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REVIEW ARTICLE

Thrombin generation and the pharmacodynamics of parenteral anticoagulants

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ABSTRACT

Parenteral anticoagulants are mainstay therapies in critical care and perioperative settings because of their unique pharmacological properties, but they have a narrow therapeutic window. This emphasizes the need to thoroughly understand their pharmacodynamics effects on hemostasis. Thrombin generation assays provide a comprehensive measure of coagulation and can characterize class-specific anticoagulant effects. We reviewed the literature with a focus on parenteral anticoagulants to define the role of thrombin generation as a pharmacodynamic measure of anticoagulation status. We contextualize these results in light of findings from a prior review on the effects of oral anticoagulants on thrombin generation and consider our findings in view of results stemming from comparative anticoagulation-focused epidemiologic research. This review provides proof of principle that, from a pharmacodynamics perspective, not all anticoagulants are the same. They exert class-specific effects on thrombin generation, and these divergent effects reflect differing mechanisms of action on coagulation initiation, amplification, and propagation. Anticoagulants with multiple downstream effects, such as unfractionated or low-molecular-weight heparins, are better able to suppress thrombin generation than selective direct inhibitors, such as bivalirudin, argatroban, direct oral anticoagulants, or factor XI(a) inhibitors. We propose a conceptually valid theoretical framework, grounded in mechanistic rationale, supported by experimentation, and leveraging thrombin generation as a common measure to compare and examine the pharmacodynamic impact of different anticoagulants. The knowledge reviewed herein may support the future development of more personalized approaches to anticoagulation treatment. Our findings contribute a foundation upon which future anticoagulation research can be based and warrant further investigation.

Significance Statement: From a pharmacodynamic perspective, not all anticoagulants are the same. Oral and parenteral anticoagulants exert class-specific effects on thrombin generation, and these divergent effects reflect differing mechanisms of action on coagulation initiation, amplification, and propagation. Anticoagulants with multiple downstream effects are better able to suppress thrombin generation than selective direct inhibitors. This review proposes a theoretical framework, grounded in mechanistic rationale, and leveraging thrombin generation as a common measure to compare and examine the pharmacodynamic impact of different anticoagulants.

I. Introduction

Parenteral anticoagulants, including unfractionated heparin (UFH), low-molecular-weight heparins (LMWHs), fondaparinux, and the parenteral direct thrombin inhibitors (DTIs) argatroban and bivalirudin, are used frequently in perioperative and critical care settings. Each anticoagulant serves an important role, given its unique pharmacokinetic and/or pharmacodynamic properties (Table 1).[1–16]

UFH is the mainstay agent for cardiovascular surgery, extracorporeal membrane oxygenation, and catheter-based procedures such as percutaneous coronary intervention (PCI) and catheter ablation of atrial fibrillation (AF). UFH is ideally suited to these applications because of its short half-life, low dependence on renal elimination, titratability, and reversibility, despite the high doses required for cardiopulmonary bypass (CPB). LMWHs have a relatively short half-life and a fast onset of action, and are useful as bridging anticoagulation in patients at high risk of perioperative thromboembolic events. The parenteral nonheparin anticoagulants fondaparinux, danaparoid, argatroban, and bivalirudin are used in the management of critically ill patients with heparin-induced thrombocytopenia (HIT).[17] Bivalirudin is used in patients with acute coronary syndrome (ACS) and non-ST-segment-elevation myocardial infarction undergoing PCI, and for extracorporeal membrane oxygenation.[18]

Parenteral anticoagulants are also used in clinical settings that carry substantial bleeding risk because of underlying comorbidities (eg, advanced age, chronic kidney disease, and frailty), acute illness (infection and inflammation), concurrent antiplatelet therapy (eg, cardiac surgery and PCI), recent invasive procedures (eg, surgery and procedural interventions in the intensive care unit), or thrombocytopenia with consumptive coagulopathy (eg, HIT, sepsis, and cardiac surgery). Many of these patients also face a high risk of thrombosis (eg, postoperative venous thromboembolism [VTE] and ACS).

UFH, argatroban, and bivalirudin require laboratory-based monitoring and dose titration because of wide variability in drug response and a narrow therapeutic window.[19,20] UFH is monitored using anti-Xa activity or clot-based assays, including the activated partial thromboplastin time or activated clotting time.[11] Although conventional measures such as anti-Xa testing or clotting times can provide an indirect sense of an anticipated anticoagulant pharmacodynamic effect, they do not necessarily reflect the net impact on the coagulation cascade. Coagulation assays fail to account for what could be important differences across anticoagulant classes (eg, direct vs indirect antithrombin-mediated inhibition, factor Xa vs thrombin inhibition), or even between anticoagulants within the same class (eg, LMWH chain length). Moreover, they do not account for competing prothrombotic effects, which can contribute to “anticoagulation failure” or breakthrough thrombosis.

II. Thrombin generation

Thrombin plays a central role in coagulation initiation, amplification, and propagation. It converts fibrinogen to fibrin, leading

Table 1
Parenteral Anticoagulants – Pharmacological Properties.

Characteristic	Unfractionated Heparin (UFH)	Low-Molecular-Weight Heparin (LMWH)	Fondaparinux	Argatroban	Bivalirudin	Hirudin
Structure	Glycosaminoglycan	Glycosaminoglycan	Synthetic Pentasaccharide	Small Molecule	Polypeptide	Polypeptide
Molecular Weight (Daltons)	3,000-30,000	3,500-5,000 (enoxaparin) 2,000-9,000 (dalteparin) 5,500-7,500 (tinzaparin)	1,725	527	2,180	7,000
Molecular Target(s)	FIIa = FXa (1:1)	FXa>FIIa (ratio) enoxaparin = 3.9:1 dalteparin = 2.5:1 tinzaparin = 2:1	Xa	FIIa (Monovalent) Active Site	FIIa (Bivalent) Active Site + Exosite I	FIIa (Bivalent) Active Site + Exosite I
	FIXa FXIa FXIIa TF·FVIIa	FIXa FXIa FXIIa TF·FVIIa				
Cofactors	Antithrombin	Antithrombin	Antithrombin	None	None	None
Additional Effects	TFPI release Anti-inflammatory Alters fibrin structure	TFPI release Anti-inflammatory Alters fibrin structure	None	Inhibition of TM/APC pathway	Inhibition of TM/APC pathway	Inhibition of TM/APC pathway
Binding Mechanism	UFH·AT = Reversible	LMWH·AT = Reversible	Fondaparinux·AT = Reversible	Reversible	Reversible	Irreversible
	UFH·AT·FIIa = Irreversible	LMWH·AT·FXa= Irreversible				
	UFH·AT·FXa= Irreversible	LMWH·AT·FIIa = Irreversible	Fondaparinux·AT·FXa = Irreversible			
Binding Affinity (K_d)	20-60 nM (chain length-dependent)	20-60 nM (chain length-dependent)	50 nM	40 nM	2 nM	0.0001 nM
Half-Life	30-150 minutes (dose-dependent)	4-5 hours	17 hours	45 minutes	25 minutes	80 minutes
Metabolism	Reticuloendothelial	Reticuloendothelial > Renal (longer chain length LMWHs)	Renal	Hepatobiliary	Enzymatic	Renal
		Renal > Reticuloendothelial (shorter chain length LMWHs)				
Reversal	Protamine	Protamine* Andexanet alfa (off-label)†	rFVIIa (off-label)§	None	None	None

Abbreviations: UFH = unfractionated heparin; LMWH = low-molecular-weight heparin; TFPI = tissue factor pathway inhibitor; AT = antithrombin; FIIa = factor IIa (thrombin); FXa = factor Xa; TM = thrombomodulin; APC = activated protein C. [1-12]

*LMWH is only partially reversable using protamine. Protamine's reversal effects are LMWH chain-length dependent, and protamine is better able to bind and neutralize UFH and longer chain-length LMWH (e.g., tinzaparin). Protamine can fully reverse the effects of tinzaparin on the ETP, but protamine can only neutralize ~60-80% of enoxaparin's effects on the ETP.

†Andexanet alfa is not a true UFH/LMWH reversal agent. Andexanet alfa can induce heparin resistance by binding to antithrombin in the presence of UFH/LMWH, thereby abrogating UFH/LMWH's antithrombin-dependent anticoagulant effects. [202, 203]

§rFVIIa might function as a bypassing agent by accelerating the kinetics of thrombin generation in the presence of fondaparinux. rFVIIa is generally not used as a true fondaparinux "reversal agent" but its use could be considered as a general hemostatic agent in the setting of serious FVIIa-associated bleeding or prior to urgent surgery [141, 142].

to the formation of a fibrin clot. Thrombin activates platelets and regulates both coagulation and fibrinolysis through its effects on the activated protein C system and thrombin-activatable fibrinolysis inhibitor, respectively. Given its central role in clotting processes, quantifying thrombin activity provides an opportunity to more accurately define the net effects of anticoagulation and hypercoagulability.[21]

Measurement of thrombin generation was first introduced in the 1950s and has been subject to consistent technological innovation and methodological standardization over the past 70 years.[22–27] Modern thrombin generation assays (TGAs) continuously measure the production of thrombin using either a fluorogenic or chromogenic thrombin-specific substrate.[28,29] Thrombin generation can be measured in platelet-poor plasma (PPP) or platelet-rich plasma (PRP) and is triggered using varying concentrations of tissue factor (TF; 0.5–20 pM) or a contact activator (eg, silica). The endpoint of conventional coagulation testing is fibrin clot formation. Fibrin formation starts after only 5% of the thrombin in a given plasma sample has been generated; thus, most of the thrombin potential in a sample cannot be measured using conventional assays.[30] By using a fluorogenic or chromogenic measure, TGAs can better quantify thrombin generation and "see past the clot." TGAs measure both kinetic and quantitative parameters of thrombin generation (Fig. 1). Kinetic parameters include the lag time (LT; time in minutes when thrombin generation first occurs) and the time to peak (TTP; time in minutes to maximal thrombin generation), whereas quantitative parameters include the peak thrombin generation ("peak" or "peak height"; which is the maximal amount of thrombin generated, in nanomolar), as well as the endogenous thrombin potential (ETP); the area under the thrombin generation curve, in nM·min). The mean velocity rate index (mVRI) is a composite parameter whereby peak thrombin generation is divided by the time difference between TTP and LT ([peak/(TTP-LT)].

