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Review Article

The pleiotropic effects of antithrombotic drugs in the metabolic–cardiovascular–neurodegenerative disease continuum: impact beyond reduced clotting

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Antithrombotic drugs are widely used for primary and secondary prevention, as well as treatment of many cardiovascular disorders. Over the past few decades, major advances in the pharmacology of these agents have been made with the introduction of new drug classes as novel therapeutic options. Accumulating evidence indicates that the beneficial outcomes of some of these antithrombotic agents are not solely related to their ability to reduce thrombosis. Here, we review the evidence supporting established and potential pleiotropic effects of four novel classes of antithrombotic drugs, adenosine diphosphate (ADP) P2Y12-receptor antagonists, Glycoprotein IIb/IIIa receptor Inhibitors, and Direct Oral Anticoagulants (DOACs), which include Direct Factor Xa (FXa) and Direct Thrombin Inhibitors. Specifically, we discuss the molecular evidence supporting such pleiotropic effects in the context of cardiovascular disease (CVD) including endothelial dysfunction (ED), atherosclerosis, cardiac injury, stroke, and arrhythmia. Importantly, we highlight the role of DOACs in mitigating metabolic dysfunction-associated cardiovascular derangements. We also postulate that DOACs modulate perivascular adipose tissue inflammation and thus, may reverse cardiovascular dysfunction early in the course of the metabolic syndrome. In this regard, we argue that some antithrombotic agents can reverse the neurovascular damage in Alzheimer's and Parkinson's brain and following traumatic brain injury (TBI). Overall, we attempt to provide an up-to-date comprehensive review of the less-recognized, beneficial molecular aspects of antithrombotic therapy beyond reduced thrombus formation. We also make a solid argument for the need of further mechanistic analysis of the pleiotropic effects of antithrombotic drugs in the future.

Introduction

Hemostasis is maintained through various physiological mechanisms to preserve normal blood flow at the site of vascular injury [1]. However, multiple triggers might lead to pathological activation of thrombotic mechanisms. For instance, endothelial insult might evoke clot formation in arteries and veins manifesting in acute coronary syndrome (ACS) and venous thromboembolism (VTE), respectively [1]. Accordingly, antithrombotic agents, including antiplatelet and anticoagulant drugs, are geared at the prevention and treatment of cardiovascular diseases (CVDs), the leading global cause of morbidity and mortality [2]. Historically, anticoagulation was first achieved through the use of either naturally occurring unfractionated heparin (UFH) polymers or their breakdown derivatives called low-molecular weight heparins [3]

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or vitamin K antagonists [4]. While some of these drugs might have other effects unrelated to clotting factor inhibition, the polar macromolecular nature of heparins dictated that these effects be confined to the vasculature [3], and the inhibition of a specific hepatic target limited the action of vitamin K antagonists elsewhere. Newer classes of antithrombotic drugs have been introduced including adenosine diphosphate (ADP)-P2Y12 receptor antagonists, Glycoprotein (GP) IIb/IIIa receptor inhibitors and the direct oral anticoagulants (DOACs) comprising direct Factor Xa (FXa) and direct thrombin inhibitors. These classes not only have solid indications in various cardiovascular disorders, but also have gained recent attention in the management of thrombotic complications related to the coronavirus disease of 2019 (COVID-19) pandemic [5]. With the majority of these drugs being small molecules with potential significant extravascular distribution [6–8], it is not surprising that an accumulating body of evidence has demonstrated pleiotropic effects of these drugs beyond the inhibition of thrombus formation in various organs and disease conditions including CVDs such as stroke, atherosclerosis, cardiac injury, and arrhythmia. Importantly, antithrombotic agents were also shown to exert various effects unrelated to hemostasis in metabolic diseases. Despite the fact that the antithrombotic activity of these agents is well-characterized, their pleiotropic effects require further investigation in order to elucidate their underlying molecular mechanisms and clinical implications. The term pleiotropic effects can be used for a broad description of outcomes varying from actions on targets other than those for which a given drug was designed, to outcomes of the original target pathway but unrelated to the main labeled indication. In the present context, we use this term to describe the effects of antithrombotic drugs that are not direct consequences of interfering with hemostasis. Indeed, while the reporting of these effects has surged in the past decade [9,10], no structured translational effort has been forthcoming. Herein, we touch bases on the fundamentals of hemostasis and antithrombotic therapy before summarizing the present knowledge on the potential pleiotropic effects of these novel antithrombotic drugs. We propose a framework whereby these drugs might modulate a common underlying mechanism that originates as an early consequence of metabolic impairment and possibly contributes to the initiation of cardiovascular and neurological involvement in metabolic syndrome.

Hemostasis

Hemostasis encompasses various well-maintained physiological processes to preserve normal blood flow in an interplay between platelets, coagulation factors, and vascular endothelium [1]. Indeed, this is achieved through a tight balance between opposing, procoagulant and anti-coagulant systems. Vascular endothelial injury results in the exposure of the highly thrombogenic subendothelial matrix containing collagen, von Willebrand factor (vWF), and adhesion proteins to blood. Subsequently, platelet activation and adhesion to the site of vascular injury form a platelet plug culminating in primary hemostasis. During secondary hemostasis, platelet surface functions as a platform driving the assembly of the activated coagulation cascade resulting in the conversion of fibrinogen into fibrin and stabilizing the platelet plug [11]. The activated coagulation cascade includes 13 proteolytic coagulation factors that are implicated in the intrinsic, extrinsic, and common coagulation pathways [12]. These factors are initially synthesized as inactive zymogens and are sequentially and catalytically activated by cleavage [13]. The hemostatic pathway is summarized in Figure 1.

Tissue factor (TF) is constitutively expressed in extravascular components such as fibroblasts and is inducible in subsets of leukocytes including monocytes, T cells, eosinophils, neutrophils, and macrophages as well as endothelial cells (ECs) and platelets [14]. Nevertheless, the exact cellular origin of TF and the contribution of cell-specific release of TF is controversial particularly due to the contribution of intercellular microvesicle-mediated transport of TF [14]. Following vascular injury, TF, also known as factor IIIa is released and FVII (Proconvertin) is activated into FVIIa initiating the extrinsic pathway of coagulation. FVIIa complexes with TF, where in the presence of calcium, the FVIIa/TF complex activates FX into FXa. Subsequently, FXa generates thrombin, also known as FIIa from prothrombin (FII) in the presence of the cofactor FVa [15,16].

In the intrinsic pathway, FXII, also known as Hageman factor, is activated by activated platelet-released, negatively charged polyphosphates into FXIIa [17]. FXIIa cleaves plasmathromboplastin (FXI) to form FXIa, which in the presence of calcium, activates Christmas factor (FIX) into FIXa. Additionally, FIX can be activated through the extrinsic pathway by FVIIa. The antihemophilic factor (FVIII) is a cofactor produced by hepatic and ECs and is found bound to circulating vWF. Following vascular injury, FVIIIa separates from vWF and interacts with FIX in the presence of calcium and negatively charged membrane phospholipids, forming a complex that activates FX into FXa [18,19].

The intrinsic and extrinsic pathways culminate at FXa, which cleaves prothrombin, yielding thrombin. Thrombin is a serine protease that cleaves fibrinogen into fibrin and thus plays a crucial role in the coagulation cascade [20]. Moreover, thrombin activates platelets by activating protease-activated receptors (PARs), PAR1 and PAR4 [21].

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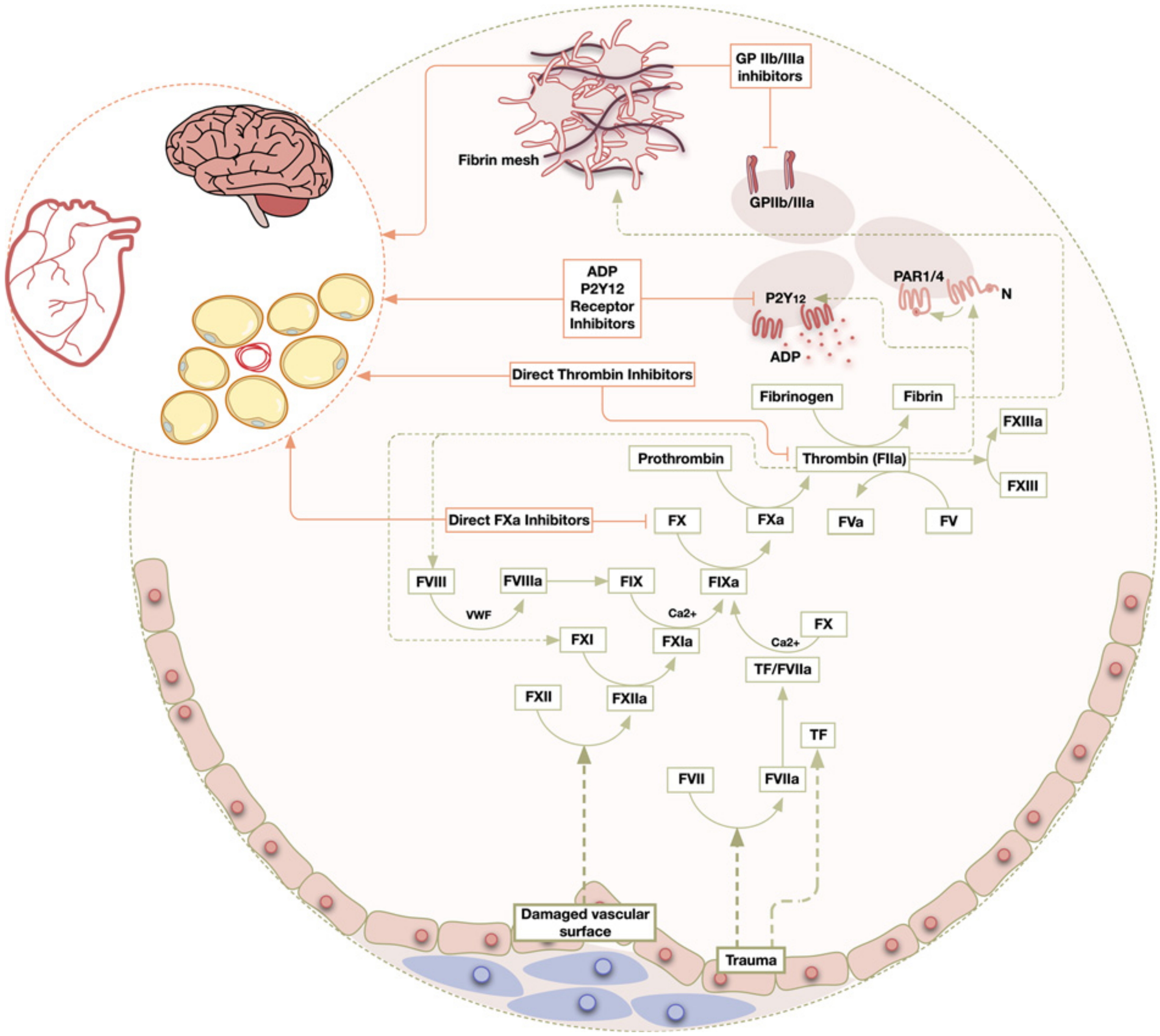

Figure 1. The hemostatic pathway including platelet and the clotting cascade

Several stimuli including damaged vascular surfaces and vascular trauma induce both platelet aggregation and clotting factor activation culminating in the production of the fibrin mesh and blood clot. Newer antithrombotic drugs including ADP P2Y12-receptor antagonists, GPIIb/IIIa receptor Inhibitors, and DOACs block different sites of the hemostatic pathways. Owing to their unique pharmacokinetic properties, these drugs can exert extravascular effects in different tissues and organs expressing their receptors. These effects can produce a positive pleiotropic impact in cardiovascular, metabolic, and neurodegenerative disease conditions unrelated to their effect on blood clotting. Abbreviations: F, factor; PAR, protease-activated receptor.

Thrombin also activates FXI, which in turn activates FIX through the intrinsic pathway. Therefore, thrombin is responsible for a positive feedback activation of coagulation and for clot propagation [20]. Nevertheless, this scheme explained *in vitro* does not explain the latest clinical advances in the past few decades and thus, the cell-based model of coagulation was introduced to elaborate on the complex interactions between platelets, the coagulation system, and the vessel wall [22]. In this model, the scheme is integrated, restructured, and is subcategorized into three phases, initiation, amplification, and propagation [23]. Although being quite similar to the intrinsic and extrinsic pathways, a level of complexity to coagulation initiation is present in the cell-based model of coagulation. In fact, the TF pathway inhibitor (TFPI) can halt the production of thrombin through TF/FVIIa complex inhibition [24]. Additionally, thrombin can be inhibited by anti-thrombin III (ATIII), which can also inhibit FXa, FIXa, and FXIIa [25]. During the amplification phase, the small amount of thrombin produced during the initiation phase activates platelet PARs and enhance its binding to collagen [26,27]. After thrombin activates FV, it dissociates from fully activated platelets

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[28]. Simultaneously, thrombin activates FXIa to activate FIX. Then, thrombin activates FVIII through its dissociation from vWF. Both FVIIIa and FIXa form a complex that activates FX, which binds to FVa to activate more prothrombin [29,30]. This thrombin feedback loop is known as the thrombin burst [31–33]. In addition to the cleavage of fibrinogen into fibrin [34], thrombin activates fibrin stabilizing factor (FXIII) into FXIIIa, which connects fibrin polymers together, forming the fibrin mesh [35].

