



Potential Therapeutic Roles for Direct Factor Xa Inhibitors in Coronavirus Infections

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Published online: 12 September 2020
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Abstract

Human factor Xa (FXa) is a serine protease of the common coagulation pathway. FXa is known to activate prothrombin to thrombin, which eventually leads to the formation of cross-linked blood clots. While this process is important in maintaining hemostasis, excessive thrombin generation results in a host of thrombotic conditions. FXa has also been linked to inflammation via protease-activated receptors. Together, coagulopathy and inflammation have been implicated in the pathogenesis of viral infections, including the current coronavirus pandemic. Direct FXa inhibitors have been shown to possess anti-inflammatory and antiviral effects, in addition to their established anticoagulant activity. This review summarizes the pharmacological activities of direct FXa inhibitors, their pharmacokinetics, potential drug–drug interactions and adverse effects, and the details of clinical trials involving direct FXa inhibitors in coronavirus disease 2019 (COVID-19) patients.

Key Points

Factor Xa is a serine protease in the common coagulation pathway, the excessive stimulation of which leads to a host of thrombotic conditions.

Factor Xa has also been linked to inflammation as well as viral infections.

In addition to their established anticoagulant properties, factor Xa inhibitors have exhibited significant anti-inflammatory and antiviral effects in several testing settings.

Currently, there are > 10 clinical trials for evaluating the potential of factor Xa inhibitors in coronavirus disease 2019 (COVID-19) patients.

Strategies to administer these drugs parenterally may facilitate their use in critically ill COVID-19 patients.

1 Introduction

The coronavirus disease 2019 (COVID-19) pandemic continues to impact the whole world. Since December 2019, there have been more than 23 million reported confirmed cases worldwide and more than 800 thousand human lives lost to the viral infection and/or its complications [1]. While most patients appear to develop a mild illness, the elderly (> 65 years of age) and patients with comorbidities of cardiovascular diseases, diabetes, hypertension, or cancer are at a higher risk of death [2]. Despite recommendations and approvals of compassionate use of few potential therapeutics, no vaccines or highly effective therapeutics are available; however, knowledge pertaining to the virus lifecycle and the disease pathogenesis continues to evolve.

Considering clinical reports, some COVID-19 patients have exhibited a hypercoagulable state, as indicated by the elevated levels of D-dimer and fibrinogen and the prolonged prothrombin time [3]. Furthermore, a number of inflammatory markers have been reported to significantly increase during the severe stage of the disease, including C-reactive protein, ferritin, interleukin (IL)-1 β , IL-6, monocyte chemoattractant protein-1 (MCP-1), granulocyte colony-stimulating factor (G-CSF), C-X-C motif chemokine ligand-10 (CXCL-10), chemokine C–C motif ligand 3 (CCL3), tumor necrosis factor (TNF)- α , and others [4]. Such excessive inflammation response has been described as a cytokine release syndrome, which appears to have led to acute lung

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injury/acute respiratory distress syndrome, multiple organ failure, and ultimately death [4]. Moreover, the immune response to the viral infection appears to have led to an over-activation of the coagulation pathways, which can further aggravate inflammation in a crosstalk that has substantially complicated the disease [5]. Together, coagulopathy and excessive inflammation appear to severely worsen the clinical outcomes of the viral infection, as demonstrated by the critically ill patients [5].

Considering the previous coronavirus outbreaks, i.e. severe acute respiratory syndrome coronavirus (SARS-CoV) [6] and Middle East respiratory syndrome coronavirus (MERS-CoV) [7], and the clinical presentations of the current outbreak, i.e. COVID-19 (also reported as SARS-CoV-2) [8], treating severe coronavirus cases appears to need therapeutics possessing (1) antiviral activity to block any stage of the viral lifecycle; (2) anticoagulant activity to manage the patient's hypercoagulable state; and/or (3) anti-inflammatory/immunomodulatory activity to mitigate the excessive inflammation. In this direction, factor Xa (FXa), a serine protease in the common coagulation pathway, is known to play a crucial role in coagulation by inducing the formation of thrombin, which eventually leads to the formation of cross-linked blood clots [9–11]. FXa has also been linked to inflammation [12] and viral infections [13]. In fact, direct FXa inhibitors have been reported to promote a host of pharmacological effects, including anticoagulant activity, anti-inflammatory activity, and antiviral activity (Fig. 1). Thus, direct FXa inhibitors may carry a significant therapeutic potential for coronavirus patients, particularly critically ill patients in the ongoing COVID-19 pandemic.