The LT might best reflect coagulation initiation, whereas peak thrombin generation and the mVRI capture the processes involved in coagulation propagation.[31,32] The ETP is the most frequently reported TGA parameter because it is perceived to be a comprehensive measure of coagulation and provides information on what cannot otherwise be quantified using conventional coagulation testing.[24,33]

Hypercoagulability and thrombotic complications have been linked most consistently to a higher peak thrombin generation and high ETP.[34–37] In contrast, low peak, ETP, and mVRI values have been associated with bleeding in various contexts.[38–42] A delayed LT might be the most conceptually relevant measure for anticoagulation-associated bleeding.[31,43]

IC50 values and related "doubling concentration" metrics are frequently reported in the anticoagulant pharmacology and thrombin generation literature as standardized summary measures of concentration-effect relationships under defined experimental conditions.[32,44–50] In this context, an IC50 refers to the drug concentration that produces a 50% reduction in a specified quantitative TGA parameter (eg, peak thrombin generation, ETP, or mVRI) relative to a drug-free control within the same assay conditions; for kinetic parameters (eg, LT or TTP), investigators commonly report the concentration that doubles the parameter (eg, 2 × LT or 2 × TTP) as an analogous summary metric. These measures are useful because they provide a standardized benchmark for effect, enabling comparability of relative pharmacodynamic potency across anticoagulant classes even when drugs produce different TGA signatures (eg, preferential effects on kinetic vs quantitative parameters). However, IC50/doubling concentrations are assay-dependent (TF trigger concentration, sample matrix, reagents, and platform) and therefore should not be interpreted as clinical therapeutic targets or dosing thresholds. This assay dependence is particularly relevant when comparing PPP with platelet-containing matrices (PRP/whole blood), because platelets provide a procoagulant phospholipid surface and release platelet granule contents (eg, FVa and polyphosphates) that amplify thrombin generation.[51,52] In addition, platelet activation releases platelet factor 4, which can bind/neutralize heparins and may attenuate apparent UFH effects in platelet-containing systems.[53] Accordingly, we use IC50 values (and related doubling concentrations) here primarily as comparative, mechanistically informative metrics to summarize relative anticoagulant potency on specific TGA parameters, recognizing that no single parameter fully captures effects across all anticoagulant classes.

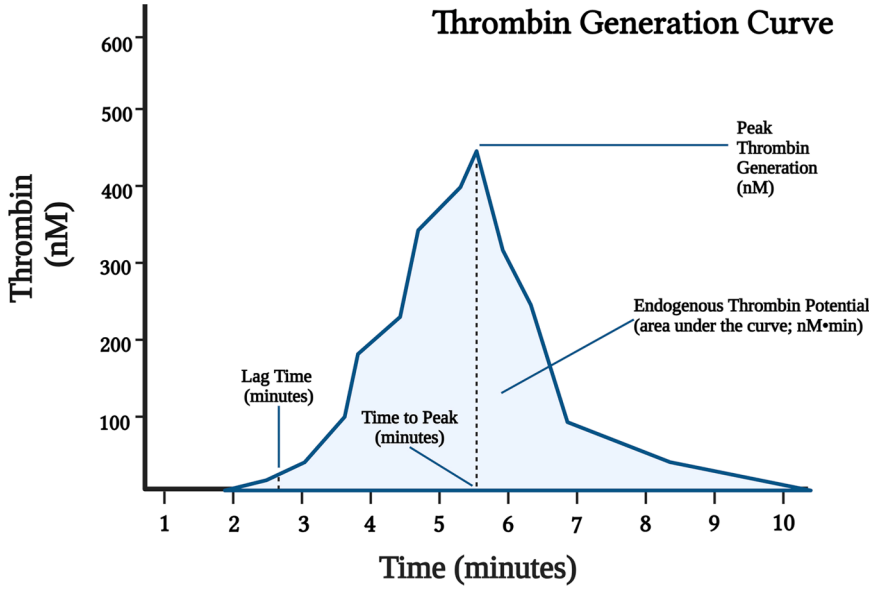

Fig. 1. The thrombin generation curve. Illustrative representation of the thrombin generation curve measured using calibrated automated thrombography with 5 pM TF as the trigger for coagulation initiation.

III. Oral anticoagulants and effects on thrombin generation

A recent review of the direct oral anticoagulants (DOACs) highlighted consistent class-specific effects on the thrombin generation curve.[33] On-treatment factor Xa inhibitor (FXaI) levels tend to produce a flat and protracted thrombin generation curve, with prolongation of kinetic parameters, suppression of peak thrombin generation, but a preserved ETP (Fig. 2).[44,45,47] Oral DTIs delay TGA kinetic parameters, with little effect on peak, ETP, and the mVRI at lower concentrations.[54] The result is a sharp, right-shifted TGA curve.[55] In contrast, vitamin K antagonists (VKAs, eg, warfarin) produce substantial, international normalized ratio-proportional suppression of the ETP.[56–59] Whereas VKAs suppress the ETP to a greater extent than the DOACs, the novel oral FXIa inhibitors (eg, asundexian and milvexian) are less able to suppress TF-triggered thrombin generation than the DOACs, and their effects are mostly measurable using contact activation or very low TF trigger concentrations (eg, <1 pM).[60–62]

Accumulating evidence stemming from translational research indicates that DOACs might confer an inadequate anticoagulant effect in some settings when compared with VKAs or UFH/LMWHs, as measured using thrombin generation. This seems to be particularly the case under highly prothrombotic conditions. DOACs cannot suppress thrombin generation triggered using mechanical heart valve material or catheter segments in vitro,[63–66] or in patients with antiphospholipid antibodies.[36] Hardy et al[67] recently demonstrated that heparins were by far the most effective anticoagulants in an in vitro model of catheter-initiated thrombin generation in PRP. Factor XI(a) and XIIa inhibitors did not adequately prevent coagulation on the studied artificial surface. Likewise, DOACs and fondaparinux showed limited efficacy. Orfeo et al[68] used thrombin generation and simulation analyses to demonstrate that fondaparinux, rivaroxaban, and dabigatran "were unable to recapitulate all features of warfarin anticoagulation when defined in terms of the pattern of the propagation phase of thrombin formation."[69] This observation has been proposed as one potential explanation for why DOACs have consistently been associated with a lower risk of major bleeding than warfarin.[70] This translational research is backed by growing clinical data suggesting a potentially inferior antithrombotic effect of the DOACs relative to VKAs in patients with mechanical heart valves,[71,72] antiphospholipid antibody syndrome,[73–75] valvular AF,[76] left ventricular thrombi,[77] or after thromboendarterectomy.[78,79]

IV. Literature review

Parenteral anticoagulants have a narrow therapeutic window. Patients receiving parenteral anticoagulation are at high risk of not only bleeding but also breakthrough thrombosis. This justifies the need to thoroughly understand their pharmacodynamic effects on hemostasis. To fill this important knowledge gap, we reviewed the literature to define the role of thrombin generation as a pharmacodynamic measure of parenteral anticoagulation and its reversal. We also aimed to contextualize the clinical relevance of thrombin generation in light of studies focused on the clinical antithrombotic efficacy and bleeding risk of parenteral anticoagulants in critical care and perioperative settings.

Our search strategy was established with the assistance of a medical library information specialist (Risa Shorr, The Ottawa Hospital) using keywords and MeSH terms detailing the population, concept, and context of interest (Supplemental Appendix). Embase and MEDLINE were searched from inception to October 9, 2024. English language articles that reported the use of a fluorometric or chromogenic TGA to measure the effects of a parenteral anticoagulant on an ex vivo (regardless of indication) or in vitro basis were eligible for inclusion. In this review, we use in vitro to describe studies where the anticoagulant was added to plasma or whole blood outside the body (ie, "spiking" experiments), whereas ex vivo refers to studies where anticoagulants were administered to participants and thrombin generation was subsequently measured in samples obtained during treatment. Parenteral anticoagulants that were considered by our search strategy included UFH, LMWH (enoxaparin, dalteparin, tinzaparin, semuloparin, bemiparin, certoparin, parnaparin, reviparin, and nadroparin), danaparoid, synthetic pentasaccharides (fondaparinux and idraparinux), argatroban, bivalirudin, and hirudin/lepirudin. Abstracts

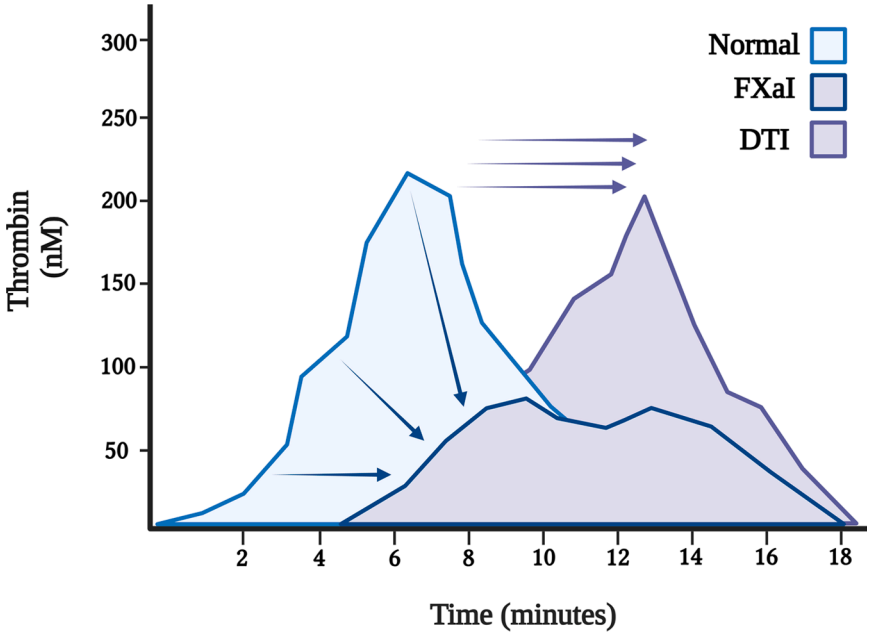

Fig. 2. Thrombin generation and class-specific DOAC effects. Illustrative representation of changes to the thrombin generation curve induced by both direct FXaIs (eg, apixaban) and DTIs (eg, dabigatran) at typical on-treatment concentrations (~200 ng/mL), as measured in PPP using calibrated automated thrombography and a 5 pM TF trigger.[44–47]

were reviewed for potential inclusion by the lead author (J.R.S.). Eligible articles were supplemented by additional relevant publications as suggested by project coauthors.

Our search strategy identified 619 abstracts for review (Supplemental Fig. 1), of which 159 underwent full-text review. Given the volume of literature identified, we focused on articles whose primary objective was to evaluate the pharmacokinetics, pharmacodynamics, or reversal of parenteral anticoagulants using TGA parameters, and on patient populations most directly relevant to critical care and perioperative medicine (cardiac surgery, dialysis, HIT, general intensive care unit population, and ACS). We excluded studies primarily focused on antiphospholipid antibody syndrome, cancer, COVID-19, hemolysis, liver disease, prothrombin complex concentrate (PCC) heparin content, pediatrics, pregnancy, and VTE prophylaxis/treatment, because these areas represent substantial additional subdomains where thrombin generation was often not the primary pharmacodynamic endpoint, and full inclusion would have broadened the scope beyond a feasible synthesis (Supplemental Fig. 1).