As mentioned above, a balance between procoagulant and anti-coagulant pathways exists in hemostasis. Like the coagulation cascade, fibrinolysis, that is the disruption of the fibrin mesh, is tightly regulated by different sets of receptors, inhibitors, and cofactors. Plasmin is an anti-coagulation proteolytic enzyme that cleaves fibrin into soluble fibrin degradation products (FDPs). Urokinase plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) activate plasminogen into plasmin. The uPA is produced by monocytes, macrophages, and urinary epithelium, while tPA is produced by ECs. PAs have a short half-life in the general circulation due to the presence of plasminogen activator inhibitors (PAIs). Activated plasmin evokes PA activity by converting single chain PA into their double-chained counterparts allowing for a positive feedback on plasmin activation [36–38]. Fibrinolysis can also be halted by the thrombin-activatable fibrinolysis inhibitor (TAFI). TAFI is a zymogen that gets activated by the thrombomodulin I/thrombin complex to form TAFIa [39]. TAFIa down-regulates fibrinolysis by inhibiting the formation of tPA, plasminogen/fibrin complex, and the formation of new plasmin [40].

Antithrombotic drug classes with potential pleiotropic effects

As mentioned in the 'Introduction' section, several classes of antithrombotic drugs have been introduced in the past two decades. The peculiar properties of these agents make them more likely to produce off-target actions that might be of beneficial outcomes. In this section, we summarize the basic pharmacological properties of four of these classes, which might assist in the understanding of the underlying molecular pathways involved in their pleiotropic effects. The pleiotropic effects of these drugs in different disease conditions are summarized in Figures 2-4.

ADP P2Y12-receptor antagonists

ADP-P2Y12 signaling in platelets stimulates their activation and aggregation, leading to thrombus formation [41]. The pharmacological targeting of P2Y12 receptors has proven effective in the treatment and prevention of thrombosis. Indeed, dual antiplatelet therapy (DAPT), comprising aspirin and a P2Y12 receptor antagonist is a cornerstone therapy in the treatment of recurrent ischemic events in patients with coronary artery disease (CAD) [42,43]. Clinically used P2Y12 receptor antagonist are either reversible or irreversible receptor inhibitors. Thienopyridine derivatives, including clopidogrel, prasugrel, and ticlopidine are prodrugs, that upon enzymatic activation, yield irreversible blockade of the receptor [44]. On the other hand, directly acting, non-thienopyridines, elinogrel, cangrelor, and ticagrelor, produce reversible inhibition of the receptor [45,46]. The localization of the Gi-protein-coupled receptor P2Y12 is not platelet-restricted, as this receptor is expressed in certain regions of the brain, vessels, heart, and blood cells and hence the off-target effects arising from the receptor blockade [47]. Indeed, these pleotropic effects may also be linked to P2Y12-independent actions. Particularly, ticagrelor was shown to augment plasma adenosine levels either by the inhibition of adenosine uptake by red blood cells or through the inhibition of the sodium-dependent equilibrative nucleoside transporter 1 [48–50], or through the induction of adenosine triphosphate (ATP) release from blood cells and its subsequent degradation to adenosine [51].

GIPIIb/IIIa receptor inhibitors

GPIIb/IIIa, also known as integrin αIIbβ3, is a calcium-dependent integrin complex localizing to the surface of platelets. Under resting conditions, GPIIb/IIIa exhibits low affinity for soluble fibrinogen, which is the primary ligand for GPIIb/IIIa. Yet following vascular injury, platelet activation triggers a conformational change leading to an enhancement of the binding affinity of GPIIb/IIIa to fibrinogen. These events eventually result in cross-linking of platelets, thereby producing a platelet plug, which is followed by the coagulation cascade that converts soluble fibrinogen into insoluble fibrin, leading to thrombus formation [52].

The identification of GPIIb/IIIa as a therapeutic target has led to the development of drugs that include abciximab, and the small-molecule GPIIb/IIIa inhibitors (GPIs) (eptifibatide, tirofiban), which are parenterally administered agents that target different binding sites on GPIIb/IIIa. Abciximab, a monoclonal antibody formerly known as C7E3 Fab, is a non-competitive irreversible GPI with a short half-life. Tirofiban and eptifibatide, which are non-peptide and peptide GPIs respectively, are competitive inhibitors with a relatively longer half-life [53]. GPIs are indicated for the short-term prevention of thrombosis in ACSs and percutaneous coronary interventions (PCIs). The pleiotropic effects of GPI have been investigated in several diseases, and these effects either stem from a non-hemostatic activity

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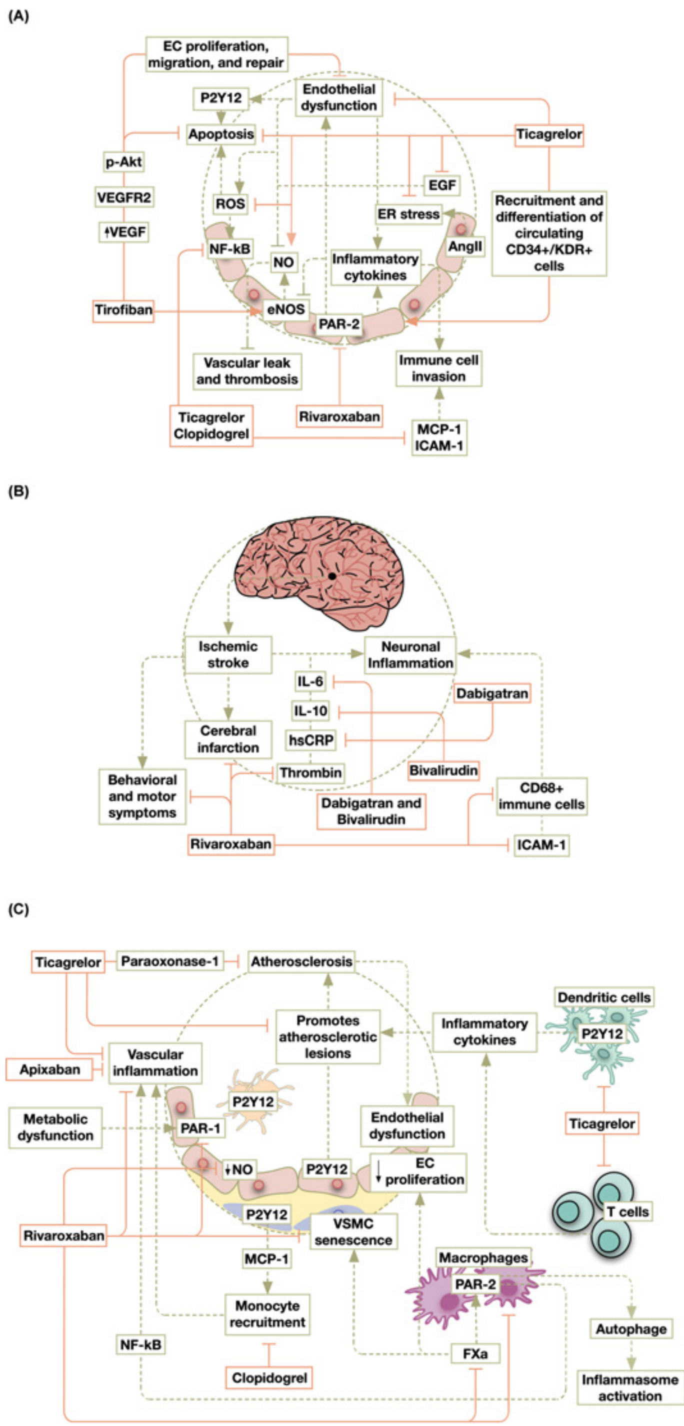

Figure 2. The proposed pleiotropic effects of novel antithrombotic drugs in different vascular disorders

(**A**) Endothelial dysfunction: different classes of novel antithrombotic drugs mitigate endothelial impairment by ameliorating inflammation, blocking PAR signaling, or induction of endothelial repair. (**B**) Ischemic stroke: inhibitors of thrombin and FXa mitigate focal and behavioral outcomes of stroke by interfering with neuronal inflammation. (**C**) Atherosclerosis: FXa and P2Y12 inhibitors interfere with inflammatory pathways promoting atherosclerosis. Abbreviations: AngII, angiotensin II; CD34, cluster of differentiation 34; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; FXa, activate factor Xa; hsCRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; KDR, kinase insert domain receptor; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor κB; NO, nitric oxide; p-Akt, phospho-protein kinase B; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2, VSMC, vascular smooth muscle cell.

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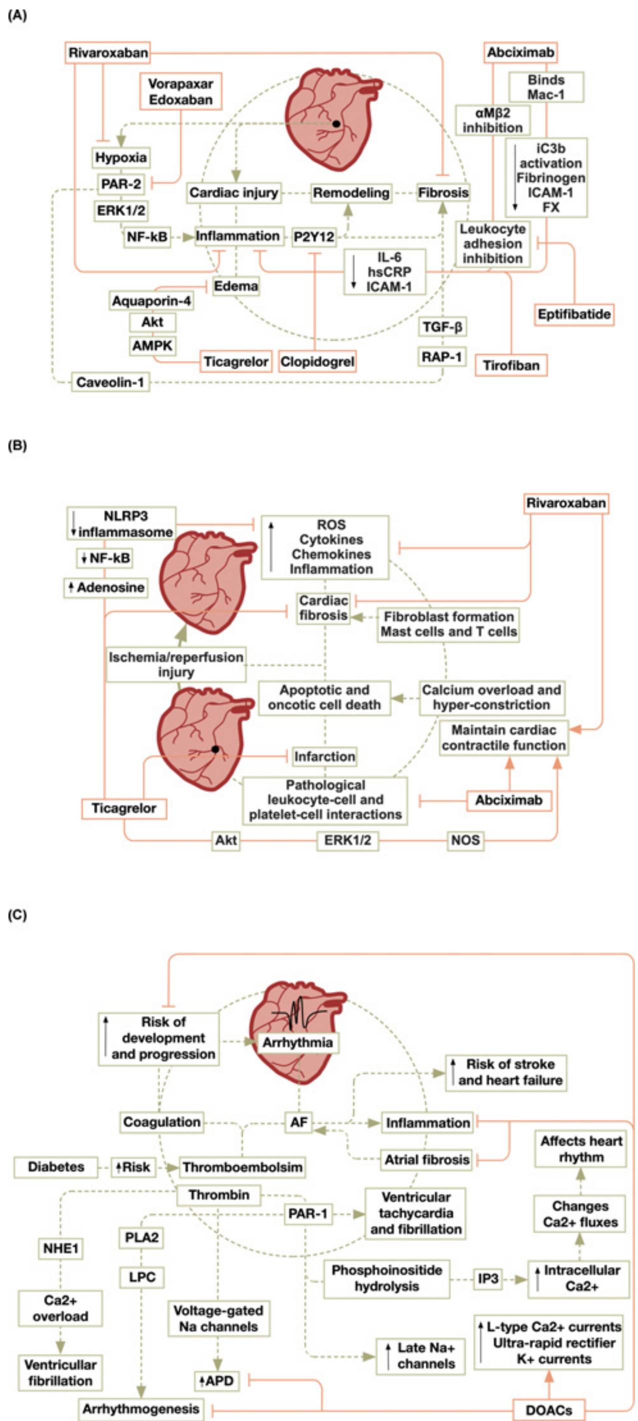

Figure 3. The proposed pleiotropic effects of novel antithrombotic drugs in different cardiac disorders

(**A**) Cardiac injury: several classes of novel antithrombotic drugs modulate a number of inflammatory and signaling pathways to ameliorate cardiac remodeling following injurious stimuli. (**B**) Ischemia–reperfusion injury: antithrombotic drugs reduce inflammation and oxidative stress post-reperfusion. (**C**) Arrhythmia: DOACs might harbor an anti-arrhythmogenic effect owing to the interference with ionic currents and cardiac inflammation. Abbreviations: αMβ2, integrin α M β 2; AF, atrial fibrillation; Akt, protein kinase B; APD, action potential duration; AMPK, AMP-activated protein kinase; ERK1/2, extracellular signal-regulated kinase 1/2; FX, factor X; hsCRP, high-sensitivity C-reactive protein; iC3b, opsonin; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; IP3, inositol triphosphate; LPC, lysophosphatidylcholine; Mac-1, macrophage-1 antigen; NLRP3, NLR family pyrin domain containing 3; NHE1, sodium-hydrogen antiporter 1; NOS, nitric oxide synthase; PLA2, phospholipase A2; RAP-1, Ras-related protein 1; ROS, reactive oxygen species; TGF-β, transforming growth factor β.

