is being reconsidered, as double inhibitors of thrombin and factor Xa are being developed [66]; it is, however, still indispensable that new inhibitors be selective with regard to coagulation factors, as compared with such digestive serine proteases as trypsin or chymotrypsin).
(3) Chemical and metabolic stability.
(4) No toxicity. Use of anticoagulants in patients with liver function insufficiency is particularly important and complicated; they currently require individual therapy adjustment, because normal therapeutic concentrations can be toxic, and too low concentration can be ineffective.
(5) Weak (or not too strong) binding to plasma proteins.
(6) High bioavailability in oral administration.
(7) Sufficiently long biological life of the drug that would allow, following oral administration, to maintain a therapeutic plasma concentration for a time enough to limit the dosing frequency to once or twice daily.
(8) Feasibility of simple monitoring of drug levels. Monitoring is essential for new inhibitors, because there are problems reported with some of the currently used clotting assays. Alternative testing, or development of well tolerated inhibitors that require no monitoring, is of particular importance.
(9) Preferably, availability of an antidote capable of preventing drug effects following an overdose or an hemorrhagic complication.

A large number of reviews describing the development of low molecular weight thrombin and factor Xa inhibitors have been published in scientific literature to date [38,49,66,67]. In reality, however, only a small number of these drugs have been approved for clinical practice by now: argatroban (a direct thrombin inhibitor for intravenous administration) [57,68,69], dabigatran etexilate (a direct oral thrombin inhibitor) [70], as well as a number of Xa factor inhibitors: fondaparinux and indraparinux (in clinical trials) that work in complex with ATIII (for intravenous administration) [71–74], and rivaroxaban (a direct oral factor Xa inhibitor) [75,76].

Thrombin inhibitors

The chemical structures of the clinically employed low molecular weight direct thrombin inhibitors can be found in Fig. 2d–f.

Argatroban (Fig. 2d) was developed more than 30 years ago in Japan [69]. Apart from that country, to date its use has been approved in the USA, Canada, China, Korea and many European countries, such as Germany, Sweden, Austria, Italy and so on. This agent has a very low bioavailability in oral administration and can, thus, be administered only parenterally.

Argatroban is a highly selective, reversible, competitive thrombin inhibitor with an inhibition constant of 39 nmol/l (relative to human thrombin).

The plasma lifetime of argatroban is 30–50 min. Argatroban differs from other inhibitors in that it undergoes metabolization by liver enzymes (mainly CYP3A4/5) and hepatic elimination, which allows avoid drug dose adjustment in patients with renal impairment. Argatroban is

most frequently administered by infusion because of its short half-life. Its anticoagulant activity starts to manifest immediately after the administration is begun and reaches a stationary level in 1 or 2 h. After the end of infusion, anticoagulant activity returns to baseline within 2–4 h [77].

The major advantage of argatroban, as compared with heparins, is that argatroban induces no antibody production and can be used in the treatment of HIT, in which anticoagulant therapy is necessary because of the high risk of thrombosis, but administration of heparins is impossible. In the treatment of immune-mediated HIT, this agent dramatically decreases mortality and occurrence of thrombotic complications, particularly limb gangrene [78]. Besides, argatroban is highly effective not only against free thrombin in solution but also against thrombin adsorbed on blood clots. This permits a reduction in the risk of recurrent occlusion of blood vessels following dissolution of previously formed thrombi. Reviews of some clinical studies of argatroban can be found in publications [57,68,78–80].

Dabigatran etexilate is the only oral thrombin inhibitor currently used in clinical practice (Fig. 2f). Large-scale clinical studies of this agent are still being conducted at present. It received approval for limited use in clinical practice for some disorders only in 2009.

Dabigatran etexilate is a prodrug. This is a rather hydrophobic compound that exhibits a relatively high ability to cross the gastrointestinal mucous membrane. After entering the bloodstream, dabigatran etexilate is hydrolyzed by plasma esterases to dabigatran (Fig. 2e), which is the active thrombin inhibitor [81]. Even after that, however, the overall bioavailability of dabigatran etexilate is only 6–7%.

Dabigatran is a competitive, reversible, direct thrombin inhibitor with an inhibition constant of 4.5 nmol/l [82]. Dabigatran undergoes essentially renal excretion (80%). It is not metabolized by the CYP450 enzymes or oxidoreductases.

One disadvantage of this inhibitor is the absence of a direct antidote to this drug. If necessary, elimination of this drug from the plasma may be accelerated by plasmapheresis, which clears up to 60% of the drug amount in 2–3 h [83].