After restricting included articles to the topics listed above, 114 articles were included in the review (Supplemental Appendix), and data were abstracted from all 114 articles.[15,16,24,32,39,40,48–50,65,66,68,80–181] All 114 articles informed the review; 70 were highlighted by the lead author (J.R.S.) for narrative synthesis because they contained methodologically rigorous comparisons, key mechanistic findings, or insightful interpretation. These included 35 articles on anticoagulant pharmacokinetics/pharmacodynamics,[24,32,48–50,65,80,83,84,86,91–93,95,97–101,104,106,108,115,116,121,124–131,133] 10 on cardiac surgery,[39,40,154,158,160,161,163–166] 2 on dialysis,[170,171] 2 on HIT,[173,174] 1 on intensive care unit patients,[175] 1 in silico study,[68] 4 on TGA methodology,[145–147,149] 4 on myocardial infarction,[176–179] and 11 on the reversal of parenteral anticoagulants.[15,16,136–144]

Out of the 114 articles included in the review, 76 used an in vitro experimental design, 19 were prospective cohort studies, 8 used a randomized interventional design, 7 used a randomized crossover design, 2 were cross-sectional studies, and 2 used in silico models. Full details of the search strategy, study selection, and TGA methodology (Supplemental Fig. 2) are reported in the Supplemental Appendix.

V. Parenteral anticoagulants and effects on thrombin generation

Among the 114 studies included in this review, 68 reported the effects of UFH on TGA parameters (59.6%), 49 on LMWH (43.0%), 24 on fondaparinux (21.1%), 23 on hirudin (20.2%), 12 on argatroban (10.5%), and 6 on bivalirudin (5.3%) (Supplemental Fig. 3). Unless otherwise specified, UFH and LMWH therapeutic ranges cited throughout refer to calibrated anti-Xa activity (IU/mL) as measured by anti-Xa assays (rather than protamine titration or other heparin quantification methods).

A. Unfractionated heparin

In vitro UFH experiments typically evaluated anti-Xa activity ranges of ~0.1 to 1.0 IU/mL[32,98,101,106,124]; 3 studies assessed concentrations > 1.0 IU/mL.[49,88,92] Among studies reporting numerical values for TGA parameters, all reported that UFH produced a dose-dependent prolongation of TGA kinetic parameters and suppression of quantitative TGA parameters (Fig. 3). Most studies reported profound effects on both kinetic and quantitative TGA parameters at very low UFH anti-Xa levels (<0.3–0.5 IU/mL), irrespective of the activator or TF concentration used to trigger thrombin generation, or whether the study was conducted on an in vitro or ex vivo basis.[49,65,91,92,95,101,106,124,130,142,149] Seven studies found that UFH concentrations within the typical therapeutic range (0.3–0.7 IU/mL) completely abolished thrombin generation, whether measured in vitro[49,65,95,101,124,142] or ex vivo.[130]

Three studies using PPP and TF trigger concentrations of ~1 to 5 pM reported UFH IC50 values ranging from 0.03 to 0.06 IU/mL for peak, 0.04 to 0.05 IU/mL for ETP, whereas doubling concentrations ranged from 0.01 to 0.02 IU/mL for the LT (2 × LT)] and 0.04 to 0.05 IU/mL for the TTP (2 × TTP).[49,146,181] Gerotziafas et al[32] used PRP and a TF trigger concentration of 6 pM and reported IC50 values of 0.30 IU/mL for the ETP, 0.25 IU/mL for peak, 0.10 IU/mL for the mVRI, and a doubling concentration of 0.10 IU/mL for both the LT and the TTP. Vermeiren et al[92] reported high in vitro correlation coefficients of |>0.90| for the relationship between UFH anti-Xa values and LT, TTP, peak, and ETP.

B. Low-molecular-weight heparin

Nine in vitro LMWH experiments evaluated anti-Xa activity levels ranging from 0.1 to 1.0 IU/mL[32,82,83,95,96,104,111,112,124]; 6 studies assessed concentrations from > ~1.0 to 2.0 IU/mL.[48,66,86,88,92,108] Some studies focused exclusively on a single LMWH (eg, enoxaparin and dalteparin)[83,93,97], whereas others compared the impact of LMWH type and chain length on TGA parameters (eg, enoxaparin vs tinzaparin).[32,86,124,149] Similar to UFH, all studies providing numerical values for TGA parameters reported a measurable anticoagulant effect on both kinetic and quantitative TGA parameters, with prolongation of the LT and TTP, and suppression of peak thrombin generation, ETP, and mVRI (Fig. 3). Studies comparing the effects of UFH versus LMWH at similar anti-Xa activity levels generally found that UFH was more

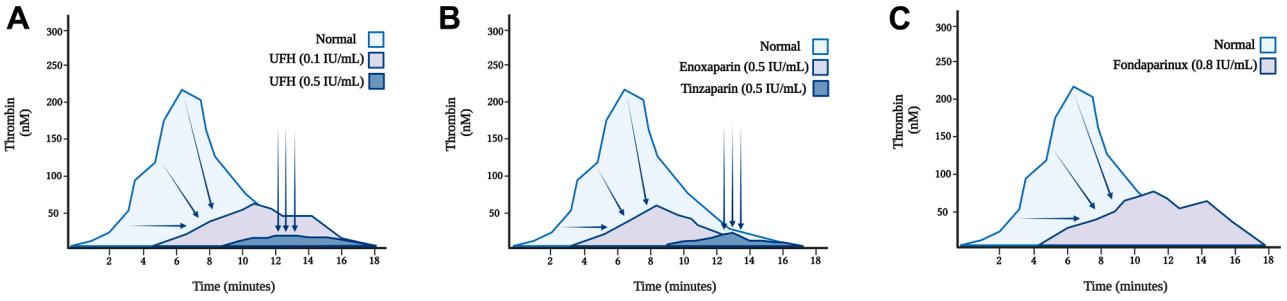

Fig. 3. Thrombin generation and class-specific UFH/LMWH/fondaparinux effects. Illustrative representation of changes to the thrombin generation curve induced by UFH, LMWH, or fondaparinux, as measured in PPP using calibrated automated thrombography and a 5 pM TF trigger. (A). Effects of increasing concentrations of UFH (anti-Xa 0.1 and 0.5 IU/mL; typical therapeutic range is 0.3–0.7 IU/mL).[88,101,102,106,111,124,130,181] (B) Effects of LMWH (enoxaparin vs tinzaparin, anti-Xa 0.5 IU/mL; typical target range is 0.5–1.0 IU/mL).[32,83,88,108,124,130,142,146,181] (C) Effects of fondaparinux (anti-Xa 0.8 IU/mL; typical target range is 0.8–1.2 IU/mL).[32,108,137]

effective at suppressing peak thrombin generation and the ETP as compared with LMWH.[32,88,92,93,124,149,181] Studies evaluating LMWH effects on thrombin generation based on chain length consistently reported that LMWHs with a longer average chain length (eg, tinzaparin) showed greater suppression of thrombin generation as compared with shorter chain length LMWHs (eg, enoxaparin) when compared at similar anti-Xa activity levels.[32,86,124,142,146,149] Tinzaparin was the most inhibitory LMWH and showed a similar effect on thrombin generation to that of UFH.[32,124] On the other hand, studies comparing the efficacy of LMWH to that of the DOACs found that LMWH was generally more effective at suppressing thrombin generation than FXaIs or DTIs.[83] Vermeiren et al[92] reported high correlation coefficients of |>0.90| for the relationship between LMWH anti-Xa values and LT, TTP, peak, and ETP, whereas ex vivo correlation measurements were lower and more variable, ranging from ~0.3 to 0.5 for LT and TTP, and ~0.5 to 0.7 for peak and ETP.[92,126,127]

IC50 values for LMWH measured using PPP ranged from 0.09 to 0.11 IU/mL for peak, and 0.10 to 0.16 IU/mL for ETP, while doubling concentrations for the LT (2 × LT) and for TTP (2 × TTP) both ranged from 0.07 to 0.09 IU/mL.[142,146,181] Gerotziafas et al[32] compared the IC50 values for various chain length LMWHs using PRP and a TF trigger concentration of 6 pM. For tinzaparin and enoxaparin, respectively, IC50 values were 0.25 IU/mL versus 0.45 IU/mL for peak, 0.35 IU/mL versus 0.55 IU/mL for ETP, 0.20 IU/mL versus 0.40 IU/mL for the mVRI, and doubling concentrations of 0.35 IU/mL versus 0.60 IU/mL for the LT and 0.28 IU/mL versus 0.60 IU/mL for the TTP.

C. Fondaparinux

Most in vitro experiments evaluated fondaparinux concentrations ranging from 0 to 3.0 μg/mL,[95,108,124,137,142,143] or 0 to 1.0 IU/mL anti-Xa units.[32,83,91] These studies demonstrated that fondaparinux was able to delay both the LT and TTP, and suppress peak, ETP, and the mVRI (Fig. 3).[32,50,83,91,96,97,104,108,124,137,141,143,176] Studies comparing the effects of on-treatment levels of UFH (anti-Xa 0.3–0.7 IU/mL) or LMWH (anti-Xa 0.5–1.0 IU/mL) to fondaparinux (~1.0 μg/mL, ~0.8–1.2 IU/mL) generally reported that fondaparinux had the least impact on both kinetic and quantitative parameters.[32,83,91,95,96,104,108,124] Salta et al[83] found that tinzaparin was better able to suppress thrombin generation than fondaparinux. Both Gerotziafas et al[108] and Hacquard et al[124] reported that fondaparinux showed a plateau effect on the ETP at concentrations > 1.0 IU/mL or > 1.0 μg/mL, respectively, whereas enoxaparin completely abolished thrombin generation at anti-Xa values > 1.0 IU/mL.

Gerotziafas et al[32] reported that the ETP IC50 value using PRP for fondaparinux (0.74 ± 0.05 IU/mL) was ~2-fold higher than UFH (0.30 ± 0.05 IU/mL) or tinzaparin (0.35 ± 0.02 IU/mL), but the mVRI IC50 value for fondaparinux (0.093 IU/mL) was significantly lower than for LMWHs (~0.2–0.7 IU/mL). Fondaparinux appears to induce similar effects on the thrombin generation curve as rivaroxaban or edoxaban, with an outsized effect on the velocity of thrombin generation (mVRI), but a relatively preserved ETP.[32,50,83]

D. Argatroban, bivalirudin, and hirudin

In vitro studies evaluating argatroban used concentrations ranging from 0 to 1.0 μg/mL, or 0 to 6 μM.[80,97,99,100,106,110,114,146] Argatroban prolongs the LT and TTP, and suppresses peak thrombin generation, ETP, and the mVRI (Fig. 4).[48,80,97,99,106,107,110,114] Studies comparing the effects of argatroban to other anticoagulants found that it induced changes to the thrombin generation curve that mirrored dabigatran, with a more pronounced effect on kinetic parameters at lower concentrations, and greater suppression of peak and ETP at higher concentrations.[48,80,97,106,110,114] At equimolar concentrations, argatroban had less effect on peak and ETP than direct FXaIs[80,97] or UFH/LMWH.[48,97,106,110]

In vitro spiking experiments tested bivalirudin concentrations ranging from ~0 to 30 μg/mL or ~0.3 to 10.0 μM,[49,84,100,101,103] whereas experiments evaluating hirudin tested concentrations ranging from 0 to 1.5 μg/mL or 0 to 250 nM.[48,49,89,95,97,99,100,104,111,112,114,116,119,121] Xu et al[49] defined target concentrations for bivalirudin and hirudin of 2.8 to 7.3 μM and 80 to 200 nM, respectively. Compared with argatroban, bivalirudin had a marked effect on kinetic parameters, with little impact on quantitative parameters, leading to substantial prolongations of the LT and TTP without any impact on peak or ETP at low concentrations (Fig. 4).[49,84,100,101] Compared with bivalirudin, hirudin had an even more pronounced and isolated effect on the kinetics of thrombin generation. Hirudin caused dramatic prolongations in the LT and TTP without any clear impact on peak or ETP.[48,49,89,95,97,99,100,104,109,111,112,114,116,121] Xu et al[49] reported LT doubling concentrations of 0.53 μM for bivalirudin and 20 nM for hirudin, whereas Samama et al[48] measured hirudin LT and TTP doubling concentrations of 55 nM and 95 nM, respectively.