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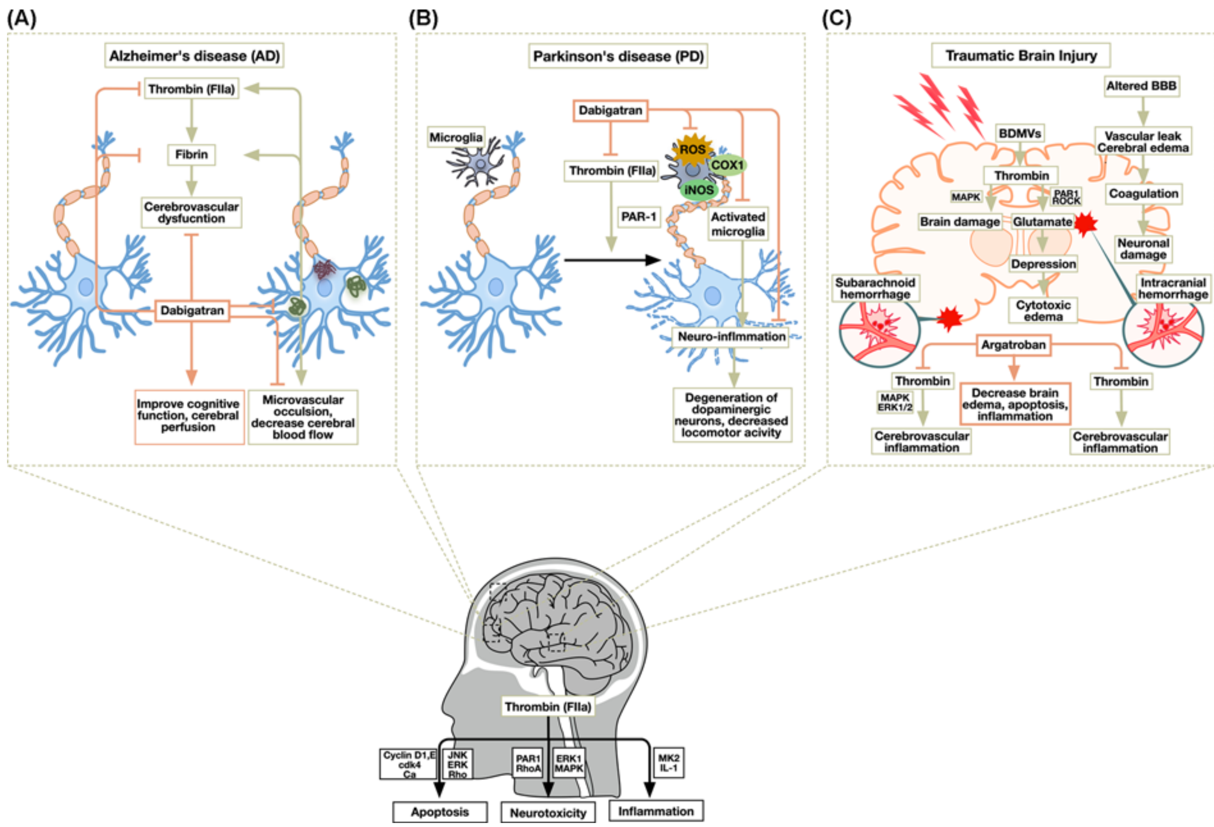

Figure 4. The proposed pleiotropic effect of novel antithrombotic drugs in neurodegenerative disorders
(**A**) Alzheimer's disease: Dabigatran inhibits the thrombin-induced fibrin production, cerebrovascular dysfunction, and neuronal toxicity. (**B**) Parkinson's disease: dabigatran decreases thrombin-mediated PAR1 signaling leading to reduced microglial activation and neuronal inflammation. (**C**) Traumatic brain injury: thrombin inhibitors can potentially mitigate post-TBI neuronal inflammation as they improve cerebrovascular function following different types of brain hemorrhage. Abbreviations: BBB, blood–brain barrier; BDMV, brain-derived cellular microvesicle; CDK4, cyclin-dependent kinase 4; COX1, cyclooxygenase 1; ERK1/2, extracellular signal-regulated kinase 1/2; IL, interleukin; iNOS, inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MK2, MAPK-activated protein kinase 2; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species.

of fibrinogen receptors, a complex modulation by other factors, or the presence of GPIIb/IIIa receptors on various cells [54].

DOACs

Aside from the traditional options for anticoagulation including UFH, low molecular weight heparin, and the vitamin K antagonist, warfarin, a dire need for novel anticoagulants was driven by the drawbacks of these drugs. Heparin and UFH are exclusively parenterally administered, and patients on heparin treatment require strict monitoring rendering long-term treatment inconvenient [55]. Moreover, warfarin has a narrow therapeutic index, along with a delayed onset of action, and multiple drug and food interactions [56]. Novel DOACs were approved for the prophylaxis and management of thrombosis, replacing heparin and warfarin. DOACs offer major advantages such as oral bioavailability, rapid onset and offset of action, reduced bleeding risk, wide therapeutic index, minimal drug and food interactions, and predictable coagulation response requiring less need for frequent monitoring [57–59].

Direct FXa inhibitors

FXa is a vitamin K-dependent coagulation serine protease that cleaves prothrombin into thrombin resulting in the conversion of fibrinogen into fibrin and subsequent clot formation. Indeed, the intrinsic and extrinsic pathways of coagulation culminate in the activation of FXa, thereby constituting an effective target of anticoagulant therapy [60]. A novel class of small molecules that reversibly inhibits FXa has been developed, consisting of rivaroxaban, apixaban, and edoxaban. Indeed, the predictable pharmacokinetic and pharmacodynamic profiles of direct FXa inhibitors

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in addition to their oral bioavailability and superior efficacy and safety in comparison with traditional anticoagulants have urged their utility as the main stay for the prophylaxis and treatment of deep vein thrombosis (DVT) and surgery-associated embolism [61–63]. Importantly, these molecules display high selectivity towards FXa in comparison with other coagulation proteases. In particular, rivaroxaban binds free, prothrombinase-bound, and clot-bound FXa [64,65].

In addition to the activation of prothrombin, FXa proteolytically cleaves the N-terminus of cellular receptors. Indeed, this process is of particular significance to PARs, which are G-protein coupled receptors (GPCRs) activated as a result of the cleavage of their N-terminus, revealing a tethered ligand [66]. PAR-mediated FXa signaling was shown to be implicated in a myriad of diseases, such as atherosclerosis, neurodegeneration, and inflammatory disorders [67]. Therefore, the coagulation-independent activities of FXa and the widely reported functions of PARs have driven investigations into a wide range of pleiotropic effects of FXa inhibitors.

Direct thrombin inhibitors

Direct thrombin inhibitors, including hirudin, argatroban, bivalirudin, and dabigatran, inhibit both free and clot-bound thrombin. Hirudin and bivalirudin have been FDA approved for the management of heparin-induced thrombocytopenia, while bivalirudin is used as an alternative to heparin in PCIs [68]. Moreover, Dabigatran is used for the prevention of venous thromboembolism, stroke in patients with atrial fibrillation (AF), and in long-term coagulation treatment [68–70]. As discussed before, the interaction between exposed TF and plasma FVII triggers the activation of the coagulation cascade and the production of thrombin following vascular injury. Thrombin is a serine protease that catalyzes the conversion of soluble fibrinogen into insoluble fibrin [71], activates FV, FVIII, FXI, and FXIII, and stabilizes the clot. Similar to FXa, thrombin activity is mediated by its interactions with PARs [72]. In particular, thrombin-induced activation of PAR-1 triggers multiple transcription-regulated, cell-specific events [9]. Therefore, directly inhibiting thrombin formation, activation, or interaction with its substrates will diminish cellular signaling beyond the attenuation of fibrin clot formation [73].

Pleiotropic effects of anticoagulant drugs in CVDs

Endothelial dysfunction: a prodrome for CVD

The endothelium has emerged as a key regulator of vascular homeostasis whereby it acts as an active paracrine, endocrine, and autocrine organ of high importance in regulating vascular function [74,75]. Endothelium activation results in the expression of chemokines and cytokines as well as adhesion molecules that in turn attracts and provokes immune cells extravasation [75]. Endothelial dysfunction (ED) reflects a pathogenic phenotype prone to atherogenesis and cardiovascular events [76]. ED is characterized by a reduction in the bioavailability of vasodilators (as nitric oxide, NO) and increase in endothelium-derived contracting factors [77]. As such the endothelium switches from NO to reactive oxygen species (ROS) signaling. Sufficient NO bioavailability is required for proper vascular function, inhibition of pro-inflammatory cytokine production, immune cell invasion, thrombosis as well as prevention of vascular leakage [78]. On the other hand, ROS production promotes nuclear factor κB (NF-κB) signaling and therefore an inflammatory profile [79]. Knowing that ED lies at the origin of the different atherosclerotic CVDs (ASCVDs) [80], it is important to shed light on the effect of anticoagulant agents on halting endothelial insult and enhancing overall endothelial function. The beneficial effects of the different drug classes on these processes are summarized in Figure 2A.

ADP $P2Y_{12}$-receptor antagonists

The significant superiority of ticagrelor over clopidogrel in patients with ACS in the Platelet Inhibition and Patient Outcomes (PLATO) trial, in addition to the ticagrelor-specific side effects, including dyspnea, bradycardia, bradyarrhythmia, and asymptomatic ventricular pauses, that are known to be mediated by adenosine, suggested that ticagrelor could possibly exhibit extra-platelet effects [81]. As such, the ability of ticagrelor to enhance adenosine plasma levels developed an interest to investigate the off-target effects of ticagrelor, particularly on vascular function [82]. The observed clinical benefit for ticagrelor in ED could possibly be mediated by adenosine, whereby the activation of adenosine receptor A2A on ECs was associated with an improvement in vasorelaxation responses and NO production [83]. The adenosine-mediated effects of ticagrelor were supported by a study conducted on healthy male subjects where ticagrelor enhanced coronary vasodilatory responses, which was reversible by theophylline, a nonselective competitive adenosine-receptor antagonist [84]. Other studies confirming the endothelial effect of ticagrelor are summarized in Table 1. However, a study demonstrating that ticagrelor, at certain plasma levels, does not always affect plasma adenosine transport, triggered the search for other potential mechanisms for treatment of ED [85].

Table 1 Main findings of studies reporting pleiotropic effects of *ADP P2Y$_{12}$-receptor antagonists* in cardiovascular disorders

	Molecule	Conducted in	Main findings	References
ED	Ticagrelor	Patients with stable CAD and concomitant chronic obstructive pulmonary disease	↓ Apoptosis of ECs ↑ NO production	[340]
		Diabetic patients with NSTEMI ACS requiring coronary stenting	↑ Brachial artery flow-mediated dilation	[341]
		Healthy male subjects	↑ Adenosine-induced coronary blood flow	[84]
		Patients with stable CAD and concomitant chronic obstructive pulmonary disease, HUVECs	↓ EGF, ↑ eNOS activity	[342]
		Angiotensin II-infused rats	↓ ER stress, ↓ ROS	[343]
		Patients with ACS	↑CD34$^+$/KDR$^+$ progenitor cells, endothelial recovery	[344]
		New Zealand white rabbits	Endothelial regeneration	[345]
	Clopidogrel	HUVECs	Inhibition of NF-κB	[88]
		HUVECs	↓ MCP-1, ↓ ICAM-1	[89]
		Healthy male and female subjects	↑ EDH	[90]
Atherosclerosis	Ticagrelor	Western diet-fed ApoE$^{-/-}$ mice	↑ ACh-mediated relaxation, ↓ inflammatory markers, ↓ atherosclerotic lesions	[115]
		HFD-fed LDLr$^{-/-}$Apobec1$^{-/-}$ mice	↑ Paraoxonase-1	[116]
		Mouse bone marrow-derived dendritic cells (DCs)	↓ DC activation by inhibition of P2Y12-dependent PI3K activity	[346]
	Clopidogrel	Atherogenic diet-fed ApoE$^{-/-}$ mice	↓ Atherosclerotic lesions, ↓ leukocyte infiltration	[114]
Cardiac Injury	Ticagrelor	Pigs with coronary artery occlusion-induced MI	↓ Structural/functional changes in myocardial tissue, ↑AMPK and Akt/PKB activation, ↓ aquaporin-4 and ↓ edema	[136]
		Pigs with coronary artery occlusion-induced MI	↓ Edema, adenosine-mediated	[137]
		Patients with STEMI, HEALING-AMI trial	Ticagrelor superior to clopidogrel for LV-remodeling	[138]
	Clopidogrel	Patients undergoing stenting	↓ Inflammatory markers	[134]
		Angiotensin II-infused mice	↓ Inflammatory markers, ↓ fibrosis	[135]
Ischemia/reperfusion injury	Ticagrelor	Rat model of I/R	↑ Adenosine, ↑activation of pro-survival Akt and ERK1/2, ↑ eNOS, ↓ infarct size	[160]
		Diabetic ZDF rats with induced I/R	↑ Adenosine, ↓ NLRP3 inflammasome	[161]
		Rat model of I/R	↓ NF-κB signaling	[162]
		Rat model of I/R	↑ regenerative cells, cardiac healing	[163]
	Clopidogrel	Patients with acute STEMI	↓ Reperfusion Injury	[164]
		New Zealand white rabbits	↓ Infarct size in reperfused heart, cardioprotective	[165]
		Healthy volunteers	Protective against induced I/R in upper arm, no change in flow-mediated dilation	[166]

Several such effects including reducing circulating epidermal growth factor (EGF), attenuating the expression of endoplasmic reticulum stress molecular markers and ROS, improving endothelial recovery by enhancing the number of CD34$^+$/KDR$^+$ progenitor cells thereby promoting endothelial regeneration are also summarized in Table 1.