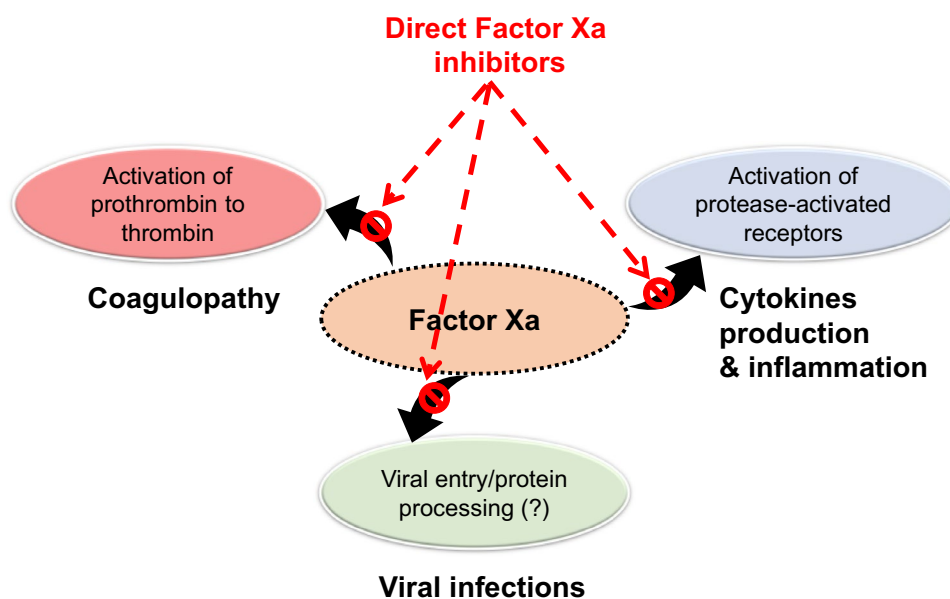
Currently, there are four clinically approved direct FXa inhibitors for use as anticoagulants, in addition to many

others under investigation. The four approved FXa inhibitors are rivaroxaban (approved in 2011; Xarelto) [14], apixaban (2012; Eliquis) [15], edoxaban (2015; Savaysa) [16], and betrixaban (2017; Bevyxxa) [17]. The chemical structures of the four drugs are provided in Fig. 2. In the following sections, we briefly review the literature that supports the various pharmacological activities of direct FXa inhibitors so as to catalyze their use to combat the ongoing coronavirus pandemic.

2 Anticoagulant Activity of Direct FXa Inhibitors

Human FXa is a trypsin-like serine protease of the common coagulation pathway. Its zymogen, i.e. factor X, is activated either via the intrinsic pathway (factor IXa) or the extrinsic pathway (factor VIIa/tissue factor) [9, 18]. FXa is the first serine protease in the common pathway leading to clot formation. The resulting FXa typically complexes with factor Va, calcium, and phospholipid to form prothrombinase, which subsequently activates prothrombin (also known as factor II) to thrombin (factor IIa) [19]. In turn, thrombin converts fibrinogen (factor I) to fibrin (factor Ia) monomers. Factor XIII, which is also activated by thrombin, converts the soluble fibrin monomers to insoluble cross-linked fibrin polymers on the surface of activated platelets, leading to the formation of a hemostatic plug [20]. Thrombin also positively feedbacks the cascade by activating factor XI [21], factor VIII [22] in the intrinsic pathway, and factor V [23] in the common pathway. This is to ensure the efficient generation of a burst of thrombin that amplifies and propagates the clotting response [24–27].

Fig. 1 Potential therapeutic benefits of direct FXa inhibitors in coronavirus infections. Four FXa inhibitors are approved anticoagulants, and several approved and experimental inhibitors have exhibited anti-inflammatory and antiviral effects in different testing settings. FXa factor Xa