Ten studies reported that low concentrations of either bivalirudin or hirudin induced a paradoxical increase in peak thrombin generation, ETP, or the mVRI.[48,49,84,89,95,97,99,101,103] This phenomenon was less commonly reported for argatroban.[100]

E. An integrated summary of class-specific anticoagulant effect patterns

Across the included studies, all parenteral anticoagulants produced a measurable anticoagulant effect on thrombin generation, with the magnitude and parameter profile varying by drug class and exposure concentration. Under commonly used TF-initiated conditions, UFH and longer-chain LMWHs (particularly tinzaparin) consistently produced the most pronounced suppression of quantitative TGA parameters (including peak thrombin generation and the ETP), and in several reports, UFH abolished thrombin generation within standard therapeutic anti-Xa ranges. Shorter-chain LMWHs (eg, enoxaparin) remained strong inhibitors but generally showed comparatively less inhibition of total thrombin generation than UFH or tinzaparin at similar anti-Xa activity. Fondaparinux typically had smaller effects on ETP than heparin-based agents, and some studies reported a plateau effect on ETP at higher concentrations. Parenteral DTIs predominantly prolonged kinetic parameters; argatroban demonstrated more concentration-dependent reductions in peak and ETP at higher concentrations, whereas bivalirudin and hirudin more often produced a marked right-shift of the thrombin generation curve with comparatively limited effects on ETP at lower exposures. Where assessed, in vitro dose-response correlations were generally stronger than correlations observed in treated patients. Mechanistic determinants of these class-specific patterns are discussed below.

VI. Mechanistic determinants of thrombin generation

A. Pharmacodynamics of unfractionated heparin, low-molecular-weight heparin, and fondaparinux

UFH and LMWHs bind to antithrombin, inducing a conformational change and increasing antithrombin's affinity for both thrombin (FIIa) and FXa by ~1000-fold (Fig. 5).[19,182,183] Through their effects on antithrombin, both UFH and LMWHs exert an indirect multitargeted anticoagulant effect with inhibition of FIIa

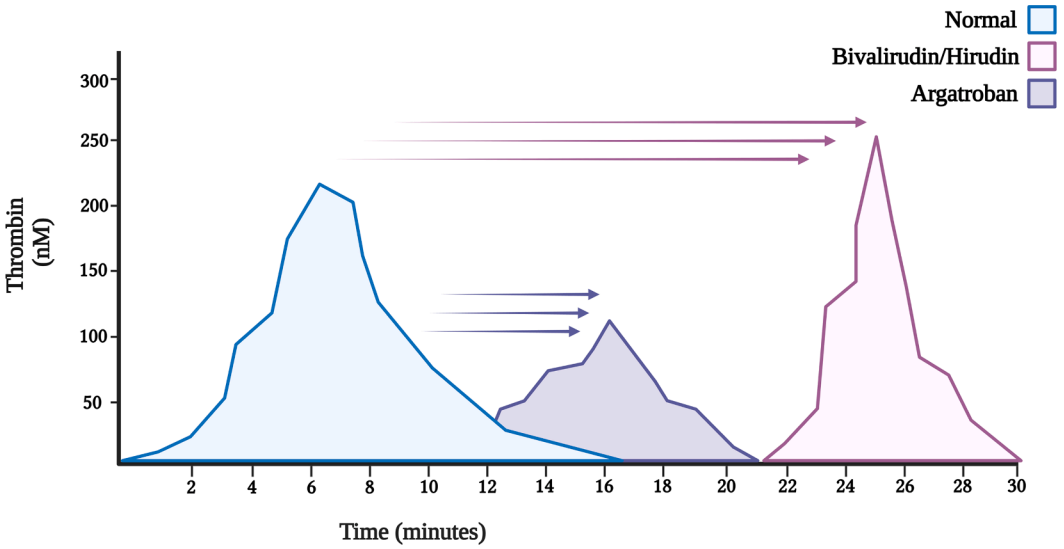

Fig. 4. Thrombin generation and class-specific parenteral direct thrombin inhibitor effects. Illustrative representation of changes to the thrombin generation curve induced by argatroban[80,97,99,106] and bivalirudin/hirudin,[48,49,84,89,101] as measured in PPP using calibrated automated thrombography and a 5 pM TF trigger.

and FXa, as well as FIXa, FXIa, FXIIa, and the extrinsic tenase complex TF·FVIIa.[68,93,184,185] In fact, Tanaka et al[106] demonstrated that, unlike argatroban, which suppresses thrombin generation independently of antithrombin, UFH's inhibitory effects are markedly reduced in the presence of antithrombin deficiency.

LMWH is made by depolymerizing UFH to varying degrees. Average LMWH chain length varies by type of LMWH (Table 1). The pharmacodynamic properties of the LMWH in question are determined by LMWH chain length. Tinzaparin has a longer average chain length, and enoxaparin/dalteparin/nadroparin/semuloparin have shorter average chain lengths. Shorter chain-length LMWHs are more dependent on renal elimination (eg, enoxaparin), whereas longer chain-length LMWHs display greater dependence on reticuloendothelial clearance (eg, tinzaparin). Fondaparinux, in contrast, is a synthetic pentasaccharide modeled after the key pentasaccharide moiety of heparin. It is an indirect antithrombin-mediated and selective inhibitor of FXa.

Antithrombin-mediated thrombin inhibition requires a heparin chain length of at least 18 saccharide units. Because of its shorter chain length, LMWH has less anti-IIa activity than UFH, and greater anti-Xa activity. Although the anticoagulant effects of UFH and LMWH are primarily mediated by inhibition of IIa and Xa, respectively, data support that the effects of both drugs on thrombin generation are determined by their anti-IIa activity.[32,67,130] UFH is better able to suppress thrombin generation than LMWH at equivalent anti-Xa concentrations.[32,92] When thrombin generation was measured using PRP and comparisons made at equivalent anti-Xa activity levels, UFH had the greatest impact on thrombin generation, followed by tinzaparin, then enoxaparin and dalteparin.[32] UFH/LMWH-induced ETP suppression and LT prolongation were mainly determined by anti-IIa activity, whereas associated anti-Xa activity results in suppression of coagulation propagation and the mVRI.[32,130] Shorter chain LMWHs with greater anti-Xa activity and fondaparinux appear to exert an effect on thrombin generation distinct from UFH; coagulation propagation is suppressed, and the mVRI is reduced, with greater preservation of the ETP, similar to what is observed with oral FXaIs (eg, rivaroxaban). In vivo, these pharmacodynamic differences might be mitigated, at least in part, by the release of platelet factor 4 from activated platelets, which preferentially binds and neutralizes longer-chain heparins (eg, UFH and tinzaparin).[32,133] This phenomenon might attenuate some of the differences seen with respect to suppression of thrombin generation. On the other hand, similar findings were observed in studies using PRP.[32,83]

In addition to antithrombin-mediated inhibition of FIXa, direct inhibition of FIXa may play an important role in determining both UFH's and LMWH's effects on thrombin generation.[93,186] UFH exerts a direct effect on the intrinsic tenase complex (FVIIIa·FIXa), and inhibits FIXa in an antithrombin-independent fashion at subtherapeutic concentrations (anti-Xa < 0.2 IU/mL).[186] UFH also binds to an exosite on FIXa and interferes with the formation of the tenase complex (FIXa·FVIIIa).[185,186] Similarly, LMWH might partially exert its antithrombotic effects through an interaction with a heparin-binding exosite on FIXa, leading to antithrombin-independent inhibition of the intrinsic tenase complex.[93] Coagulation amplification and propagation through the intrinsic tenase complex are key determinants of peak thrombin generation, the ETP, and the mVRI.[187–189] It follows that UFH and LMWH's multi-targeted mechanism of action and effects on coagulation initiation, amplification, and propagation might explain their greater ability to suppress the ETP at therapeutic concentration ranges as compared with targeted inhibitors such as the oral FXaIs or fondaparinux (Fig. 5).[32,50,83]

UFH and LMWH trigger the endothelial release of tissue-factor pathway inhibitor (TFPI)[190,191] an important physiological anticoagulant. TFPI is an endogenous inhibitor of FXa and a major inhibitor of the TF·FVIIa extrinsic tenase complex, inhibiting the early phases of the procoagulant response.[192] TFPI might be an important contributor to the in vivo anticoagulant pharmacodynamic effects of both UFH and LMWH.[98,131,193,194] Brodin et al[98] showed that administering a UFH bolus to healthy volunteers promoted a 10-fold increase in TFPI levels. Increasing concentrations of TFPI modulated the antithrombotic effects of low levels of UFH (0.1 IU/mL) by amplifying its effects on LT prolongation and suppression of the ETP.[98]

B. Pharmacodynamics of argatroban, bivalirudin, and hirudin

Argatroban is a synthetic monovalent small molecule (~509 Da) parenteral DTI that reversibly binds to thrombin's active site and has a short half-life of <1 hour (Fig. 6; Table 1).[195] Hirudin is a polypeptide and irreversible inhibitor of thrombin, with a high