P25Y12-receptor antagonism was shown to suppress inflammation, which is a characteristic of ED that triggers endothelial apoptosis [86,87]. In this context, incubation of ticagrelor and clopidogrel with human umbilical vein ECs (HUVECs) was associated with an attenuation of LPS-induced cellular dysfunction by inhibition of NF-κB signaling

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pathway and enhancement of cell viability [88]. In TNF-α-induced HUVECs, clopidogrel decreased the expression of monocyte chemotactic protein-1 (MCP-1) and intercellular adhesion molecule 1 (ICAM-1), which mediate the recruitment and adhesion of inflammatory cells to the endothelium [89]. Interesting findings by Dahmus et al. proposed a possible effect of clopidogrel on endothelium-dependent hyperpolarization (EDH) [90]. In order to evaluate cutaneous microvascular function in healthy human subjects, both cutaneous reactive hyperemia and slow local skin heating methods were used. Each method recruits a distinctive mechanism for vasodilation. Whereas reactive hyperemia induces cutaneous vasodilation that is EDH-dependent with little contribution from NO, the exact opposite is true with regard to local skin heating. In this study, oral clopidogrel produced a significant increase in cutaneous reactive hyperemia response, suggesting a potential benefit for clopidogrel on EDH-mediated cutaneous microvascular function. Altogether, evidence supporting that the pleiotropic effects of P2Y12-receptor inhibitors, beyond their antiplatelet activity, could potentially justify their preferential clinical selection in patients with vascular diseases.

FXa inhibitors

FXa and PAR signaling are implicated in ED, a hallmark of many diseases including diabetes [91]. In a mouse model of diabetes, it was shown that PAR-2 expression increased in the aorta and that FXa promoted JNK phosphorylation and reduced endothelial nitric oxide synthase (eNOS) phosphorylation levels. Interestingly, mice deficient in PAR-2 did not exhibit ED. Rivaroxaban administration for 3 weeks to diabetic mice attenuated ED and promoted eNOS phosphorylation in the aorta [92].

GPIIb/IIIa receptor inhibitors

Given the beneficial effects of GPIIb/IIIa blockade on coronary stenting safety, studies have focused on identifying whether these drugs can prevent ED [93]. A clinical study conducted on patients with ED due to PCI confirmed the ability of tirofiban to enhance endothelial function [94]. Similar conclusions were reached for another GPI, abciximab, which was shown to enhance coronary blood flow response to acetylcholine, hence microvascular endothelial function, in patients who underwent coronary stenting [95]. The mechanism by which GPIIb/IIIa inhibition can improve endothelial function depends on the enhancement of the Akt/eNOS signaling pathway, hence endothelium-dependent NO-cGMP signaling [96,97]. On the other hand, multiple lines of evidence support an endothelial regenerative role for tirofiban, besides its antiplatelet effects, via the vascular endothelial growth factor (VEGF)/VEGF receptor 2 (VEGFR2)/pAkt axis that counteracts endothelial apoptosis [98] as summarized in Table 2.

Ischemic stroke

Not only is stroke the leading cause of death and disability worldwide, but also its prevalence is expected to increase dramatically by 2030 [99,100]. As such, proper care and improved recurrent stroke prevention strategies are prudent. Ischemic stroke results from reduced cerebral blood flow due to several causes such as cardiac embolism, occlusion of cerebral vessels, or atherosclerosis affecting the cerebral circulation [101]. Thrombin levels increase following ischemia which could further induce neural damage [102]. Moreover, several inflammatory biomarkers are identified as predictors of stroke incidence and prognosis thereafter [103,104]. High-sensitivity C-reactive protein (hsCRP), a marker of inflammation, is strongly associated with stroke and is considered an independent predictor for acute ischemic stroke [105,106]. Potential pleiotropic effects of antithrombotic drugs on these pathways are depicted in Figure 2B.

Direct thrombin inhibitors

The pleiotropic effects of dabigatran were assessed in acute ischemic stroke, a condition where inflammatory responses and platelet activity are amplified [107]. Significantly, when given to patients post-stroke, dabigatran produced an anti-inflammatory effect where both levels of IL-6 and hsCRP were reduced in patients' serum [107]. Interestingly, an additional study on bivalirudin, another direct thrombin inhibitor, confirmed this anti-inflammatory effect. Bivalirudin attenuated hemostatic activation in rats following cardiopulmonary bypass with a trend towards lower IL-6 and IL-10 levels [108].

FXa inhibitors

In light of the favorable outcomes in stroke patients receiving oral anticoagulants, researchers investigated the potential pleiotropic effects of FXa inhibitors administration prior to or in the early phase of stroke occurrence. The results of studies conducted on several animal models in this context are summarized in Table 3.

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Table 2 Main findings of studies reporting pleiotropic effects of *GPIIb/IIIa receptor inhibitors* in cardiovascular and metabolic disorders

Disease	Molecule	Conducted in	Main findings	References
ED	Tirofiban	Patients with CAD undergoing PCI	↑ Flow-mediated vasodilation in forearm vessels	[94]
		Patients with CAD undergoing PCI	↑ Acetylcholine-induced vasodilation	[96]
		Male Wistar rats, HUVECs	↑ PI3K/Akt/eNOS pathway	[97]
		HUVECs	↑ VEGF, ↑ endothelial layer proliferation/migration	[347,348]
		HUVECs	↑ VEGF/VEGFR2/pAkt, ↓ endothelial apoptosis	[98]
		HUVECs	↑ Growth on vascular scaffolds	[349]
	Abciximab	Patients undergoing PCI	↑ Coronary blood flow response to acetylcholine	[95]
Cardiac injury	Tirofiban	Patients with ACS	↓ Serum hs-CRP, IL-6 and sICAM-1	[142,143]
		Patients with STEMI	↑ Cardiac function	[144]
		Sprague–Dawley with ischemia/reperfusion	↑ Activation of cardiac PKCε, PI3K, Akt, p38 MAPK, and ERK1/2	[145]
	Abciximab	Patients with acute MI	↓ Mac-1 on leukocytes	[139]
		THP-1 cells	Binds to Mac-1/prevents Mac-1 interaction with different ligands	[140]
	Eptifibatide	ACS patients undergoing PCI	↓ sCD40L and RANTES	[146,147]
Ischemia/reperfusion injury	Abciximab	Isolated perfused rat heart model of ischemia	↑ Cardiac contractile function, inhibition of inhibition of leukocyte–EC interactions	[167]
		Isolated rat heart model of ischemia/reperfusion	↓ Platelet-mediated myocardial injury following I/R	[168]
Metabolic diseases	Eptifibatide	Human aortic smooth muscle cells (HASMCs)	↓ Insulin-induced VSMC proliferation, suppress insulin-mediated increase in focal adhesions	[211]

Atherosclerosis

Previously regarded as a simple obstruction of the arterial wall by fatty deposits, atherosclerosis is now viewed as a disease characterized by chronic inflammation that involves interactions between platelets, leukocytes, vascular smooth muscle cells (VSMCs), and ECs [109]. Figure 2C depicts the effects of different antithrombotic drugs on the atherogenic processes.

ADP P2Y$_{12}$-receptor antagonists

Platelets were identified as key players in immunity linking inflammation to atherogenesis [110], a process that is highly driven by platelet P2Y12 receptor [111]. Nonetheless, extensive evidence supported that vascular P2Y12 receptors also play a role in regulating the inflammatory response that contributes to the progression of atherosclerosis. P2Y12-deficient vessels demonstrated a protective phenotype against the development of atherosclerotic lesions in comparison to P2Y12-expressing vessels, independent of platelet activation [112]. Moreover, ADP-P2Y12 signaling in VSMCs is responsible for up-regulation of MCP-1 and enhancing monocyte recruitment, which eventually leads to vascular inflammation [113]. Therefore, various studies on P2Y12-receptor antagonists exhibited marked efficacy in reducing atherogenesis. Clopidogrel significantly prevented the development of atherosclerotic lesions and leukocyte infiltration in aortic vessels of ApoE$^{-/-}$ mice on a high-fat diet [114]. However, the use of such antiplatelet therapy alone might not be effective in reversing the remodeling of atherosclerotic lesions, hence suggesting a prophylactic benefit of clopidogrel. Likewise, treatment with ticagrelor reduced ED and atherogenesis in ApoE$^{-/-}$ mice manifested by an enhancement in acetylcholine-mediated relaxation and a reduction in the number of atherosclerotic lesions, respectively. Such effects were thought to be mediated by a down-regulation of inflammatory molecules [115]. The superiority of ticagrelor compared to clopidogrel in decreasing atherogenesis was attributed to its unique ability to up-regulate paraoxonase-1, an anti-atherosclerotic molecule, even though platelet inhibition was exerted equally by both drugs [116]. Additional anti-inflammatory effects of ticagrelor in the context of atherosclerosis are summarized in Table 1.

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Table 3 Main findings of studies reporting pleiotropic effects of *FXa inhibitors* in cardiovascular and metabolic disorders

Disease	Molecule	Conducted in	Main findings	References
ED	Rivaroxaban	STZ-induced diabetic and $PAR2^{-/-}$ mice and HCAECs	↓ ED, ↑ aortic eNOS phosphorylation	[92]
		Glucose-stimulated HUVECs	↑ Cell viability, ↓ glycemic excursion-induced apoptosis and ROS production	[207]
Ischemic stroke	Apixaban	Acute ischemic stroke patients newly prescribed anti-thrombotic agents	↓ Serum IL-6 and hsCRP	[107]
		Mouse model of focal cerebral ischemic stroke through occlusion of the middle cerebral artery	↓ Thrombin activity in the ipsilateral hemisphere and the infarct area	[102]
	Rivaroxaban	Prophylactic treatment prior to transient cerebral artery occlusion in a rat model of brain I/R injury	↓ Thromobin generation, infarct size, ICAM-1 expression, $CD68^{+}$ immune cell infiltration, ↑ behavioral and motor symptoms post-stroke	[350]
Cardiac injury	Edoxaban	Canine model of congestive heart failure	Suppresses ventricular expression of fibrotic markers	[152]
	Rivaroxaban	Intermittent hypoxia in microvascular ECs	↓ PAR-2 expression, ERK-1/2 and NF-κB signaling	[148]
		Paigen diet (21.4% protein, 27.4% fat, and 51.2% carbohydrate)-fed SR-BI KO/$ApoeR61^{h/h}$ mice subjected to ischemic cardiomyopathy	↑ Survival rate, ↓ heart failure, cardiac fibrosis, and cardiac expression of pro-inflammatory genes	[149]
		Angiotensin-II-induced cardiac fibroblasts	↓ Cellular migration, proliferation and production of inflammatory cytokines, ↓ NF-κB and MAPK/AP-1 signaling pathways	[150]
		HfpEF patients	↓ Circulating markers of fibrosis and diastolic dysfunction	[151]
Atherosclerosis	Apixaban	Randomized, double-blinded, placebo-controlled, phase 2 study in patients with either ST-segment elevation or non-ST-segment elevation ACS	↓ Inflammation mediated by platelets and leukocytes	[122]
	Rivaroxaban	$ApoE^{-/-}$ atherosclerotic mice	↓ Aortic expression of pro-inflammatory cytokines and adhesion molecules and stabilizes atherosclerotic plaques	[123]
		$ApoE^{-/-}$ mice on pro-atherosclerotic Western diet (15% cocoa butter, 1% corn oil, 0.25% cholesterol, 40.5% sucrose, 10% cornstarch, 20% casein, free of cholate, total fat content 16%)	↓ Plaque burden and positively influences ECM post-infarction remodeling, ↓ onset of atherosclerosis, ↑ plaque regression and enhances plaque stability	[351]
		$ApoE^{-/-}$ mice on pro-atherosclerotic Western diet	↓ Aatherosclerotic lesion progression, lipid deposition, collagen loss, macrophage accumulation and MMP-9 expression in plaques of the aortic root and ↓ the expression of inflammatory markers in the abdominal aorta	[352]
		HUVECs and mouse model of ischemic hindlimb	↓ FXa-induced senescence and FXa-mediated impaired angiogenesis	[127]
		High glucose-treated HUVECs and spontaneous type 2 diabetic rats (ZFDM $LepR^{fa/fa}$)	↓ HG-induced expression of SA-β-gal, p53, p21 and $p16^{INK4a}$, restores telomerase activity, ↓ O_2^{-}, $p22^{phox}$ and ICAM-1 and restores NOX and eNOS activity, ↓ PAR-1 up-regulation	[128]

Continued over

Table 3 Main findings of studies reporting pleiotropic effects of *FXa inhibitors* in cardiovascular and metabolic disorders (Continued)

Disease	Molecule	Conducted in	Main findings	References
		HFD-fed rat model of atherosclerosis obliterans	↓ Irregular narrowness of femoral artery lumen, disordered smooth muscle cells, ECs and internal elastic plates ↑ HDL levels and ↓ total cholesterol, LDL, and TG levels ↓ TLR-4/NF-κB signaling	[129]
		FXa-stimulated VSMCs	↓ VSMCs senescence and pro-inflammatory cytokines production	[130]
Ischemia/reperfusion injury	Rivaroxaban	Rat model of peripheral I/R	↓ Systemic oxidant damage	[169]
		Mice subjected to I/R	↑ Survival rate, cardiac function, ↓ inflammation and left ventricular fibrosis	[170]
Arrhythmia	Rivaroxaban	Rabbit left atrium and isolated single left atrium cardiomyocytes	↓ Left atrial action potential duration and ↑ L-type Ca^{2+} current and ultrarapid rectifier K^+ current without affecting transient outward K^+ current	[189]
		NVAF patients	Anti-inflammatory activity, ↑ soluble thrombomodulin levels	[124,192,193]
	Edoxaban	Mouse model of AF and vascular ECs	↓ Expression of pro-inflammatory cytokines, ↑ the expression levels of prostacyclin and prostaglandins, and regulates the Wnt-β-induced PI3K/ATK-activated protein C system	[194]
	Apixaban	NVAF patients	Anti-inflammatory activity, ↑ soluble thrombomodulin levels	[192]
Diabetic nephropathy	Edoxaban	$Ins2^{Akita/+}$ $eNOS^{+/+}$, $eNOS^{+/-}$ and $eNOS^{-/-}$ mice, $F2rl1^{-/-}$; Ins2Akita/+ : $eNOS^{+/-}$ mice, and human ECs	↓ PAR expression, glomerulosclerosis, renal pro-inflammatory markers, albuminuria, mesangial proliferation, and fibrosis	[202]

FXa inhibitors

Platelets significantly contribute to atherogenesis and atherothrombosis, either through direct cell-to-cell interaction or via platelet-derived signaling molecules [117]. In addition to EC injury, PAR-2 signaling plays a pivotal role in the early events leading to the development of atherosclerosis [118–120]. It was also shown that FXa aggravates atherogenesis through PAR-2-mediated inhibition of macrophage autophagy, and thus the activation of inflammasomes [121]. Moreover, apixaban attenuated inflammation mediated by platelets and leukocytes in patients suffering from ACS, a major complication of atherosclerosis [122]. Rivaroxaban reduced the aortic expression of pro-inflammatory cytokines and adhesion molecules in a model of advanced atherosclerosis [123,124].