To date, dabigatran etexilate has already passed large-scale clinical studies and has been approved in a number of countries for clinical application in some disorders, predominantly in the prevention and treatment of venous thrombosis after orthopedic surgery and in the prevention of stroke and systemic embolism in patients with atrial fibrillation. Clinical trials have shown that dabigatran etexilate is as effective in these conditions as enoxaparin (LMWH) or warfarin, but it causes fewer clinically important episodes of adverse bleeding. A detailed review of these publications presenting results of clinical studies of dabigatran etexilate, as well as of new oral factor Xa inhibitor preparations, can be found [38,84].

Selective factor Xa inhibitors

Two different types of specific factor Xa inhibitors are utilized in clinical practice: indirect inhibitors working only in coadministration with ATIII and direct low molecular weight inhibitors.

Indirect inhibitors include fondaparinux (Fig. 2i) and idraparinux (as well as biotinylated idraparinux). Both these drugs were synthesized on the basis of the pentasaccharide incorporated in the heparin molecule part responsible for the ATIII binding [3]. Both fondaparinux and idraparinux can be administered only parenterally. Idraparinux is different from fondaparinux in its additional methylation of the hydroxyl groups of the polysaccharide molecule. As a result, the half-life of the drug is significantly prolonged (to approximately 80 h, as compared with 17 h for fondaparinux [3]), thus allowing once weekly subcutaneous administration of idraparinux, whereas fondaparinux should be given once daily [85].

Clinical studies have demonstrated that fondaparinux (administered once daily) is as effective in the primary treatment of DVT as LMWH [86] and is not inferior to unfractionated heparin in the treatment of pulmonary embolism as well [87].

If idraparinux is proved to be well tolerated and highly effective, it may become a good alternative to vitamin K antagonists in the long-term treatment and prevention of DVT and pulmonary embolism. Besides, this drug may enable replacement of the two-stage therapy with LMWH followed by a vitamin K antagonist, which is now the standard, with single-drug treatment. This can significantly decrease the cost of treatment, because no dose adjustment and constant monitoring will be necessary anymore at the vitamin K antagonist therapy stage. Results of already completed clinical studies have demonstrated that idraparinux is well tolerated and equally effective in the treatment of DVT as the two-stage therapy with LMWH followed by a vitamin K antagonist (in 6-month therapy), but it performed worse than the same two-stage standard regimen in the prophylaxis of recurrent thrombosis in patients with pulmonary embolism. And most of the recurrent thrombosis episodes occurred within the first 2 weeks of treatment [88].

The biotinylated form of idraparinux was developed recently. It has been shown that the addition of biotin to the inhibitor molecule does not interfere with its binding to ATIII and linking of this complex with factor Xa. This inhibitor form, however, can present an opportunity of rapid neutralization of the active inhibitor in the plasma after administration of avidin, which becomes fast

bound to biotin. The resulting complex is then eliminated from the body. The use of an antidote may be necessary in patients with developing complicating bleeding or in those requiring urgent surgery. At present, this agent is undergoing clinical investigation.

Out of all direct factor Xa inhibitors, only rivaroxaban has been approved to date for clinical application (in Canada and in Europe, for prophylaxis of venous thrombosis, primarily after orthopedic surgery) (Fig. 2g). Apixaban (Fig. 2h), edoxaban and some other medications are now in their phase III clinical studies [84,89,90].

Direct factor Xa inhibitors exhibit good bioavailability following oral administration (from 50 to 80%). Renal elimination varies from 25 to 65% between different inhibitors, and the half-life from 5 to 12 h. As a result, these agents can be administered once or twice a day while no regular coagulation monitoring is required. The most important interactions with other medicinal products include potent inhibition of CYP3A4 and the p-glycoprotein transporter [38]. The shortcoming of these inhibitors is the absence of appropriate antidotes.

New directions in the development of effective anticoagulants

Major causes of thrombosis include the following:

(1) Vessel wall damage (from mechanical or chemical impact, rupture of an atherosclerotic plaque).
(2) Imbalance of blood coagulation factors and inhibitors (such as elevated fibrinogen concentration, decreased antithrombin level, altered activities of mutant forms of some factors, etc.).
(3) Pronounced activation of coagulation (for instance, following a trauma or infection).