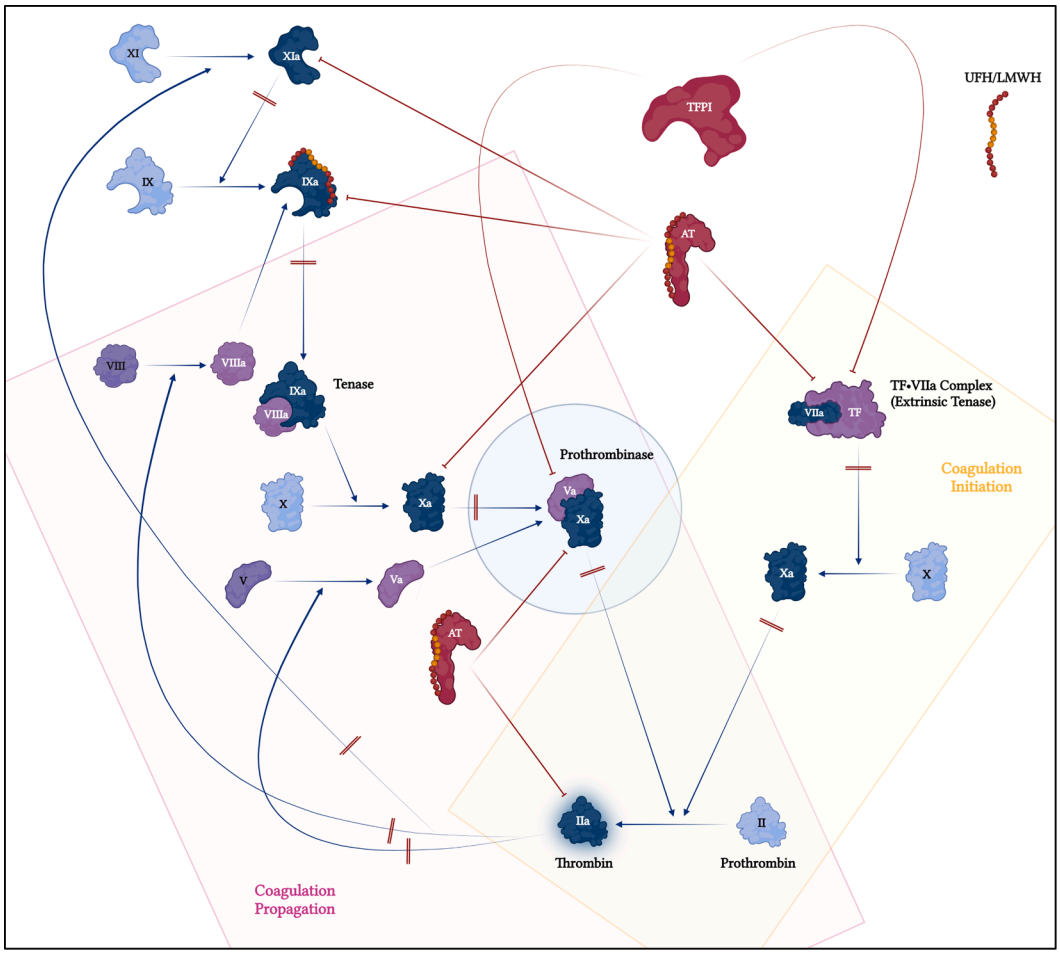

Fig. 5. Effects of UFH/LMWH on coagulation initiation and propagation. UFH and LMWH both have antithrombin-dependent and antithrombin-independent broad-based effects on factors involved in both coagulation initiation and propagation.[7,10,68,93] Heparins bind to an exosite on FIXa and interfere with formation of the tenase complex (FIXa·FVIIIa).[185,186] Heparins also induce the endothelial release of TFPI, which is an important inhibitor of early forms of the prothrombinase complex and the extrinsic tenase complex.[190,191] The result is profound suppression of both kinetic and quantitative parameters of thrombin generation (LT, TTP, peak, ETP, and mVRI) at relatively low anti-Xa activity levels (<0.3 IU/mL). AT, antithrombin.

affinity (inhibition constant in the picomolar range; Ki = ~0.1 pM).[5] Bivalirudin is a synthetic oligopeptide analog of hirudin. Whereas hirudin is renally excreted and argatroban is eliminated by hepatobiliary clearance, bivalirudin undergoes predominantly proteolytic cleavage (~80%), with the remainder by renal elimination (~20%).

Hirudin and bivalirudin are bivalent thrombin inhibitors, binding to thrombin's active site and exosite 1. Bivalirudin differs from hirudin in that it is less potent and is a functionally reversible thrombin inhibitor, with a binding affinity (Ki = 2 nM) that is intermediate between that of hirudin (Ki = 0.1 pM), dabigatran (Ki = 4.5 nM), and argatroban (Ki = 40 nm).[6,196] Thrombin's exosite 1 mediates binding to fibrinogen, FXI, FXIII, and thrombomodulin.[49,197–200] It also mediates important interactions with FV and FVIII[200]; thrombin-mediated activation of FV and FVIII plays a crucial role in the feedback amplification and propagation phases of coagulation.

The contrasting TGA patterns observed for argatroban (and dabigatran) versus bivalirudin and hirudin (Figs. 2 and 4) might be partly attributable to their respective pharmacodynamic effects on coagulation initiation and propagation. The monovalent DTIs argatroban and dabigatran induce progressive prolongation of TGA kinetic parameters at lower concentrations, but eventually reduce both peak thrombin generation and the ETP at higher concentrations.[54,80,97,99,106] This contrasts with hirudin and bivalirudin's significant prolongation of the LT and TTP without affecting TGA quantitative parameters (Fig. 4).[49,99,101]

Lindhout et al[121] evaluated hirudin's mechanism of action by monitoring prothrombin consumption and the sequential generation of activated clotting factors (thrombin, FXa, FVIIIa, and FVa) using chromogenic measurements in the absence and presence of hirudin. They demonstrated that the dramatic prolongation of thrombin generation induced by hirudin occurs because hirudin blocks thrombin-mediated feedback activation of FV and FVIII (coagulation amplification), thereby delaying formation of the intrinsic tenase complex (FIXa·FVIIIa), the prothrombinase complex (FXa·FVa), and the onset of the propagation phase of coagulation. Once all hirudin is bound to generated thrombin, trace amounts of thrombin result in an "escape" event, leading to thrombin-mediated feedback activation of FV, FVIII, and sequential FX activation, coagulation propagation, and ensuing explosive thrombin generation. The result is a profound delay to an otherwise sharp TGA curve.[49,99] This same pattern is seen with bivalirudin.[49,101]

These findings might explain why anticoagulants that selectively target thrombin exert a greater effect on kinetic TGA parameters as compared with FXaIs or multitargeted anticoagulants (eg, UFH, LMWHs, and VKAs). Monovalent and bivalent DTIs may primarily function by delaying thrombin-mediated coagulation

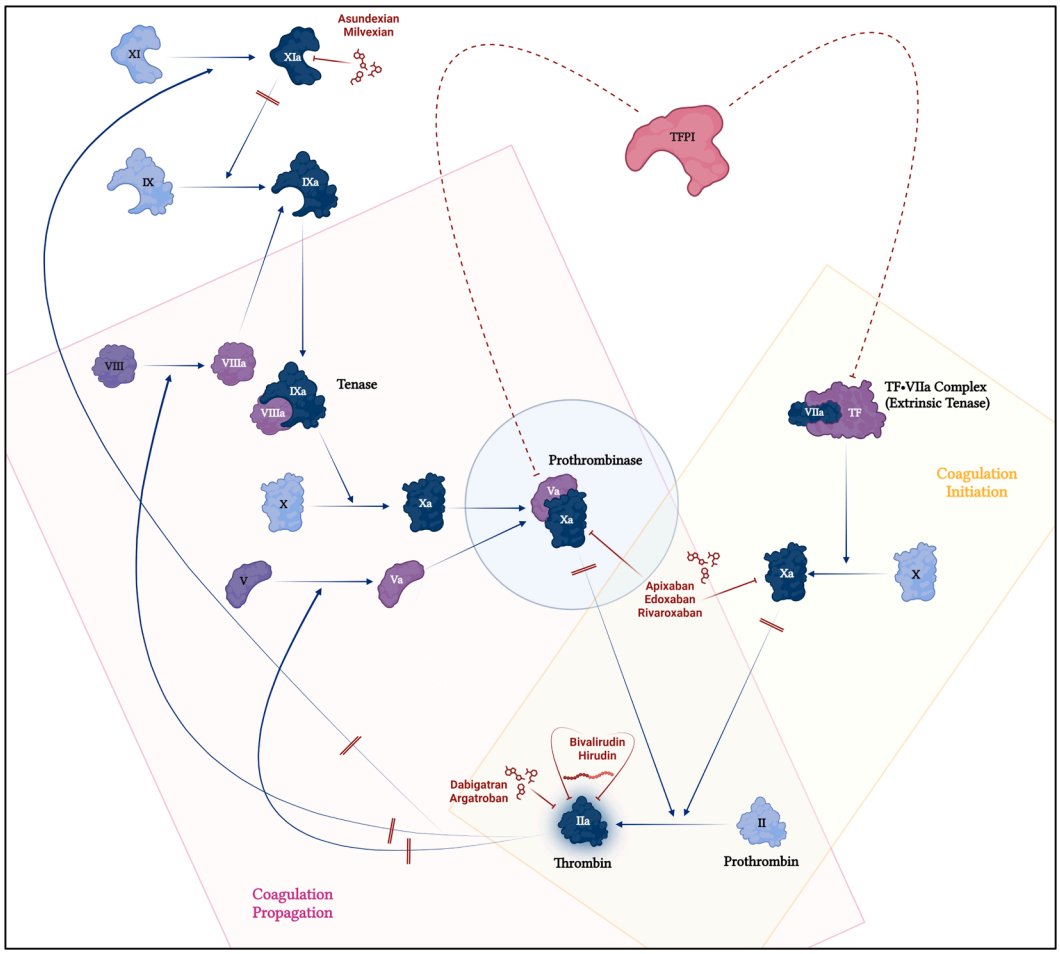

Fig. 6. Effects of DTIs, FXaIs, and FXIa inhibitors on coagulation initiation and propagation. DTIs selectively inhibit the effects of thrombin on both coagulation initiation and propagation. Given thrombin's key role as an initiator of coagulation, DTIs primarily delay the onset of thrombin generation and create a block between coagulation initiation and propagation.[45,49,80,97,99,106] The result is a right-shifted but sharp TGA curve with delayed LT and TTP.[33] These effects are more pronounced for the bivalent DTIs (bivalirudin and hirudin) as compared with monovalent DTIs (argatroban and dabigatran). FXaIs (apixaban, edoxaban, and rivaroxaban) selectively block the action of trace amounts of FXa generated during coagulation initiation by the extrinsic tenase complex (TF·FVIIa), as well as the action of the prothrombinase complex (FXa·FVa). The result is a delay in coagulation initiation and suppression of coagulation propagation, with a flat and protracted thrombin generation curve.[33,55] FXIa inhibitors (asundexian and milvexian) prevent FXIa-mediated activation of coagulation. FXIa inhibitors inhibit contact-activated thrombin generation, but do not affect TF-triggered thrombin generation.[60]

initiation and subsequent propagation (ie, "thrombin retardants"). The differing TGA patterns observed for dabigatran/argatroban versus bivalirudin/hirudin (Figs. 2 and 4) might relate to bivalirudin and hirudin's bivalent mechanism of action (targeting both thrombin's active site and exosite I). Bivalent inhibition of thrombin's active site and exosite I might lead to a complete shutdown of thrombin activity. Blocking thrombin's exosite I function interferes with its feedback activation of FV and FVIII, and, by extension, delays coagulation propagation.[121] Hirudin's irreversible binding and greater binding affinity may exaggerate these effects relative to bivalirudin.