The participation of PAR-2 in the pathogenesis of vascular inflammation and atherosclerosis has been demonstrated in $ApoE^{-/-}$ mice. PAR-2-associated vascular inflammation and macrophage activation included the activation of the NF-κB signaling pathway. $ApoE^{-/-}$ $PAR2^{-/-}$ mice exhibited reduced atherosclerotic lesions in the aortic arch along with features of stabilized atherosclerotic plaques compared to $ApoE^{-/-}$ mice [121]. FXa inhibition with rivaroxaban was shown to have an anti-atherosclerotic effect. Rivaroxaban treatment of $ApoE^{-/-}$ mice on pro-atherosclerotic Western diet decreased plaque burden and positively influenced ECM post-infarction remodeling [125,126]. Similar findings are also summarized in Table 3.

Moreover, incubation with FXa significantly decreased HUVECs proliferation and increased the expression of insulin-like growth factor binding protein-5, EGR-1, p53, and $p16^{INK4a}$. Rivaroxaban reduced FXa-induced senescence. Rivaroxaban also reversed the FXa-mediated impaired angiogenesis in an ischemic hindlimb mouse model [127]. Maeda et al. recently showed that Rivaroxaban reversed the high glucose-induced increased expression of SA-β-gal, p53, p21, and $p16^{INK4a}$ in HUVECs. FXa inhibition by rivaroxaban also restored telomerase activity, suppressed O_2^-, $p22^{phox}$, and ICAM-1 and restored NOX and eNOS activity. Incubation with high glucose also caused PAR-1 elevation that was reversed by Rivaroxaban. These results were also shown *in vivo* in dyslipidemic diabetic mice. As such, rivaroxaban restored endothelial function and prevented the progression of atherosclerosis [128]. In addition, a rat model of atherosclerosis obliterans showed an irregularly narrowed femoral artery lumen, disordered smooth muscle cells, ECs, and internal elastic plates. These vascular derangements were reversed by rivaroxaban intragastric administration. In addition, rivaroxaban treatment increased HDL levels and decreased total cholesterol, LDL, and TG levels. Rivaroxaban exhibited its anti-atherosclerotic effects via the toll-like receptor 4/NF-κB signaling pathway [129].

Within atherosclerotic plaques, FX is produced in VSMCs, inflammatory cells and ECs. Chronic FXa stimulation induced VSMCs senescence accompanied by p53 and IGFBP-5 up-regulation *in vitro*. FXa inhibition with rivaroxaban reduced FXa-induced VSMC senescence and pro-inflammatory cytokines production. Thus, locally produced FX may contribute to atherosclerosis progression and its inhibition partially ameliorates atherosclerotic plaque deposition [130]. Given these effects of FX in the initiation and progression of atherosclerosis, the anti-atherosclerotic pleiotropic potential of FXa inhibitors requires further in-depth investigation.

Cardiac injury

Cardiac remodeling occurs in response to cardiac pathophysiological stimuli. Chronic structural and molecular alterations result from several factors including myocardial infarction (MI), ischemia/reperfusion (I/R), and pressure and volume overload [131]. Inflammation is a critical mediator of cardiac remodeling. Indeed, following cardiac injury, the inflammatory state is sustained through continuous cytokine release [132]. Figure 3A demonstrates the possible modifying effects imparted by the different drug classes.

ADP $P2Y_{12}$-receptor antagonists

P2Y12-receptor-dependent platelet activation promoted cardiac remodeling and dysfunction through inducing inflammatory changes in a pressure overload-triggered cardiac remodeling mouse model [133]. In fact, P2Y12 knockout mice exhibited suppressed cardiac remodeling including changes in cardiac hypertrophy, collagen synthesis, inflammation and cardiac dysfunction [133]. Previous reports also revealed that clopidogrel treatment in patients undergoing stenting reduced the release of inflammatory and cardiac markers [134]. Clopidogrel treatment also alleviated markers of cardiac inflammation and fibrosis in a mouse model of hypertension [135]. Likewise, ticagrelor demonstrated beneficial effects in alleviating structural and functional changes in myocardial tissues and improving cardiac healing following left anterior descending coronary artery ligation in pigs [136]. The cardioprotective mechanism of ticagrelor was attributed to enhancement of AMPK and Akt/PKB activation and reduction of aquaporin-4 expression, which culminate in alleviating the formation of edema [136]. As mentioned previously ticagrelor may exert its cardioprotective effects via adenosine-mediated mechanisms [83]. These adenosine-related off-target effects of ticagrelor may play a role in decreasing necrosis in heart tissue and alleviating edema formation [137]. The observed benefit of ticagrelor in myocardial recovery is supported by the results of the HEALING-AMI trial, in which it was shown to be superior to clopidogrel for left ventricular remodeling after reperfusion in patients with ST-segment elevation MI [138].

GPIs

The potential cardioprotective effects of GPIs reported by many studies are brought about by their ability to modulate inflammation that is at the basis of pathogenesis of cardiac injury. Abciximab, which represents the most widely studied GPI, was found to interrupt platelet–leukocyte interaction in MI patients and the up-regulation of leukocyte receptor Mac-1 (αMβ2) on monocytes, an integrin that mediates leukocyte adhesion [139]. Given the detrimental outcomes of the interaction of leukocytes with the microvasculature on reperfusion following MI, the use of abciximab may be a favorable approach for such condition. Not only does abciximab hinder platelet interaction with other cell types, it can also bind to Mac-1 itself on monocytes and prevent its adhesion to multiple ligands, including fibrinogen, ICAM-1, inactivated complement factor 3b (iC3b) and FX [140]. These anti-inflammatory effects were consistent irrespective of the route of administration during PCI, i.e. whether administered via intracoronary or intravenous routes [141].

Another GPI, tirofiban, demonstrated similar effects as well, where a significant reduction in the serum levels of hs-CRP, IL-6, and sICAM-1 was noted in patients with acute MI undergoing PCI [142,143]. Also, perioperative administration of tirofiban in ST-segment elevation MI patients undergoing PCI was accompanied by an enhancement in cardiac parameters and endothelial function [144]. These cardioprotective effects of tirofiban are at least partially mediated by the activation of several signaling pathways, including PKCε, PI3 kinase, Akt, p38 MAPK, and extracellular signal-regulated kinase 1/2 (ERK1/2) [145]. The third GPI, eptifibatide, was shown to elicit similar effects to that of abciximab, where it significantly decreased circulating leukocyte-platelet aggregates and soluble CD40 ligand, which are implicated in the progression of atherosclerosis [146,147]. Thereby, GPIs may be considerable therapeutic options for suppressing the inflammatory reaction in patients with cardiac injury due to MI.

FXa inhibitors

The emerging pleiotropic effects of direct FXa inhibitors prompted researchers to investigate the potential effects of FXa inhibition on cardiac remodeling. Indeed, rivaroxaban reversed the increases in PAR-2 expression as well as ERK-1/2 and NF-κB signaling induced by intermittent hypoxia in microvascular ECs via a PAR-2 dependent pathway [148]. Rivaroxaban also improved survival rate in HFD-fed ApoE$^{-/-}$ mice that developed MI. In addition, Rivaroxaban attenuated heart failure, cardiac fibrosis, and cardiac expression of pro-inflammatory genes [149].

As cellular migration, differentiation, and proliferation of cardiac fibroblasts are major drivers of cardiac fibrosis, Hashikata *et al.* investigated the potential ability of rivaroxaban to modulate angiotensin-II-induced cardiac fibroblasts function. Rivaroxaban significantly reduced cellular migration, proliferation, and production of inflammatory cytokines. In addition, rivaroxaban inhibited inflammatory signaling via NF-κB and MAPK/AP-1 pathways inhibition [150]. In one study, 1-year old PAR-2$^{-/-}$ mice suffered from diastolic dysfunction with an increased expression of α-smooth muscle actin, collagen deposition, endothelial activation and inflammation. This was attributed to a PAR2-dependent caveolin-1 down-regulation leading to an enhanced pro-fibrotic PAR-1 and TGF-β signaling [151]. The treatment with vorapaxar, a PAR-1 antagonist reversed these derangements and reduced inflammation. Upstream PAR inhibition in patients treated with FXa inhibitors also reduced circulating markers of fibrosis and diastolic dysfunction [151]. Moreover, in a canine model of congestive heart failure edoxaban suppressed ventricular expression of fibrotic markers including atrial fibronectin and PAR-2 [152]. Thus, the modulation of the FXa-FIIa/PAR-1/PAR-2/TGF-β is a promising therapeutic approach in heart failure with preserved ejection fraction (HfpEF) [151].

I/R injury

Tissues and organs behave differently in their susceptibility following I/R. However, they share common characteristic responses which include increases in the generation of ROS, cytokines, chemokines, and other immune-modulatory components [153,154]. ROS formation is induced following I/R leading to an increased intracellular calcium concentration and a hypercontractile state causing subsequent apoptotic and oncotic cell death [155–157]. Myocardial cells are exquisitely sensitive to ischemia. Indeed, I/R stimulates fibroblast formation, mast cell and T-cell modulation which induce cardiac fibrosis [132]. The immune response initiates inflammatory reactions that play a prominent role in the reperfusion component of total tissue in I/R following MI [158]. Reperfusion following cardiac ischemia is paradoxically associated with cardiac injury, and prompt revascularization remains the cornerstone therapeutic approach to prevent cardiac damage [159]. Some of the modulatory effects exerted by antithrombotic drugs under these circumstances are shown in Figure 3B.

ADP $P2Y_{12}$-receptor antagonists

Ticagrelor has been identified as a promising molecule to prevent cardiac defects following reperfusion. In a rat model of I/R injury, an acute single dose of ticagrelor prior to reperfusion was shown to augment adenosine levels, enhance the activation of Akt, ERK1/2, and eNOS. These mechanisms led to an improvement in cardiac function accompanied by a significant reduction in cardiac fibrosis and infarct size [160]. Similarly, treatment of rats undergoing coronary artery ligation with ticagrelor led to a cardioprotective effect against I/R through enhancement of adenosine levels in cardiac tissue, leading to an attenuation of the expression of the NLRP3 inflammasome components and an increase in anti-inflammatory markers [161]. In the same rat model, ticagrelor actions were documented to be mediated by inhibiting the NF-κB signaling pathway [162]. Moreover, ticagrelor reduced left-ventricular remodeling after reperfusion injury in rats. Interestingly, ticagrelor enhanced the levels of markers of regenerative cells, which is suggestive of cardiac healing [163]. Similarly, clinical evidence has supported a potential role for clopidogrel in cardio-protection against reperfusion injury [164], in a mechanism that is thought to involve signal transduction during reperfusion rather than inhibition of intravascular coagulation [165]. In fact, administration of a single dose of clopidogrel prior to an episode of I/R demonstrated protective effects [166].

GPIs

Considering the early identified anti-inflammatory properties of GPIs, their potential role in I/R has been a subject of interest to many studies. In an isolated perfused rat heart model of I/R, abciximab effectively maintained cardiac contractile function through inhibition of leukocyte–EC interactions and platelet–EC interaction [167]. Consistently, another study reported that abciximab suppresses platelet-mediated myocardial injury following I/R [168].

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FXa inhibitors

Rivaroxaban has been proven to be as effective as LMWH in protecting against systemic oxidant damage caused by I/R [169]. Furthermore, rivaroxaban improved survival rate in mice subjected to I/R. As well, rivaroxaban improved cardiac function, inflammation and inhibited the left ventricular fibrosis [170].

Arrhythmia

There exists a multidirectional relationship between coagulation, inflammation, and AF, as coagulation itself is associated with an increased risk of arrhythmias development and progression [171]. AF is an abnormal heart rhythm that is characterized by chaotically irregular beating of the atria that increases the risk of strokes, heart failure, and other cardiac complications. The most significant complication of AF is thromboembolism, a risk that is further increased with coexisting type 2 diabetes [172,173]. Additionally, AF promotes a hypercoagulable state that is associated with pro-inflammatory pathways [174]. AF-related stroke has been recently treated with DOACs including dabigatran, rivaroxaban, apixaban, and edoxaban [175,176]. Although these drugs did not alter AF-induced augmentation of serum prothrombotic markers in comparison with conventional anticoagulants [175,176], emerging lines of evidence suggest pleiotropic, disease-modifying antiarrhythmic actions of DOACs on the electrical and structural remodeling and inflammation in AF [174] (Figure 3C). Therefore, DOACs may provide therapeutic benefits in AF exceeding thrombo-prophylaxis, by preventing cardiac arrhythmogenesis and progression into persistent arrhythmia forms and suppressing inflammation.