Further activities in the development of anticoagulants should include a search for drugs counteracting individual causes of thrombosis and inhibiting respective elements in the blood coagulation cascade mechanism. Whenever possible, the possibility of normal hemostasis should be safeguarded in order to avoid complicating hemorrhage.

Anticoagulant therapy may be targeted at different system elements:

(1) Platelets (reduction of platelet activation can decrease the ability of platelets to cooperate with active coagulation factors and thus attenuate coagulation).
(2) The interaction between coagulation factors and lipids may be decreased. This should results in a reduction in the amount of procoagulant complexes attached to the lipid surface (antibodies may be used to block this binding).
(3) The activities of individual blood coagulation factors (IIa, Xa, IXa, VIIa, XIIa, XIa) or cofactors (Va, VIIIa, tissue factor) may be inhibited or the extrinsic tenase (tissue factor /VIIa) complex suppressed.
(4) One could also try to accelerate the dissolution of already formed blood clots by activating the fibrinolysis system. Strictly speaking, this approach is not anticoagulant therapy, but it does help counteract thrombosis.

Both high molecular weight preparations (antibodies, DNA or RNA aptamers, carbohydrates) that can only be administered parenterally, intravenously or subcutaneously, and low molecular weight synthetic compounds that can be taken by mouth can be employed as coagulation inhibitors. Besides, anticoagulant therapy may also be based on natural blood coagulation inhibitors, such as antithrombin, tissue factor pathway inhibitor, activated protein C and thrombomodulin (thrombin cofactor in protein C activation).

All of the above options have been tested in contemporary medicine, but they have not yielded the expected result as yet – people are still dying from thrombosis. And so not only is the development of new inhibitors of traditional targets in the coagulation cascade (thrombin and factor Xa) still under way, but the search for new possible targets for anticoagulant therapy continues as well. Some studies of recent years [2,91–93] have demonstrated that inhibitors of contact activation pathway factors may prove very attractive in this respect.

The intrinsic (contact) pathway of blood coagulation works alongside with the extrinsic pathway. It is launched by the contact of plasma factor XII with any foreign surface. Such way formed factor XIIa activates the subsequent chain of reactions that involves consecutively activated factors XIa, IXa and Xa. The extrinsic and intrinsic pathways come together at the factor Xa level. The role of the intrinsic pathway in coagulation has remained somewhat unclear over a rather long period, because the extrinsic pathway has been demonstrated to be the main pathway for activation of coagulation *in vivo* [94]. Besides, individuals with intrinsic factor (XII and XI) deficiencies usually have normal hemostasis and do not suffer from bleeding. Currently, the role of the intrinsic pathway in coagulation is being reconsidered. It has been already shown that this pathway is essential for normal spatial plasma clot growth [95,96,97]. On the contrary, the absence of any pronounced influence of these factors on normal hemostasis has allowed a presumption that inhibition of this pathway may prove a promising strategy in anticoagulation. Animal thrombosis models have demonstrated indeed that administration of a factor XIIa inhibitor prevents total occlusion of damaged arterioles [91]. An experimental development of factor XIIa inhibitor was reported in 2010. Albumin-bound infestin (anticoagulant protein of the blood-sucking bugs *Triatoma infestans*) was utilized as the

inhibitor. This complex induced complete inhibition of experimental thrombosis in mice [98].

Particularly interesting studies of the intrinsic pathway of blood coagulation have been reported in the last 4 years. It has been demonstrated that platelets being activated release polyphosphates from their dense granules and that these polyphosphates serve as a potent procoagulant and proinflammatory stimulus. Coagulation activation is triggered in the intrinsic pathway, because the large polymeric polyphosphate molecules (approximately 60–100 phosphate residues) serve as the surface for factor XII activation [92]. Therefore, an additional opportunity of coagulation system activation through the contact pathway exists in arteries with high blood flow rates where platelet activation occurs quickly following an injury. Under these circumstances, inhibition of factors XIIa and/or XIa in this pathway may prove a very promising strategy for anti-coagulant therapy. It should be underlined, however, that the factor XIIa inhibitor blocks only the top of the cascade and is unable to protect the body from thrombosis due to increased function of other active factors.

Numerous experimental models have demonstrated *in vivo* (in mice and monkeys) that both factor XIIa inhibitors and factor XIa inhibitors are effective in the prophylaxis of thrombosis and entail only a minimum impact on hemostasis [91]. This show once more that the inhibitors of factors XIa and XIIa may become essential in the development of new effective antithrombotic treatments.

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Conflicts of interest

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