In contrast, prothrombinase's key positioning in the coagulation cascade as the effector of coagulation propagation might best explain the greater impact of FXa inhibition on the peak and velocity of thrombin generation (Figs. 5 and 6).[50,104,108] Indirect anticoagulants affecting multiple coagulation factors, such as UFH/LMWHs or VKAs, might provide greater suppression of peak thrombin generation, the mVRI, and the ETP because they simultaneously interfere with the actions of multiple coagulation factors needed for coagulation amplification and propagation (FII, FIX, and FX).[68,93] In support of this theory, several groups have reported that combined inhibition of thrombin and FXa produces a synergistic impact on thrombin generation that exceeds the expected additive effect of selectively inhibiting either factor.[64,83,105] Jaffer et al[64] hypothesized that this finding might explain why DOACs fail to prevent mechanical heart valve-induced thrombosis.

Several groups have reported that low concentrations of either bivalirudin or hirudin induced a paradoxical increase in peak thrombin generation, ETP, or the mVRI.[48,49,84,89,95,97,99,101,103] These findings were observed irrespective of the TGA platform or TF concentration,[49,84,95,97,99–101,103] and whether or not testing was performed with added thrombomodulin.[95] Several groups have attributed these findings to an artifactual phenomenon arising from DTI inhibition of the thrombin-based calibrator used in many TGA platforms, including calibrated automated thrombography, as well as interference with the algorithms used to subtract α2-macroglobulin-thrombin activity from total measured amidolytic activity during TGA.[201,202] Others have suggested that these findings represent de facto hypercoagulability, mediated by DTI-induced suppression of the thrombin-thrombomodulin negative feedback system and impaired protein C activation. In fact, some studies have found that these paradoxical increases in peak thrombin generation and the ETP are exaggerated by the addition of recombinant human soluble thrombomodulin to the TGA

reaction system.[87,95] Interestingly, similar paradoxical findings were also reported using in silico simulation,[203] which would not be subject to the same artifactual limitations as in vitro systems using a thrombin-based calibrator. The etiology and clinical significance of these findings remain to be understood.

C. A mechanistic synthesis of anticoagulants and thrombin generation impact patterns

Taken together, the thrombin generation signature conferred by different parenteral anticoagulants appears to be determined by (1) breadth and mode of inhibition (eg, multitargeted vs selective inhibition, monovalent vs bivalent thrombin inhibition) and (2) which phases of coagulation are predominantly affected (initiation vs propagation). The LT most clearly reflects the impact of anticoagulation on coagulation initiation, whereas effects on coagulation propagation are best captured by peak thrombin generation and the mVRI. ETP reflects the integrated area under the thrombin generation curve, but it does not capture the full time-dependent shape of thrombin generation. Thus, 2 samples may have similar ETP values yet differ substantially in kinetics (eg, LT/TTP) or propagation features (peak/mVRI), supporting parallel reporting of kinetic and quantitative parameters when comparing anticoagulant effects. UFH and longer-chain LMWHs (eg, tinzaparin) exert broad, multitargeted anticoagulant effects, including antithrombin-dependent inhibition of thrombin and FXa, antithrombin-independent interference with intrinsic tenase-dependent coagulation propagation (including effects on FIXa and on the formation/function of the FVIIIa·FIXa complex), as well as indirect effects such as heparin-mediated endothelial release of TFPI. As a result, UFH and longer-chain LMWHs (eg, tinzaparin) typically both delay the onset of thrombin generation and produce deep suppression of quantitative TGA parameters, with proportionally larger reductions in the ETP. In contrast, agents with greater anti-Xa activity (shorter-chain LMWHs and fondaparinux) exert more selective effects on propagation metrics (reduced peak and mVRI) while preserving the ETP. Parenteral DTIs primarily disrupt thrombin-driven initiation of coagulation and feedback amplification. Argatroban (monovalent active-site inhibition) shows a concentration-dependent progression from kinetic delay to reductions in peak/mVRI and ETP, whereas bivalirudin and hirudin (bivalent inhibition involving exosite I) more prominently delay the thrombin burst with comparatively smaller reductions in ETP in many experimental contexts.

VII. Clinical and translational evidence

A. Reversal of parenteral anticoagulants

The studies summarized below are primarily translational (predominantly in vitro) and are intended to contextualize thrombin generation responses in clinically relevant settings rather than to imply validated bedside TGA-guided reversal strategies. Out of 12 articles focused on the reversal of parenteral anticoagulants, 5 in vitro studies evaluated the effects of protamine,[138–140,142,144] 3 in vitro studies tested andexanet alfa,[135,136,138] 1 in vitro study evaluated inactivated antithrombin,[137] 5 studies evaluated recombinant factor VIIa (rFVIIa),[15,16,141–143] 1 in vitro study evaluated PCC,[141] 2 tested activated PCC (aPCC),[141,142] and 1 evaluated fresh frozen plasma.[142]

Andexanet alfa is not a true UFH/LMWH reversal agent, and clinically applicable data in this setting remain limited, particularly for UFH. Andexanet alfa, a recombinant nonfunctional variant of FXa, retains many of the same binding properties as endogenous FXa, including binding to antithrombin. Noncovalent binding of antithrombin to andexanet alfa is enhanced in the presence of UFH/LMWH. When this occurs, antithrombin·heparin complexes are sequestered by andexanet alfa, and free antithrombin is not available to mediate UFH/LMWH's anticoagulant effects. This induces transient heparin resistance and provides partial reversal of UFH/LMWH. Chabata et al[13] conducted mechanistic experiments using thrombin generation and equilibrium modeling to show that andexanet alfa abolishes UFH dose responsiveness. In the absence of andexanet, UFH leads to expected progressive delay and suppression of thrombin generation, with marked attenuation at UFH concentrations typical of CPB, and complete suppression as UFH is increased further.[13] In the presence of andexanet, this response is blunted. Lower UFH concentrations no longer meaningfully delay or suppress peak thrombin generation, higher UFH concentrations are required to achieve comparable suppression, and at increased andexanet exposure, suppression remains incomplete despite very high UFH levels. These findings support that the partial UFH/LMWH neutralization achieved with andexanet alfa is due to andexanet-mediated heparin resistance via sequestration of UFH-antithrombin complexes, rather than due to true reversal. This can lead to unpredictable UFH activated clotting time responses and a risk of life-threatening CPB circuit thrombosis when andexanet alfa is used before emergent cardiac surgery.[13,14,204]

Limited LMWH data nevertheless suggest that andexanet can reduce enoxaparin anti-Xa activity and rapidly restore thrombin generation. In healthy volunteers receiving steady-state enoxaparin, andexanet reduced anti-Xa activity, whereas ETP increased substantially, rising into or above the pre-enoxaparin range immediately after bolus administration.[205] Similar findings were identified in a subset of enoxaparin-treated patients with major bleeding in ANNEXA-4, where anti-Xa activity fell by a median of 75% (0.48−0.14 IU/mL) at the end of bolus administration, whereas median ETP increased from 519 nM·min at baseline to 2344 nM·min, and remained above baseline over subsequent time points.[205,206] Although these data do not establish andexanet as a predictable UFH/LMWH reversal strategy, they do provide evidence that andexanet can reduce enoxaparin anti-Xa activity and rapidly restore thrombin generation.

Protamine has long been known to have an anticoagulant effect, and excessive administration is recognized as a risk factor for increased postoperative blood loss after cardiac surgery. Ni Ainle et al[140] investigated this anticoagulant effect and its underlying molecular mechanisms using TF-initiated thrombin generation. Therapeutic doses of protamine induced statistically significant and dose-dependent prolongations of the LT and TTP, and suppression of peak thrombin generation and the ETP, suggesting that protamine affects coagulation initiation and propagation. Protamine initially reversed the anticoagulant effects of UFH when added to samples containing UFH; adding higher concentrations of protamine progressively suppressed the ETP to values ~50% of normal (ie, relative to normal pooled plasma). Ni Ainle et al[140] demonstrated that this effect occurs because of protamine's effects on coagulation initiation, specifically via interaction with FV. Protamine might bind to FV and prevent thrombin from activating FV to FVa during coagulation initiation. Protamine was better able to reverse the effects of longer chain length LMWHs (eg, tinzaparin > enoxaparin). Although protamine could fully reverse the effects of tinzaparin, reversal of enoxaparin's effects on the ETP reached a limit at around ~60% to 80% of normal.[140,142] Inadequate heparin neutralization and protamine excess are recognized contributors to perioperative bleeding. The potential clinical implications of the translational findings outlined above are discussed in detail in the subsequent section (*Cardiac Surgery*).

Several groups have investigated whether bypassing/hemostatic agents (rFVIIa and aPCC) can partially correct fondaparinux/

idraparinux-associated alterations in thrombin generation.[141–143] rFVIIa partially corrected the LT in several studies, but not peak or ETP.[15,141,143] One study found that 20 IU/kg of aPCC was able to partially correct the effects of fondaparinux on the LT, peak, and ETP without inducing hypercoagulability.[141] These effects likely occur through a bypassing mechanism, since injection of rFVIIa did not have any measurable impact on the pharmacokinetic profile of fondaparinux or idraparinux.[15,16] These results are consistent with the effects of rFVIIa and aPCC on DOAC-altered thrombin generation.[33] Since fondaparinux has no specific approved reversal agent, both rFVIIa and aPCC are recommended by neurocritical care guidance as potential hemostatic strategies for fondaparinux-associated intracranial hemorrhage.[207]

B. Cardiac surgery

Most studies measuring TGA in the context of cardiac surgery compared measurements before CPB to values obtained after post-CPB protamine reversal.[39,40,160,161,163,164,166] A few studies measured TGA parameters intraoperatively immediately after UFH administration[158,163,165] and found that UFH completely abolished thrombin generation during cardiac surgery, irrespective of the TGA TF trigger concentration used.[163,165] UFH-induced TFPI release might contribute to UFH's in vivo anticoagulant effects during cardiac surgery. Bosch et al[158] found evidence of intraoperative coagulopathy despite neutralizing UFH using polybrene in samples measured intraoperatively. They attributed this to UFH-induced TFPI release, with a 13.9-fold increase in free TFPI levels.