Importantly, coagulation promotes the development of AF and its progression not only through the action of thrombin on PARs, but also through the involvement of type-2 ryanodine receptors, L-type Ca^{2+} channels and voltage-gated Na^{+} channels [177]. Indeed, thrombin-mediated PAR activation was demonstrated to induce changes in Ca^{2+} fluxes and subsequently affecting heart rhythm [178,179]. Similarly, the local administration of thrombin or the PAR-1-activating peptide, TRAP, in isolated rat hearts subjected to acute MI caused ventricular tachycardia and fibrillation, which were attenuated following the treatment with a thrombin inhibitor [180,181]. Moreover, TRAP-mediated activation of PAR-1 was found to stimulate the hydrolysis of phosphoinositide, with the subsequent increase in cytosolic Ca^{2+} levels in ventricular cardiomyocytes [179]. This was reversed by the depletion of the sarcoplasmic reticulum Ca^{2+} stores with caffeine, suggesting the involvement of type-2 ryanodine receptors in thrombin-mediated effects [182]. In fact, studies carried out on ventricular cardiomyocytes suggest that thrombin promotes early afterdepolarizations and thus increased the heart rate through increasing inositol triphosphate (IP3) levels and subsequently diastolic and systolic Ca^{2+} levels [179]. Thrombin was also shown to prolong the action potential duration of guinea pig ventricular papillary muscle, which was reversed by tetrodotoxin, suggesting the involvement of voltage-gated Na^{+} channels [183]. Indeed, the action of thrombin on PAR-1 gives rise to multiple proarrhythmogenic mediators. The formation and accumulation of lysophosphatidylcholine (LPC), a proarrhythmogenic lipid mediator generated by PLA_2, are driven by the action of thrombin on PAR-1 [184]. Indeed, the formation and accumulation of LPC strongly promotes arrhythmogenesis in isolated ventricular cardiomyocytes [185–187]. Similarly, plasmalemmal N^{+}–H^{+} exchanger-1 (NHE-1) activity is stimulated by thrombin which leads to Ca^{2+} overload and thus, contributes to ventricular fibrillation [188]. Nevertheless, whether this thrombin-mediated Ca^{2+} overload can be overcome by DOACs remains to be investigated. Moreover, thrombin-mediated PAR-1 activation in freshly isolated human atrial cardiomyocytes directly modified atrial electrophysiological properties through augmenting late Na^{+} currents [183]. Also, the inhibition of FXa with rivaroxaban directly reduced left atrial action potential duration and increased both L-type Ca^{2+} current and ultra-rapid rectifier K^{+} current without affecting transient outward K^{+} current [189].

In addition to the anti-arrhythmogenic effect, FXa inhibition was shown to possess an anti-fibrotic activity [190,191]. As atrial fibrosis is a major contributor to AF, researchers were prompted to investigate the potential of FXa inhibitors to suppress AF [190,191]. As such, both apixaban and rivaroxaban were shown to exert an anti-inflammatory activity in non-valvular AF (NVAF) patients [192]. Indeed, rivaroxaban treatment in patients with NVAF resulted in an increase in soluble thrombomodulin, which was suggested to partly account for the observed anti-inflammatory effect [124]. Clinically, there was no significant difference in the anti-inflammatory potential of dabigatran and rivaroxaban in NVAF patients [193]. In a mouse model of AF, edoxaban down-regulated the expression of pro-inflammatory cytokines and increased the expression levels of prostacyclin and prostaglandins. Notably, edoxaban treatment up-regulated PI3K and Akt expression and significantly stimulated Wnt-β phosphorylation. Thus, edoxaban exerted a beneficial effect on venous thromboembolism in mice with AF through the regulation of the Wnt-β-induced PI3K/ATK-activated protein C system [194]. The inhibition of FXa-mediated thrombin generation in goats with persistent AF decreased endomysial fibrosis, and thus reduced the complexity of AF substrate

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[171]. Additionally, thrombin was recently shown to enhance Akt and Erk-mediated pro-fibrotic signaling in isolated rat atrial fibroblasts, in addition to increasing the expression of TGF-β1 and MCP-1 and collagen deposition [171]. All of these thrombin-provoked derangements were abrogated by dabigatran. This provides evidence for the pleotropic effects of both classes of DOACs not only in mitigating the thrombotic risk associated with AF, but also reducing its substrate and hindering its pro-fibrogenic and pro-inflammatory consequences when present.

Pleiotropic effects of anticoagulant drugs in metabolic diseases

The development of diabetes and its complications is greatly driven by inflammation and imbalanced coagulation [195]. Complications of diabetes include diabetic nephropathy, retinopathy, cardiomyopathy, and foot ulceration. Several studies highlighted that the inhibition of FXa by direct FXa inhibitors and, thus, suppressing subsequent downstream PAR signaling ameliorates diabetic complications. Indeed, studies have shown an increased tissue PAR expression in different forms of metabolic dysfunction in humans and experimental animals including obesity, obese and non-obese diabetes, produced due to genetic predisposition, high-fat diets, or immune-mediated β-cell destruction [196]. In this regard, not only was PAR signaling shown to promote inflammatory responses [197], it was also implicated in reinforcing the metabolic insult where it promoted obesity and insulin resistance in high-fat fed mice [198]. Consistently, *in vivo* inhibition of PAR2 delayed weight gain in a rat model of diet-induced obesity where elevated free fatty acids were thought to induce PAR2 expression in adipocytes [199].

Regarding diabetic complications, several studies demonstrated that the expression of renal FX and FXa activity as well as the expression of FX in glomerular macrophages increases in type 2 diabetes. Importantly, PAR-2 cleavage by FXa and subsequent PAR-2 signaling was shown to up-regulate inflammation and renal fibrosis through NF-κB and MAPK signaling [9,200,201]. Interestingly, edoxaban was shown to ameliorate diabetic nephropathy and down-regulated the expression of PARs [202]. Moreover, accumulating evidence suggests that the lack or reduction of renal eNOS exacerbates diabetic nephropathy and is associated with an increased expression of renal TF [203,204]. In this context, diabetic $eNOS^{-/-}$ mice demonstrated increased renal expression of TF and FX [205,206]. Importantly, edoxaban corrected glomerulosclerosis, decreased renal pro-inflammatory markers expression, attenuated albuminuria, mesangial proliferation, and fibrosis not only in $eNOS^{-/-}$ but also in $eNOS^{+/-}$ diabetic mice. Additionally, edoxaban reduced renal PAR expression in $eNOS^{-/-}$ diabetic mice. It was also demonstrated that the lack of PAR-2 attenuated inflammation and tissue fibrosis in diabetic nephropathy [206]. Therefore, targeting PAR-2 signaling must be further investigated as a potential valuable target in the management of diabetic nephropathy. As FXa and PAR signaling are implicated in ED, a hallmark of diabetic complications, Pham *et al.* demonstrated an increased expression of PAR-2 in the aorta of diabetic mice [92]. Moreover, FXa was shown to promote JNK phosphorylation and reduce eNOS phosphorylation levels. Interestingly, $PAR\text{-}2^{-/-}$ mice exhibited no ED and rivaroxaban-treated wildtype mice exhibited attenuated ED and enhanced eNOS phosphorylation in the aorta [92]. Another study highlighted that glucose-stimulated HUVECs treated with rivaroxaban at varying concentrations, exhibited increased cell viability and inhibited glycemic excursion-induced apoptosis and ROS production [207]. On the other hand, diabetes was shown to increase PAR expression in mouse aorta [208]. In this model, dabigatran treatment improved endothelium-dependent relaxation and inhibited thrombin-mediated endothelial inflammation.

Diabetic cardiomyopathy also represents a major complication of diabetes. Indeed, the expression levels of PAR increases during the course of diabetes development and the activation of PAR-1 by thrombin in cardiac fibroblasts increased the expression of TGF-β and matrix metalloproteinase 2 and 9 promoting cardiac injury [209]. Argatroban was demonstrated to significantly reduce plasma glucose and improve plasma insulin levels in the animal model. Importantly, argatroban inhibited the expression of PAR-1, PAR-4, TGF-β, and cyclooxygenase 2 (COX-2), and up-regulated the sarco/endoplasmic reticulum calcium ATPase through the inhibition of PAR-1, which in turn improved cardiac contractility [210].

Since early stages of metabolic dysfunction involve hyperinsulinemia, enhanced proliferation of smooth muscle cells through insulin signaling might be observed [211]. Indeed, αvβ3 integrins present on vascular cells influence insulin-dependent proliferative signaling. As insulin displayed a dose-dependent proliferation of human smooth muscle cells, this effect was completely abolished following the pre-treatment with eptifibatide, but not tirofiban [211]. Moreover, targeting αvβ3 integrins with eptifibatide inhibited insulin-stimulated increase in the number of vinculin-containing focal adhesions [211] highlighting the inflammatory role of GPIIb/IIIa in VSMC dysfunction in this early stage of metabolic dysfunction. In the same context, soluble dipeptidyl peptidase 4, a novel adipokine whose levels are increased in obesity and metabolic syndrome [212], appeared to induce human VSMC proliferation and inflammation in a PAR2-dependent manner [213]. Upon PAR2 silencing, increased cell proliferation, ERK1/2

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activation, and NF-κB-mediated IL-6 and IL-8 production were reversed [213]. These observations highlight the importance of the inhibition of FXa/PAR signaling axis and the potential vascular and metabolic impact of drugs inhibiting these factors in metabolic disorders.

Pleiotropic effects in neurodegenerative diseases

Cardiovascular dysfunction as well as metabolic impairment with the accompanying inflammatory changes constitute stressors that elicit microvascular brain responses. Indeed, inflammation is at the core of neurodegenerative and cardiometabolic disease where indicators of both vascular and cognitive dysfunction co-exist not only in type 2 diabetic patients [214], but also in the prediabetic stage preceding the diagnostic features of diabetes [215]. In this regard, it is crucial to recognize the extensive crosstalk between inflammation and coagulation pathways that are synchronously triggered in response to injury [216]. Metabolic dysfunction leads to endothelial activation triggering the secretion of pro-inflammatory cytokines [208] and coagulation factors inducing thrombin production [217], which in turn exacerbates the secretion of pro-inflammatory mediators [218] and leads to a closed cycle of continuous endothelial activation, vascular inflammation and deterioration of the neurovascular function. This is particularly relevant to the action of DOACs, which would uniquely fit as pharmacological tools to interrupt this loop and ameliorate thrombin-mediated neurovascular inflammation in conditions such as Alzheimer's disease (AD) [219], traumatic brain injury (TBI) [220], and Parkinson's disease (PD) [221,222]. While direct studies examining the DOAC brain–blood barrier penetration have not been forthcoming, a recent study examined the association between molecular correlations of different pharmacokinetic properties of DOACs and the reported adverse effects [223]. This study demonstrated that rivaroxaban is the most likely to demonstrate brain–blood barrier penetration, whereas dabigatran is the least despite being associated with a higher risk of intra-cranial hemorrhage. On the other hand, literature shows that during TBI and neurodegenerative diseases such as AD and PD the integrity of the brain–blood barrier is altered [224,225]. Such a disruption might contribute to therapeutic delivery whereby DOACs gain entry into the affected CNS and ameliorate neuronal inflammation and toxicity [226,227]. Thrombin-induced neurotoxicity and neuroinflammation are orchestrated by multiple pathways and its effects on neuronal cells are concentration-dependent, being protective at lower concentrations and detrimental in higher ones [227]. In the mechanisms listed below, we focused on the detrimental effects of thrombin in various neurological conditions.

As mentioned above, thrombin is cleaved from prothrombin by FXa. The major source of thrombin is blood; however, transcripts of prothrombin and FX are present in the central nervous system [228,229]. Similar to the effects reported in the peripheral vasculature, thrombin has a significant effect on the cerebrovascular endothelium where it induces endothelial contraction, an effect that is suggested to be mediated via PARs and identified in the rat brain vascular ECs [230,231]. Apart from the vascular damaging effect, it has also been documented that following CNS injury prothrombin mRNA is up-regulated, suggesting that thrombin could be produced by neurons themselves [232,233]. PARs, mainly PAR-1, -3 and -4 are activated by thrombin and not only are they expressed in the CNS, but they also undergo up-regulation following brain ischemia [234]. Thrombin induces neuroinflammation and oxidative stress leading to neuronal injury in neurodegenerative diseases [235,236]. Studies on microglial cells indicated that thrombin stimulates the microglial synthesis of pro-inflammatory mediators such as TNF-α, IL-6 and -12, and CXCL1 [218]. Along with its role in exacerbating inflammation, thrombin is capable of exerting direct neurotoxicity via activation of PAR-1 followed by RhoA activation leading to induction of apoptosis in addition to up-regulation of NADPH oxidase and increased oxidative stress [237–239]. Moreover, thrombin induces up-regulation of the pro-apoptotic pathway through activating cyclin D1 and E as well as cyclin-dependent kinase, cdk4 [240]. In this regard, it was shown that thrombin provokes a rapid influx of calcium leading to neuronal apoptosis [241]. Thrombin-induced neurotoxicity comprises sustained activation of ERK and p38 MAPK pathways [242,243]. Indeed, at the early phase of thrombin exposure, ERK activation leads to several apoptosis-like features. Furthermore, MAPK, specifically MAPK-activated protein kinases 2, participates in inflammatory responses following ischemic cerebral injury via induction of IL-1β production [244,245]. On the other hand, JNK contributes to the regulation of neuronal and microglial thrombin-induced apoptosis [246]. This thrombin-induced microglial apoptosis is also associated with sustained activation of ERK and involves the Rho signaling pathway [247]. Evidence regarding the impact of different thrombin inhibitors on models of neuroinflammatory disease is summarized in Table 4.