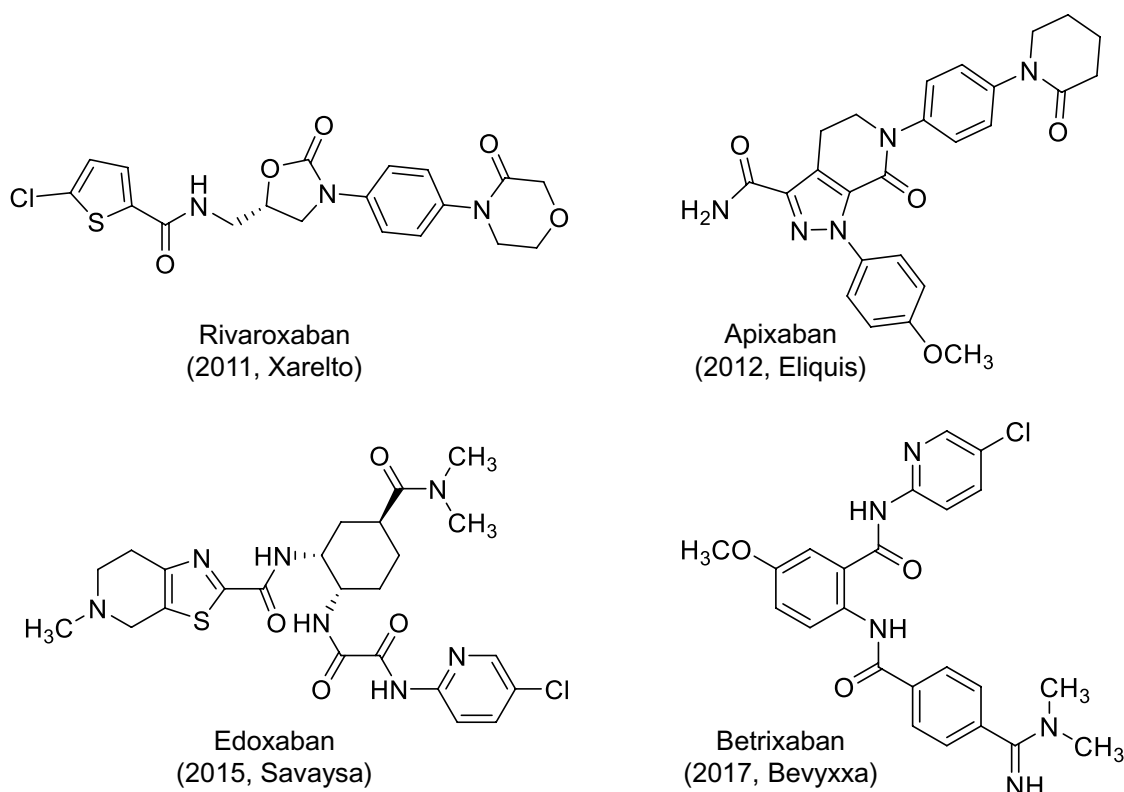


Fig. 2 Chemical structures of US FDA-approved direct FXa inhibitors, along with the year of FDA approval as well as their brand and generic names. *FXa* factor Xa

While the blood clotting process is important in maintaining hemostasis, its excessive activation typically leads to thrombotic complications. Moreover, activation of the coagulation cascade during viral infections is probably a protective mechanism to limit the spread of the infection. However, excessive clotting can lead to disseminated intravascular coagulation and subsequent hemorrhage. In fact, several reports have suggested that excessive coagulation is substantially linked to viral infections [28, 29] and, in this case, the viral infections by SARS-CoV-2, SARS-CoV-1, MERS-CoV [30]. Considering the ongoing pandemic, increasing evidence indicates that critically ill COVID-19 patients develop a hypercoagulable state that has been linked to poor outcomes of progressive respiratory failure and even death [31–36]. The hypercoagulable state of COVID-19 patients has been attributed to increased circulating prothrombotic factors, endothelial injury, and immobilization [37]. Disseminated intravascular coagulation, as well as coagulopathies of venous thromboembolism and ischemic stroke, has been described in severe cases of COVID-19 [37–41]. Interestingly, early anticoagulation in patients with severe COVID-19 infection has been found to reduce the risk of thrombotic complications and improve overall clinical

outcomes, as was demonstrated by heparins, antithrombin-based anticoagulants [32, 35, 42–44].

Along these lines, direct FXa inhibitors are therapeutically approved for the prevention and/or treatment of venous thromboembolism, including deep vein thrombosis and pulmonary embolism [45, 46]. Mechanistically, the four approved drugs in Fig. 2 are small molecule, competitive, and highly selective inhibitors of the enzyme. In contrast to heparins, these inhibitors directly (without antithrombin) inhibit both the free FXa and the clot-bound FXa. Relative to other anticoagulant classes of warfarin, heparins, and direct thrombin inhibitors, direct FXa inhibitors are associated with relatively less internal bleeding risk [47, 48]. Needless to mention, there is a US FDA-approved antidote known as andexanet alfa (Andexxa®) to treat potential bleeding events that may arise with their clinical use [49]. Importantly, FXa inhibitors are generally associated with less rebound hypercoagulation that is more common with heparins and direct thrombin inhibitors [50, 51]. In fact, while heparins appear to be gaining significant interest in treating COVID-19 patients [52, 53], a recent study has documented evidence of heparin resistance in critically ill patients [54]. This suggests that alternative anticoagulants are in urgent

need, and indeed, it encourages the consideration of direct FXa inhibitors.