The studies cited above all found evidence of ongoing postoperative coagulopathy after protamine reversal post-CPB, with prolongation of the LT and TTP, and lower peak, ETP, and mVRI values relative to pre-CPB.[39,40,158,160,161,163–166] One study compared patients undergoing cardiac versus noncardiac surgery; postoperative coagulopathy was only seen in the cardiac surgery patients, and these differences largely disappeared by postoperative day 1.[166] Factors contributing to post-CPB coagulopathy included "heparin rebound" and measurable residual post-CPB UFH anti-Xa activity,[160,161] a protamine-induced anticoagulant effect,[39,158,161] UFH-induced TFPI release,[158,161,163] and dilutional coagulopathy.[161,164]

Several studies reported a statistically significant association between TGA parameters and post-CPB bleeding.[39,40,158,164,165,169] Few studies formally defined major bleeding, and most reported postoperative estimated blood loss or chest tube output. Four studies stratified TGA parameters according to postoperative blood loss.[39,40,158,165] Lower peak thrombin generation and ETP were both associated with greater postoperative blood loss, whether measured preoperatively,[39,40,165,169] intraoperatively,[158] or postoperatively.[39,40,167] Coakley et al[40] performed receiver-operative-characteristic curve analysis and reported area under the curve values of 0.71 (95% confidence interval, 0.57–0.85) for ETP and 0.76 (95% confidence interval, 0.62–0.89) for the mVRI. Fewer studies evaluated associations with LT or TTP, and, among those that did, no statistically significant relationship was identified.[40,158]

C. Heparin-induced thrombocytopenia

Two studies used thrombin generation to characterize HIT-induced hypercoagulability[173] and the antithrombotic efficacy of anticoagulants commonly used in the setting of HIT.[174] Tardy-Poncet et al[173] showed that spiking PRP samples with 0.2 IU/mL of UFH produced an anticoagulant effect in HIT-negative patients, but a procoagulant effect was observed in HIT-positive patients.[173] When UFH was added to PRP samples from HIT-positive patients, a blunted anticoagulant response relative to HIT-negative patients was observed, with no effect on the ETP, an increase in the peak, mVRI, and a shortening of the LT and TTP.

Tardy-Poncet et al[174] also evaluated the in vitro effects of argatroban, danaparoid, and fondaparinux on thrombin generation in PPP samples from patients with HIT. Samples were incubated with UFH (0.2 IU/mL), as well as standard therapeutic concentrations of argatroban (400 and 600 ng/mL), danaparoid (0.65 IU/mL), and fondaparinux (1 μg/mL). They also found that spiking samples with UFH produced a hypercoagulable TGA profile, with a peaked, sharp, left-shifted TGA curve. Argatroban and danaparoid were better able to suppress peak thrombin generation and the ETP relative to fondaparinux. Argatroban extended the LT the most (3- to 4-fold), followed by fondaparinux (2-fold), whereas danaparoid showed only a modest effect on LT (~1.4-fold). These anticoagulant effects are consistent with the effects of argatroban, heparinoids, and fondaparinux in non-HIT patients.

D. Acute coronary syndrome

One in vitro study tested the ability of UFH and LMWH at prophylactic (0.2 IU/mL) and "curative" (0.8 IU/mL) concentrations to suppress monocyte-induced thrombin generation, given the role of monocyte-derived TF in myocardial infarction and ACS.[177] This study found that longer chain LMWHs with higher anti-IIa/anti-Xa activity ratios (eg, tinzaparin) displayed a similar ability to UFH to suppress thrombin generation, whereas shorter chain LMWHs with greater anti-Xa specificity (eg, enoxaparin) showed significantly less inhibition of the ETP compared with either UFH or tinzaparin.

One single-center prospective study of patients undergoing PCI for ACS sought to determine why patients receiving bivalirudin for PCI might experience an excess risk of early acute stent thrombosis.[178] They randomly assigned 20 patients to receive either UFH (70 IU/kg bolus) or bivalirudin (0.75 mg/kg bolus followed by an infusion of 1.75 mg/kg/h until the end of PCI). UFH and bivalirudin completely shut down thrombin generation during the procedure, with an unmeasurable peak and ETP. Bivalirudin's effects appeared to wane faster than UFH. When measured 4 hours postprocedure, peak and ETP were significantly lower among UFH-treated patients than bivalirudin-treated patients, in whom peak and ETP values had returned to baseline. The authors hypothesized that bivalirudin's shorter half-life and rapid restoration of thrombin generation might promote coagulation and protease-activated receptor-mediated platelet activation, which might explain the lower risk of major bleeding seen with bivalirudin versus UFH in PCI patients,[18,208,209] as well as the risk of early acute stent thrombosis.

VIII. Implications for anticoagulation therapy

A. Class-specific effects of parenteral anticoagulants on thrombin generation

Parenteral anticoagulants influence thrombin generation and coagulation in different ways depending on their mechanism of action. Class-specific anticoagulant TGA effect patterns have been reported consistently throughout the literature. These patterns likely reflect divergent effects from differing mechanisms of action on coagulation initiation, amplification, and propagation. Whether these patterns are clinically meaningful is unclear and warrants further investigation. The results from this review highlight that, from a pharmacodynamic perspective, not all anticoagulants are the same.

The assumption that all anticoagulants achieve their pharmacodynamic objective in the same way and to the same degree is false. Anticoagulation's "end result" may not necessarily be the same for different anticoagulant classes. Anticoagulants with different mechanisms of action have repeatedly been compared through head-to-head randomized controlled trials (RCTs) and observational research. Hypotheses used to explain observed differences in efficacy (ie, thrombosis) or safety (ie, bleeding) typically revolve around anatomic considerations (eg, valvular AF, mucosal vs nonmucosal bleeding), pharmacokinetic factors (eg, drug-drug interactions, impaired absorption, and suboptimal adherence), baseline risk factors and comorbidities (eg, age, frailty, obesity, and luminal gastrointestinal malignancy), confounding and bias, or even random chance (ie, "bad luck"). Pharmacodynamics are seldom considered.[210,211]

There are well-recognized and important differences that exist across indications in terms of antithrombotic efficacy and bleeding risk. Accumulating probabilistic data support that the DOACs might exert an inferior antithrombotic effect relative to VKAs in some high-risk patient populations.[71–79] Along the same lines, many clinicians reach for LMWH when faced with treating breakthrough VTE[212–214] or breakthrough stroke, a strategy endorsed by several guidelines.[215–217] These findings might relate to the lesser effect of DOACs seen on the ETP as compared with either UFH/LMWH or VKAs.[36,63,64] Although some of these epidemiologic associations might relate to off-target nonanticoagulant effects or clinical confounding because of improved care from routine international normalized ratio monitoring visits in VKA-treated patients,[218] the fact that improved clinical efficacy is seen for these high-risk conditions (eg, mechanical heart valves and breakthrough VTE) despite what is often suboptimal international normalized ratio control, fluctuating UFH anti-Xa levels, or poor adherence to LMWH injections, suggests that, on a mechanistic basis, anticoagulants blocking multiple coagulation pathways might confer a better anticoagulant effect. These observations may grow in importance when thrombosis occurs in vivo because of specific risk factors, such as artificial surface-induced contact activation (eg, mechanical heart valves and catheter surfaces).[63–66]

Likewise, divergent effects on thrombin generation might help clinicians decipher the reasons for known differences in bleeding risks across anticoagulant classes. DOACs are associated with a lower risk of major bleeding than VKAs, and intracranial hemorrhage in particular. Relative preservation of ETP with on-treatment DOAC levels, as compared with either VKAs or UFH/LMWHs, might partly explain this observation.[70] Our findings may also explain why different anticoagulants within the same class (eg, FXaIs) differ in terms of the risk of on-treatment bleeding complications. A growing body of evidence suggests that apixaban is associated with a lower risk of bleeding than rivaroxaban.[219,220] This may be related to a combination of achieving higher peak drug levels with rivaroxaban,[221,222] as well as rivaroxaban-specific pharmacodynamic effects. Rivaroxaban exerts a greater effect on clotting times (eg, prothrombin time) than apixaban. Moreover, rivaroxaban prolongs the LT and suppresses peak and ETP to a greater degree than apixaban at similar concentrations.[44,45,210] Kim et al[210] found that rivaroxaban inhibits the prothrombinase complex with 4-fold greater potency than apixaban, with corresponding Ki values for prothrombinase-induced thrombin production of 0.7 ± 0.3 and 2.9 ± 0.5 nM, respectively ($P < .02$), a finding that they attributed to divergent inhibitory effects on the initial activation cleavage of prothrombin by FXa.

Similarly, bivalirudin for PCI/ACS has consistently been associated with a lower risk of serious bleeding as compared with UFH (including the risk of death from iatrogenic bleeding complications), irrespective of concomitant glycoprotein IIb/IIIa inhibitor use.[18,209,223–225] Bivalirudin has also been associated with an increased risk of early acute stent thrombosis compared with heparin.[209,225,226] Although all anticoagulants affect kinetic and quantitative TGA parameters to varying degrees, bivalirudin and, by extension, hirudin, are unique in that they have a discriminating impact on the kinetics of thrombin generation (Fig. 4). This may impart a lower risk of bleeding, perhaps at the cost of lower antithrombotic efficacy relative to anticoagulation that better suppresses the ETP (eg, UFH).

B. From polypharmacologic to selective inhibition: A mechanistic framework for anticoagulant effects

On a broader level, antithrombotic effects might lie on a continuum, as determined by the ability of an anticoagulant to suppress the ETP (Fig. 7).[227] Multitargeted anticoagulants with broad-based effects on coagulation initiation and propagation lie on one end of this spectrum. This includes UFH, VKAs, and LMWHs. Bivalirudin and hirudin, highly selective DTIs, lie on the opposite end. Oral FXaIs and fondaparinux sit in the middle, whereas dabigatran and argatroban lie somewhere between bivalirudin/hirudin and the direct FXaIs. Longer chain-length LMWHs (eg, tinzaparin) would lie closer to UFH than the FXaIs; the opposite could be said of shorter chain-length LMWHs (eg, enoxaparin). Importantly, anticoagulants should not solely be compared based on the ETP (eg, bivalent DTIs function as "thrombin retardants" and primarily delay the onset of the thrombin burst). No single TGA parameter fully captures the effects of all anticoagulants.[31,56,86,105] On the other hand, the ETP represents the total amount of thrombin generated during the thrombin burst and might provide the best all-encompassing measure of antithrombotic potency on a mechanistic basis.