Alzheimer's Disease

Alzheimer's Disease (AD) is a chronic neurodegenerative disease with a progressive cognitive and functional decline. The pathological hallmarks of AD are mainly amyloid plaques and neurofibrillary tangles that are related to the production and accumulation of the protein β-amyloid (Aβ) and hyperphosphorylated tau proteins, respectively

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Table 4 Main findings of studies reporting pleiotropic effects of *FIIa inhibitors* in cardiovascular, metabolic, and neuroinflammatory disorders

Disease	Molecule	Conducted in	Main findings	References
ED	Dabigatran	STZ-induced diabetic mice and HUVECs	↑ Endothelium-dependent relaxation and ↓ thrombin-mediated endothelial inflammation	[208]
Arrhythmia	Dabigatran	NVAF patients	Anti-inflammatory activity, ↑ soluble thrombomodulin levels	[193]
		Goats with persistent AF, isolated rat atrial fibroblasts	↓ Endomysial fibrosis, thrombin-induced up-regulation of TGF-β1 and MCP-1 and collagen deposition	[171]
AD	Dabigatran	TgCRND8 AD mice	↑ Cognitive function, cerebral perfusion, ↓ toxic fibrin deposition, and neuroinflammation	[266]
PD	Dabigatran	Rotenone model of PD	↑ Striatal dopamine levels, motor function, and ↓ neurotoxic pro-inflammatory cytokine production	[280]
		LRRK2 transgenic *Drosophila melanogaster* model of PD	↑ Locomotor activity by ↓ ROS	[235]
TBI	Argatroban	Intravascular perforation rat model	↑ BBB disruption, ↓ brain edema, apoptotic brain cell death, and expression of inflammatory markers	[293]
		C57BL/6 wildtype (WT) and complement3 KO mice following the induction of intracranial hemorrhage	↓ Thrombin-induced inflammation and complement pathway activation, and ↓ C5a receptor expression	[294]
Diabetic cardiomyopathy	Argatroban	HFD (powdered normal pellet diet 365 g/kg, lard 310 g/kg, acid casein 250 g/kg, cholesterol 10 g/kg, vitamin and mineral mixture 60 g/kg, DL-methionine 3 g/kg, yeast powder 1 g/kg, sodium chloride 1 g/kg)-fed STZ-induced diabetic rats	↓ Plasma glucose, plasma insulin levels, expression of PAR-1, PAR-4, TGF-β, and COX-2, and ↑ SR/ER calcium ATPase through ↓ of PAR-1, which in turn improves cardiac contractility	[210]

[248,249]. A large body of evidence identified vascular pathology and cardiovascular risk factors in the development of AD [250]. Indeed, studies have provided evidence that certain coagulation factors may mediate and exacerbate inflammation in CNS [251–253]. Thrombin and fibrin have been suggested as possible pathological mediators in AD. Along with thrombin pro-inflammatory role, thrombin signaling pathway has been linked to two major pathological hallmarks of AD, the τ and Aβ [254,255]. Aβ has been reported as a prothrombotic factor stimulating coagulation cascade and is linked to vascular events in AD patients [256,257]. Continuous thrombin signaling in AD induces Aβ which in turn promotes thrombin and fibrin generation via activation of factor XII [257,258]. Thrombin-induced Aβ accumulation and deposition around vascular walls leads to cerebrovascular dysfunction known as cerebral amyloid angiopathy which is typical of early development of AD [259]. This will impair neurovascular coupling and trigger microvascular occlusion, microhemorrhage and vasoconstriction leading to a decline in cerebral blood flow and perfusion and an exacerbation of the neurodegenerative process of AD [260,261]. Additionally, the endogenous CNS thrombin inhibitor produced by glial cells, protease nexin 1, has been shown to be markedly reduced in AD [262].

Previous studies have identified possible treatment approaches of AD by modulating coagulation pathways. A mouse model of AD receiving the LMWH, Enoxaparin, showed a reduced Aβ deposition, cytotoxicity, and inflammation together with improved cognition [263,264]. Thus, in the context of AD, the use of direct thrombin inhibitors, such as dabigatran, might offer a more convenient alternative to heparins given its ease of administration [265]. As such, the possible role of dabigatran to counteract cerebrovascular dysfunction in AD was assessed [266]. In AD mouse model, long-term treatment with dabigatran improved cognitive function, cerebral perfusion, and prevented toxic fibrin deposition. Concomitantly, neuroinflammation and amyloid deposition in the brain were ameliorated as evidenced by the significant reduction in amyloid plaques, oligomers, phagocytic microglia, and infiltrating T cells

[266]. These effects are summarized in Figure 4A. Nevertheless, it is important to note that antithrombotic interventions are not always associated with beneficial outcomes in patients suffering from dementia. Indeed, the Aspirin in Reducing Events in the Elderly (ASPREE) trial, enrolling a total of 19114 healthy individuals of 70 years of age or older, reported no prevention or reduction in the incidence of dementia [267]. Yet, several epidemiological studies indicated a beneficial role of aspirin in dementia [268,269]. Aspirin, 75 mg for 6 months, was associated with lower risk of incident dementia in patients with late onset of depression [269]. A comparative study on 351 twins revealed that individuals on high dose aspirin had lower prevalence of AD and maintained better cognitive function than those on placebo [268]. Indeed, the neuroprotective effects of aspirin such as enhancing the memory, anti-inflammatory and enhancing the Aβ clearance have been demonstrated [270,271]. Low-dose aspirin ameliorated amyloid pathology in a mouse model of AD, the pathway was linked to PPARα activation [272]. Therefore, it is thought that the absence or low basal levels of PPARα, as in older individuals with AD and dementia, could decrease the optimal activity of aspirin. Clinical trials are needed to confirm the present hypothesis on the favorable outcomes of such interventions on AD manifestations and progression.

Parkinson's Disease

Parkinson's Disease (PD) is a neurodegenerative disease that predominantly affects the dopaminergic neurons in the substantia nigra coupled with Lewy body formation, which mainly affects motor function [273]. Thrombin has previously been identified as a possible pathological mediator in PD [274]. Moreover, in patients with PD, PAR-1 is up-fdregulated in brain tissues [275,276]. Thrombin has been shown to induce microglial activation, that involve PAR-1, and further degeneration of dopaminergic neurons [277]. Thrombin also facilitates the release of ROS and pro-inflammatory mediators including COX-1 and iNOS expressed by microglial cells induces dopaminergic neurons cell death [278,279].

The role of thrombin was further emphasized by studies reporting that treatment with the direct thrombin inhibitor, dabigatran (Figure 4B), could be neuroprotective and may possess regenerative effects in PD [235,280]. In the rotenone model of PD, dabigatran treatment restored striatal dopamine levels, improved motor function, and repressed neurotoxic pro-inflammatory cytokine (NF-κB and TNF-α) production [280]. These effects were linked to activation of nuclear receptor-related 1 (Nurr1) and thrombin inhibition [280]. In addition, dabigatran treatment improved the locomotor ability in a *Drosophila melanogaster* model of PD by reducing ROS [235]. Finally, further highlighting the importance of PAR-1 in PD neurodegeneration, a study on PAR-1 deficient mice showed that these mice were protected against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced toxicity which causes PD-like profile [281]. The extensive work that has been done implicating a role of thrombin in PD pathogenesis along with the safe profile of dabigatran highlight the importance of considering clinical experimentation and use of dabigatran in this indication.

Traumatic Brain Injury

TBI-induced coagulation is a common condition that has been overshadowed by the severity of the injury itself. The idea of a local brain injury disseminating systemic coagulopathy is intimidating and reversing this is very critical to improve clinical outcomes [282]. TBI induces mechanical alterations in blood brain barrier (BBB) at the site of injury, thus increasing vascular leakage of fluid to cause cerebral edema, release of brain-derived coagulating factors, and neuronal damage [283–285]. Studies have shown that following TBI, brain-derived cellular microvesicles (BDMVs) are rapidly released from hippocampal cells and migrate through the disrupted endothelial barrier into the circulation and induce hypercoagulation [286]. BDMVs are highly procoagulant as they express TF and phosphatidylserine, which will integrate factors V and VIII to induce thrombin generation. As such, these BDMVs have been identified as circulating molecules to initiate and amplify coagulation leading to an exaggerated and disseminated coagulation [287,288]. Thrombin itself could further contribute to vascular leakiness as it targets the cerebrovascular endothelium inducing their contraction, which is in part mediated by PAR1 leading to increases in intracellular calcium concentration [230]. Moreover, thrombin release following TBI causes increases in extracellular glutamate via PAR1 and ROCK which is linked to the depressive behavior following TBI [289]. Glutamate in turn may contribute to the formation of post-traumatic cytotoxic edema [290].

Thrombin plays an important role in brain injury after subarachnoid hemorrhage (SAH), a pathway that is linked to MAPK and further activation of ERK1/2. Once ERK1/2 and MAPK pathways are activated after SAH, the cerebral blood flow is altered, the cerebral vascular inflammatory mediators are release and the neurological function is further deteriorated [291,292]. Selective inhibition of MAPK by SB203580, significantly attenuated the neurological damage following SAH [292]. This highlights the importance of targeting thrombin to modulate the SAH neurotoxicity and a possible neuroprotective role of thrombin or MAPK inhibitors. A standard intravascular perforation rat model was used to test the effects of direct thrombin inhibitor argatroban post SAH. High doses of argatroban ameliorated BBB disruption, lowered the brain water content (brain edema) in both hemispheres, cerebellum, and the brain stem, and attenuated apoptotic brain cell death and the expression of inflammatory marker which aggravate the condition. Overall, thrombin inhibition improved neurological outcomes and provided neuroprotection against acute events following SAH [293].

The neuroprotective effects of Argatroban were further investigated in C57BL/6 wildtype (WT) and complement3 $(C3)^{-/-}$ mice after intracranial hemorrhage (ICH) induction. Thrombin is presumed to promote uncontrolled complement system activation through its action on C5a convertase that increases cleavage of complement component C5 into C5a leading to excessive tissue inflammation and damage. Thrombin likely contributes to the exacerbation of an ICH through its activation of immunocompetent microglia and the increase in the release of inflammatory cytokines which conjure acute necrosis and apoptosis of neurons. Argatroban exhibits its neuroprotective role either by inhibiting thrombin-induced inflammation or complement pathway activation or suppressing C5a receptor expression. The simultaneous blockade of thrombin and C5a receptors provided fewer neurological apoptosis, decreased brain water content and brain edema, and decreased inflammatory factors resulting in improvements in neurological function [294] (Figure 4C). Argatroban is superior to other direct thrombin inhibitors due to its short half-life, which allows rapid offset of action in case of bleeding and ease of monitoring its thrombotic effect [295]. However, this effect as well as the effect of other agents requires further systematic and clinical investigation.

The emerging pleiotropic effects of DOACs on adipose tissue inflammation: one target for all disorders?

While evidence has long implicated a significant entanglement among pathological pathways triggered or exacerbated by metabolic impairment, therapeutic modalities for vascular dysfunction and neurodegenerative disorders remained fairly independent. This is possibly in part because the common therapeutic approaches focused on the end manifestations of the disease rather than the underlying trigger. This is typically demonstrated in drug classes like calcium channel blockers, lipid lowering fibrates, insulin secretagogues, choline esterase inhibitors, and dopaminergic agonists used for the control of different cardiovascular, metabolic, and neurological manifestations. More recently, the focus of research in this field shifted to investigate the role of adipose inflammation in the underlying pathology. Not only does adipose tissue affect cardiac health through paracrine and endocrine interactions with cardiovascular tissue [296,297], low-grade adipose tissue inflammation associated with excessive calorie intake, obesity and type 2 diabetes sits at the crossroads of vascular dysfunction and cognitive impairment [298–300]. Significantly, localized adipose tissue inflammation originating in specific depots, particularly perivascular and perirenal adipose, was found to be the earliest response to metabolic challenge prior to increased body weight or blood glucose levels, and to contribute to the development and progression of metabolic dysfunction-associated CVDs [301,302]. Indeed, we have recently demonstrated that this localized low-grade inflammatory state, preceding the development of obesity, hyperglycemia, and hypertension, is associated with vascular contractile abnormalities, ED, cardiac autonomic neuropathy, renovascular impairment, and hippocampal neuroinflammation producing cognitive dysfunction [303–308]. Importantly, interventions ameliorating this localized adipose inflammation were sufficient to reverse the associated cardiovascular and cognitive manifestations.

Recent evidence has implicated the activation of thromboinflammatory processes in the low-grade adipose tissue inflammation seen in metabolic syndrome, obesity, and diabetes [309–312]. Diet-induced adipose tissue inflammation was shown to include a myriad of immune cells and inflammatory cytokines and adipokines [313]. As such, thromboinflammation could be proposed as a possible instigator of vascular derangements arising from the inflammation of these adipose depots. This is especially relevant in the context of early metabolic deterioration which precedes the development of diabetes. Of particular interest are the increased levels of thrombin, FXa and PAR1/2/4 expression and activity in different metabolic and CVDs [309–311,314]. Importantly, adipose tissue inflammation is associated with an increased production of TF eventually leading to increased thrombin and FXa activity resulting in the emergence of a hypercoagulable state [312,315,316]. As mentioned previously, both factors can affect vascular smooth muscle and ECs phenotype and function leading to vascular remodeling, vascular injury, and atherosclerosis by acting through several pathways including PARs. Quite significantly, the role of clotting factors does not seem to cease at being a transducer of the detrimental impact of adipose inflammation on vascular function, yet it appears that increased thrombin and FXa activity could further exacerbate adipose tissue inflammation in a positive feedback loop [315,317,318]. Interestingly, the progression of adipose tissue inflammation and vascular dysfunction downstream of PAR signaling appear to occur via common targets including hypoxia inducible factor 1α (HIF-1α),

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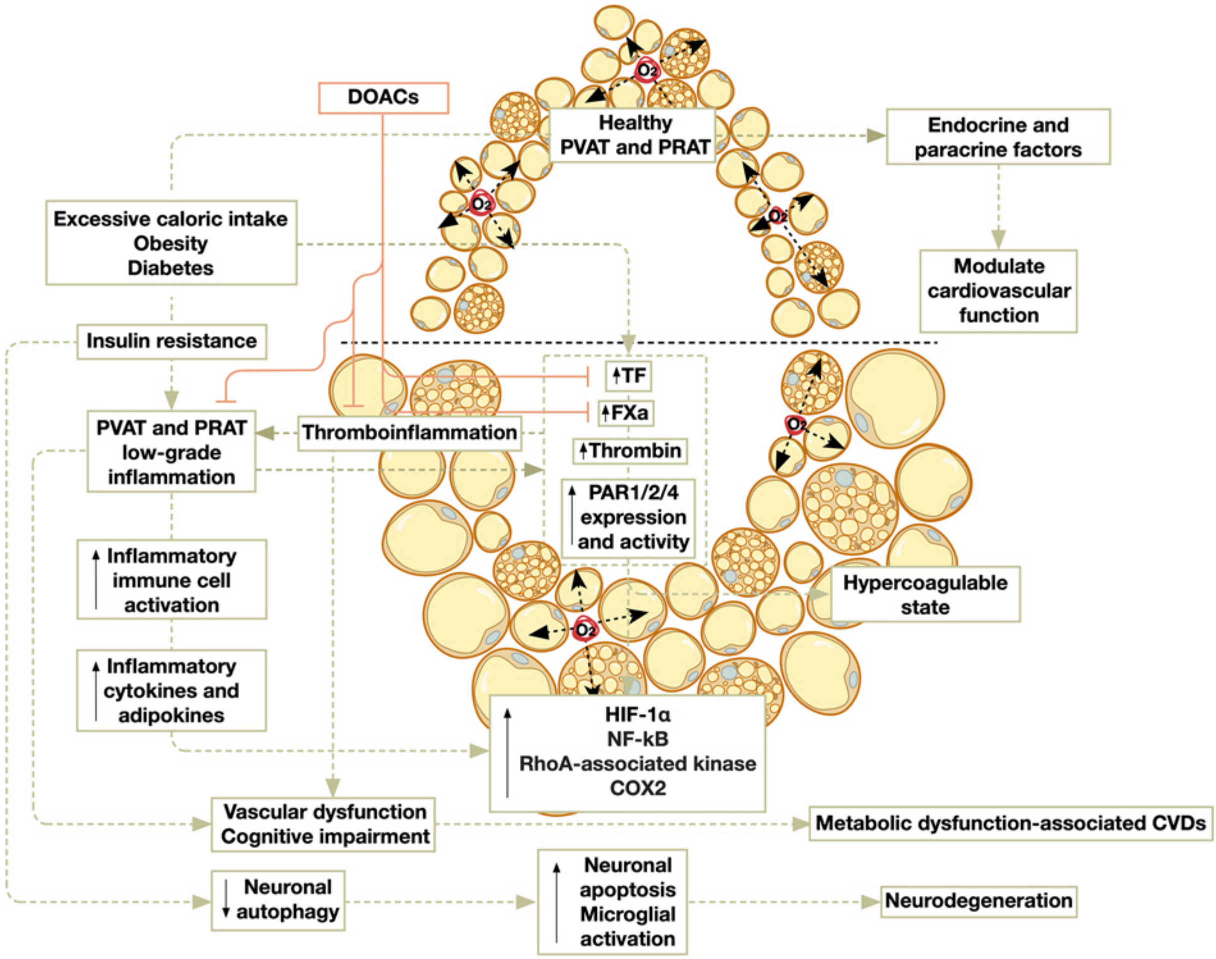

Figure 5. The interplay between perivascular and perirenal adipose tissue and thrombo-inflammation involving clotting factor and PAR signaling

Excessive calorie intake, diabetes, and obesity are associated with adipose tissue inflammation that involve increased production of inflammatory cytokines and elevated clotting factor activity. Activated clotting factors further exacerbate adipose tissue inflammation and the consequent cardiovascular and metabolic disorders, including insulin resistance, that can contribute to neuronal inflammation and cognitive dysfunction. Antithrombotic drugs offer a potential tool to interrupt this negative feedback loop early in the course of metabolic disease and confer a protective effect in the metabolic-cardiovascular-neurodegenerative disease continuum. Abbreviations: PRAT, perirenal adipose tissue; PVAT, perivascular adipose tissue.

NF-κB, RhoA associated kinase, and COX-2 [303,319–322]. Therefore, it could be suggested that adipose tissue inflammation, particularly in the perivascular depot, which leads to vascular derangements with effects extending to neuroinflammation, occurs via the contribution of increased clotting factor activity and dysfunctional PAR signaling in adipose tissue, VSMCs, ECs, and neurons. Nevertheless, such a mechanism has not yet been investigated. Importantly, DOAC treatment with either FXa or direct thrombin inhibitors could potentially counteract the increase in clotting factor-driven pathological signaling. The proposed model is summarized in Figure 5. Despite offering valuable tools to interrupt the thromboinflammatory cycle, there exists a significant knowledge gap regarding the potential pleiotropic effects of these drugs on adipose tissue inflammation and the ensuing effects. Indeed, multiple lines of evidence implicate mechanisms related to suppression of autophagic flux, increased neuronal apoptosis and microglial activation occurring downstream of metabolic impairment, particularly insulin resistance, in neurodegenerative disorders [308,323]. Amelioration of adipose inflammation helps alleviate insulin resistance [324], which offer an additional mechanism contributing to the reversal of neuroinflammation besides the correction of vascular disorders. As such, it becomes plausible that interruption of the adipose thromboinflammatory loop using DOACs might serve multiple beneficial purposes by improving the overall metabolic profile and amelioration of vascular dysfunction and neuronal inflammation. This, in addition to the direct modulatory action of DOACs on these tissues and organs, makes their pleiotropic effects a high priority for research.

Potential risks and gaps for future investigation

One of the major concerns regarding the use of DOACs, whether for their intended anticoagulant effect or pleiotropic benefits, is the enhanced risk of bleeding, which is thought to be generally directly proportional to the anticoagulant dose. Despite the wealth of evidence on the possible pleotropic effects of oral anticoagulants, there remains no clinical dose/exposure–response data for these effects. Additionally, there is no robust clinical evidence that DOACs, at their indicated doses provide clinical benefit beyond anticoagulation by virtue of their pleiotropic effects. Therefore, a dire need exists for clinical studies deriving consensus on the specific dosing of these drugs in patients outside of the usual cardiovascular indications, thus providing a proof of concept that anticoagulant therapy-mediated benefits outweigh bleeding risk in non-cardiovascular indications. Additionally, potential arising complications leading to increased mortality with prolonged anticoagulant and antiplatelet therapy, independent of enhanced bleeding may limit such an approach [267,325]. Nevertheless, the ATLAS ACS 2-TIMI trial reported that low dose rivaroxaban (2.5 mg) led to a significant reduction in cardiovascular mortality and all-cause mortality without increasing the risk of fatal bleeding in patients with recent ACS [326]. However, another study showed that the typical 20 mg dose of rivaroxaban was associated with increased intracranial and extracranial hemorrhage, including gastrointestinal bleeding in patients with nonvalvular AF [327]. Similarly, edoxaban exhibited a dose-dependent increase in bleeding risk, and low-dose regimens were non-inferior to warfarin in stroke prevention in patients with moderate-to-high risk AF [328]. Moreover, higher dabigatran plasma concentrations were shown to be correlated with worse bleeding outcomes in AF patients [329]. However, the value of the previous observations in establishing the safety of DOACs in the intended cardiometabolic indications cannot be inferred directly owing to considerable differences in the patient populations. Indeed, pharmacokinetic and pharmacodynamic subanalyses of major trials on the effect of DOACs in AF patients showed that the risk of bleeding side effects was strongly correlated with patient characteristics independent of the plasma concentration levels [330,331]. As such, one of the challenges facing the use of these and similar drugs for their cardiometabolic benefits is determining a dosing range with a reasonable risk/benefit ratio. At this stage, only parallels can be drawn regarding their potential safety in patients without active thromboembolic disorders. In this regard, DOACs emerged as an option backed by some evidence for the management of the COVID-19-related thrombosis in absence of standard guidelines [332–335]. Apixaban manifested a high safety and efficacy profile when administered to ICU patients with severe COVID-19 [336]. Furthermore, dabigatran was suggested to be used as a primary treatment option at discharge in COVID-19 patients with AF [337].

Apart from the bleeding risk, much remains to be determined regarding the potential drawbacks of chronic suppression of the FXa/thrombin/PAR1/2 signaling. Indeed, the importance of pathways triggered downstream of FXa/thrombin-mediated PAR1/2 activation in the normal physiology has long been inferred from the phenotypes displayed by transgenic mice. For instance, thrombin and FX deficient mice demonstrate a much higher *in utero* and neonatal lethality than fibrinogen knockout ones [9] indicating that the detrimental effect of their absence could not be explained solely by hemorrhagic consequences. Similarly, PAR2 knockout mice demonstrate considerable lethality [338]. Thus, a significant investigational effort is required to determine the optimal approach to mitigate these pathways. Whether DOACs (thrombin and/or FXa inhibitors) are the best tools as opposed to selective PAR antagonists with regards to the risk/benefit ratio of developmental or homeostatic outcomes is yet to be determined as well as the safe dosing ranges.

Conclusions

Several clotting factors including TF, FXa and thrombin, as well as PARs represent molecular targets that contribute to pathological pathways overlapping a number of disorders spanning metabolic, cardiovascular, and neurodegenerative diseases. In addition, ADP $P2Y_{12}$-receptors and GPIIb/IIIa receptors appear to contribute to such pathogenesis making a strong case for the use of the available drugs with the ability to modulate these targets in the peripheral tissue to modify the pathological course. In this context, DOACs emerge as a valuable tool, not only capable of directly affecting several manifestations of cardiovascular, metabolic, and neurological impairment, but also harboring significant potential to ameliorate adipose tissue inflammation effectively relieving the ensuing disorders. Indeed, the search for tailored drug therapy particularly targeting the initial localized low-grade adipose inflammation continues [339]. The available evidence from studies in both humans and animal models suggest that antithrombotic drugs could indeed

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modify the earliest events underlying vascular and cardiometabolic interactions including ED and adipose tissue inflammation. The unique pharmacological properties and safety profile of DOACs warrant future investigation of their possible role in such an indication.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ACS, acute coronary syndrome; AD, Alzheimer's disease; ADP, adenosine diphosphate; AF, atrial fibrillation; ATP, adenosine triphosphate; Aβ, β-amyloid; BDMV, brain-derived cellular microvesicle; COVID-19, coronavirus disease of 2019; COX, cyclooxygenase; CVD, cardiovascular disease; DOAC, direct oral anticoagulant; EC, endothelial cell; ED, endothelial dysfunction; EDH, endothelium-dependent hyperpolarization; EGF, epidermal growth factor; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinase 1/2; FXa, Factor Xa; GP, glycoprotein; GPI, GPIIb/IIIa inhibitor; hsCRP, high-sensitivity C-reactive protein; HUVEC, human umbilical vein EC; ICAM-1, intercellular adhesion molecule 1; ICH, intracranial hemorrhage; iNOS, inducible nitric oxide synthase; I/R, ischemia/reperfusion; LMWH, low molecular weight heparins; LPC, lysophosphatidylcholine; MCP-1, monocyte chemotactic protein-1; MI, myocardial infarction; NF-κB, nuclear factor κB; NO, nitric oxide; NVAF, non-valvular AF; PAR, protease-activated receptor; PCI, percutaneous coronary intervention; PD, Parkinson's disease; ROS, reactive oxygen species; SAH, subarachnoid hemorrhage; TAFI, thrombin-activatable fibrinolysis inhibitor; TBI, traumatic brain injury; TF, tissue factor; UFH, unfractionated heparin; uPA, urokinase plasminogen activator; VSMC, vascular smooth muscle cell; vWF, von Willebrand factor.

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