The FXI(a) inhibitors might lie even further on this spectrum than bivalirudin/hirudin (Fig. 7).[227] Emerging data suggests FXI(a) inhibitors have little effect on TF-initiated thrombin generation, and anticoagulant effects are more easily detected using contact-activated thrombin generation.[60–62] Some have put forward that this approach uncouples hemostasis from thrombosis. A more rational viewpoint might be that a literal tradeoff occurs with increasing anticoagulant selectivity, giving rise to corresponding increases in hemostatic safety but decreases in antithrombotic efficacy. Our proposed conceptual framework (Fig. 7) may partly explain the recent failure of the FXIa inhibitor asundexian during phase III testing for stroke prevention in AF (OCEANIC-AF).[228] Trying to achieve anticoagulation by blocking factors far from coagulation's fulcrum (prothrombinase, thrombin) may reduce the risk of anticoagulation-associated bleeding. However, escape mechanisms may compromise antithrombotic efficacy under highly prothrombotic conditions, where TF-driven coagulation plays a significant role (eg, cancer).[62,229] Whether FXI inhibition truly dichotomizes hemostasis and thrombosis remains to be determined.[60]

C. Remaining uncertainties: Translating pharmacodynamic inferences to clinical outcomes

Many readers might raise the concern that inferences based on in vitro data and pharmacodynamic principles are not consistently borne out by existing clinical epidemiologic and probabilistic outcome data. Results from several large RCTs appear to contradict the pharmacodynamics-based ranking above (Fig. 7). Multiple RCTs have demonstrated the noninferiority of DOACs relative to LMWH/VKAs for recurrent VTE.[230–232] Likewise, RCTs in patients with cancer-associated VTE have shown that LMWHs are superior

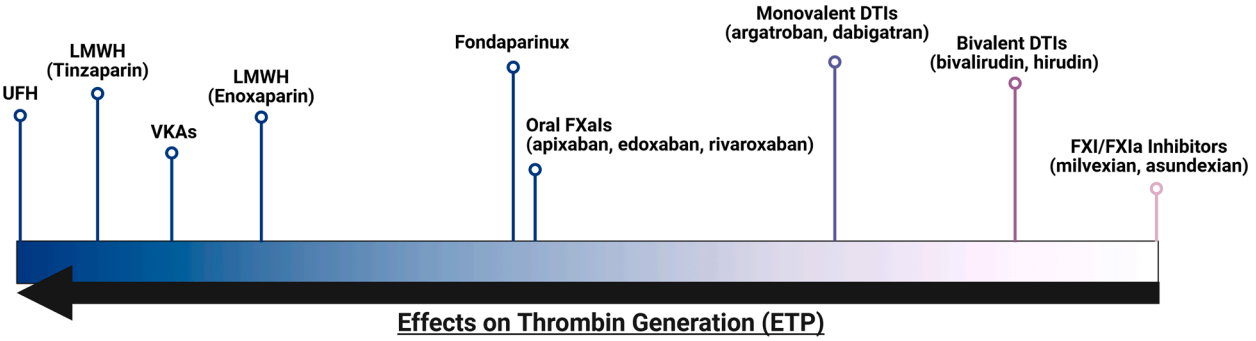

Fig. 7. Relative ability of anticoagulants to suppress TF-initiated thrombin generation. Relative ranking of anticoagulants based on their ability to suppress the ETP at on-treatment concentrations (eg, international normalized ratio 2.0–3.0 for VKAs), as measured using TF-initiated thrombin generation. This ranking reflects a qualitative synthesis of the literature, incorporating both in vivo studies in treated subjects and a larger body of in vitro pharmacodynamic thrombin generation experiments. Several between-class comparisons are inherently indirect. Accordingly, this figure represents a conceptual framework intended to guide future research and requires prospective empirical validation (and may be revised) as additional data emerge.

to VKAs for recurrent VTE,[233] and in turn, that DOACs are noninferior relative to LMWHs (albeit with LMWH dose reduction after the first month of treatment).[234–236] Some meta-analyses suggest that DOACs are superior to LMWH with respect to recurrent VTE.[237,238] Concerns surrounding these discrepancies are valid. However, it is important to consider that "real-world" clinical efficacy and safety are determined by multiple factors that contribute to an "end result" and the occurrence of a clinical event. The pharmacodynamic impact of an anticoagulant cannot be considered in a vacuum. Pharmacodynamic considerations must be viewed through their superposition over pharmacokinetics (ie, the drug level), clinical factors that contribute to drug levels (eg, absorption, half-life, drug–drug interactions, and adherence), as well as countless comorbidities and risk factors that interact in complex and synergistic ways to contribute to bleeding or thrombosis.[211]

What seems to be less clear is how different mechanisms of achieving anticoagulation might tangibly affect clinical outcomes, how pharmacodynamic parameters might interact with well established clinical risk factors for bleeding and thrombosis, and how this information can be used to make anticoagulation safer for patients. In vitro assays will never be able to perfectly recapitulate the complexity of in vivo coagulation processes. Indeed, TGAs are instruments with which to better understand anticoagulants and their effects on coagulation. They cannot account for cellular, rheological, endothelial, or fibrinolytic contributions to hemostasis and bleeding. By design, in vitro reaction systems must use an artificial trigger (eg, TF) to activate coagulation, which variably replicates in vivo hemostasis. Even so, they provide clinicians with the best possible means to isolate and contrast the effects of different anticoagulants. Mechanistic research provides insight that cannot otherwise be ascertained using traditional epidemiologic methods and might foster better, more personalized approaches to anticoagulation.[23] Many studies included in this review confirmed a statistically significant association between TGA parameters and bleeding.[39,40,158,164,165,169] This adds to existing evidence on the clinical relevance of TGA parameters in VKA/DOAC-treated patients,[41,239] patients with arterial thromboembolism or VTE,[35,240,241] and patients with congenital bleeding disorders.[242,243]

Despite substantial technological advances, TGAs have not yet been widely implemented or prospectively validated in routine clinical testing, in part because the assay is sensitive to preanalytical variables and heterogeneity in analytical conditions (eg, reagent and trigger concentrations and data processing), which can produce interlaboratory variability and limit interplatform comparability. International standardization initiatives (including guidance from the International Society on Thrombosis and Haemostasis Scientific Standardization Committee on recommended preanalytical/analytical conditions) and newer fully automated systems incorporating standardized reagents, quality controls, and normalization to reference plasma (eg, ST Genesia) are intended to mitigate these limitations and will facilitate validation for broader clinical adoption.[25,27,145,244] Although attempts to apply TGAs at the bedside have been hampered by limited availability, slow turnaround time, and preanalytical limitations, important progress continues to be made in these respects. The discrepancies observed between high in vitro and lower/more variable ex vivo correlation coefficients imply that thrombin generation may capture net anticoagulant effects in context, not simply exposure. In vivo, baseline thrombin generation, inflammation, comorbid prothrombotic drivers, and concomitant therapies contribute variance that attenuates simple bivariate correlations with measured drug levels, yet remain directly relevant to patient risk. Thrombin generation is best viewed as an integrated pharmacodynamic readout, rather than a direct surrogate for plasma anticoagulant levels.

D. Charting the future: Rational anticoagulation therapy

Future work should aim to move anticoagulation therapy further toward mechanism-informed characterization of both efficacy and safety. Interindividual heterogeneity in baseline hemostatic potential is substantial, and this baseline phenotype (and how it is perturbed by treatment) helps define each patient's therapeutic window. In this context, thrombin generation offers an integrated pharmacodynamic readout that links molecular targets to system-level coagulation behavior. Conceptually, the "on-therapy" thrombin generation profile reflects both a patient-specific baseline and a drug- (or class-) specific effect, yielding distinct response phenotypes where comparable exposure can produce meaningfully different patterns of thrombin suppression and delayed kinetics, with plausible implications for residual thrombotic risk versus bleeding propensity.[245]

Progress will depend on coupling mechanistic assays with predictive tools that explicitly model heterogeneity (rather than treating it as noise) and that connect phenotype, exposure, pharmacodynamics, and outcomes. Systems-based pharmacology, physiological modeling, and explainable machine-learning approaches can help integrate nonlinear relationships between class-specific anticoagulant effects, thrombin generation parameters, and clinical events, possibly supporting risk stratification.

Critically, for thrombin generation to meaningfully strengthen inference, it must be deliberately integrated into study design rather than being appended opportunistically. This requires an a priori conceptual framework and deliberate measurement strategy (including timing relative to anticoagulation dosing, prespecified assay conditions, and harmonized preanalytical and analytical methods), rather than being treated as an afterthought. This aligns with an "Evidence-Based Medicine+" ethos, in which mechanistic and probabilistic evidence are treated as complementary, and mechanistic endpoints are valued as contributors to causal understanding alongside (not subordinate to) clinical outcomes.[23,246,247] Under this view, mechanistic data can explain discrepant results, refine therapeutic frameworks, and guide rational experimentation across drug classes and clinical settings (including those in which parenteral agents predominate).

IX. Conclusions

Thrombin generation's future potential lies in achieving a better understanding of the interplay between the mechanisms used to achieve anticoagulation, their corresponding pharmacodynamic effects, and the net clinical result. In this sense, TGAs have become indispensable tools for anticoagulation-focused pharmacological research. The information contained in this review provides the mechanistic underpinnings needed to understand many findings stemming from anticoagulation-related epidemiological research over the past 3 decades. These data must be interpreted cautiously and cannot be applied directly to clinical care, which, nevertheless, should not undermine the value of the information outlined herein. Our findings may help rationalize anticoagulant selection and support decision-making in complex clinical circumstances where informed management is otherwise challenging because of a lack of high-quality evidence. More importantly, this body of work contributes a foundation for future anticoagulation research.

Abbreviations

ACS, acute coronary syndrome; AF, atrial fibrillation; aPCC, activated prothrombin complex concentrate; CPB, cardiopulmonary bypass; DOAC, direct oral anticoagulant; DTI, direct thrombin inhibitor; ETP, endogenous thrombin potential; FXaI, factor Xa inhibitor; HIT, heparin-induced thrombocytopenia; LMWH, low-molecular-weight heparin; LT, lag time; mVRI, mean velocity rate index; PCC, prothrombin complex concentrate; PCI, percutaneous coronary intervention; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RCT, randomized controlled trial; rFVIIa, recombinant factor VIIa; TF, tissue factor; TFPI, tissue-factor pathway inhibitor; TGA, thrombin generation assay; TTP, time to peak; UFH, unfractionated heparin; VKA, vitamin K antagonist; VTE, venous thromboembolism.

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Conflict of interest

Joseph R. Shaw has received in-kind laboratory support from Diagnostica Stago. François Mullier reports speaker fees from Stago. Jonathan Douxfils reports consultancy fees from Gedeon Richter, Estetra, and Mithra Pharmaceuticals, and is also the founder of QUALIblood sa. Kenichi Tanaka reports receiving grants from Grifols, Hikari Dx, Octapharma, and VarmX outside the submitted work, and advisory/adjudication board participation: CSL and Octapharma outside the submitted work. Bianca Rocca has received honoraria for advisory board work related to factor XI inhibitors for Bayer AG. Hugo ten Cate reports consulting fees from Synapse Research Institute, and is a shareholder with Coagulation Profile. Marc Carrier reports grants from Pfizer, and personal fees from BMS, Leo Pharma, Bayer, Pfizer, Anthos, Regeneron, and Sanofi. Jerrold H. Levy has participated on steering committees for Bayer, Grifols, Octapharma, and Werfen. All other authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Joseph R. Shaw: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review and editing. **Cheryl L. Maier**: Writing – review and editing. **Michaël Hardy**: Writing – review and editing. **François Mullier**: Writing – review and editing. **Jonathan Douxfils**: Writing – review and editing. **Kenichi Tanaka**: Writing – review and editing. **Bianca Rocca**: Writing – review and editing. **Hugo ten Cate**: Writing – review and editing. **Paul Y. Kim**: Writing – review and editing. **Marc Carrier**: Writing – review and editing. **Jean M. Connors**: Conceptualization, Supervision, Writing – review and editing. **Jerrold H. Levy**: Conceptualization, Supervision, Writing – original draft, Writing – review and editing.

Supplemental material

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