Together, given the established efficacy and safety of direct FXa inhibitors as anticoagulants, and the pathogenesis of the ongoing viral pandemic, it is anticipated that these drugs can play a major role in treating the reported coagulopathies so as to mitigate the illness manifestations and reduce the death rate of COVID-19 patients.

3 Anti-Inflammatory Activity of Direct FXa Inhibitors

In addition to coagulation, human FXa also plays a substantial role in inflammation. In this arena, it was shown that FXa, via its interaction with effector cell protease receptor-1, may function as a mediator of acute inflammation *in vivo*. This pathway may augment both coagulation and inflammatory cascades and contribute to the pathogenesis of tissue injury [55]. It was also shown that exposing human umbilical vein endothelial cells to FXa concentration-dependently stimulated the cytokine production of IL-6, IL-8, and MCP-1, as well as the expression of adhesion molecules of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 [56]. The adhesion molecules also increased the polymorphonuclear leukocyte adhesion to the endothelial cells. To confirm the contribution of FXa, active site-blocked FXa was found inactive with respect to cytokine production and adhesion molecule expression [56]. FXa additively also contributed to the thrombin action of calcium-mobilizing and proinflammatory reactions of endothelial cells [57]. In fact, FXa was found to stimulate proinflammatory and profibrotic responses in fibroblasts [58], human atrial tissue [59], and RAW 264.7 macrophages [60] via protease-activated receptor-2. An investigational FXa inhibitor was also recently reported to inhibit MCP-1 production in endothelial cells via protease-activated receptor-1 [61]. As a result, FXa-mediated signaling has been implicated in the pathogenesis of several inflammatory diseases, including fibrosis, cardiovascular diseases, diabetic nephropathy, and cancer [62, 63].

Importantly, the anti-inflammatory effects of FXa inhibitors have been demonstrated in human subjects. For example, the anti-inflammatory effects of rivaroxaban and apixaban were recently observed in Japanese patients with atrial fibrillation [64]. The anti-inflammatory effect of apixaban was also recently demonstrated in the acute phase of ischemic stroke patients [65]. A post hoc analysis of the X-VerT trial also revealed that rivaroxaban caused a significant reduction in the levels of D-dimer and IL-6 in patients with atrial fibrillation [66]. These results indicate that blocking the activity of FXa may not only be beneficial to prevent the virus-associated coagulopathies but may also dampen

the virus-triggered excessive immune response. In fact, it is plausible to assume that the current dosage regimens of the clinically available direct FXa inhibitors can be adequate to promote the much-needed anti-inflammatory activity to mitigate the cytokine storm of COVID-19, yet this remains to be properly evaluated in clinical trials.

4 Antiviral Activity of Direct FXa Inhibitors

SARS-CoV-2 is a single stranded, positive-sense RNA virus that utilizes a surface spike protein to enter the host cells, initially the epithelial cells of the respiratory system [67]. The spike protein has two important subunits linked together in the form of S1–S2. The S1 subunit carries the host receptor binding domain and the S2 subunit is responsible for the virus fusion with the host cell membrane. The S1 subunit of the viral spike protein binds to its receptor angiotensin-converting enzyme 2 (ACE2) on the host cell. A proteolytic activation mediated by the host proteases takes place to break the linkage between the S1 and S2 subunits so as to facilitate the virus fusion with the host cell membrane [67, 68]. Host proteases that are important for the activation process include furin, transmembrane protease serine 2 (TMPRSS2), and lysosomal cathepsins. Nevertheless, previous experience with SARS-CoV revealed that other host proteases may also be important for viral fusion and entry, including FXa [67, 68]. In fact, it was previously shown that an experimental FXa inhibitor blocked the viral entry of SARS-CoV into the host cells by preventing the spike protein cleavage into the S1 and S2 subunits. The inhibitor concentration-dependently blocked the SARS-CoV plaque formation in Vero E6 cells. Thus, direct FXa inhibitors may prevent coronavirus entry to human cells [69].

Furthermore, a recent study has computationally identified direct FXa inhibitors as potential inhibitors of TMPRSS2 [70]. In addition, another study suggested that FXa is essential for efficient replication of hepatitis E virus, a positive-stranded RNA virus, in cell culture and is potentially involved in ORF1 polyprotein processing [71]. Thus, direct FXa inhibitors may interfere with the replication of hepatitis E virus. Moreover, selective inhibition of FXa was recently shown to improve left ventricular function during coxsackievirus B3-induced myocarditis and appeared to lead to improved myocardial remodeling [72]. FXa inhibition also significantly reduced adeno-associated virus-2 infections in mice [73]. Lastly, in human umbilical vein endothelial cells, FXa was also found to exploit herpes simplex virus-associated tissue factor to increase infection through cellular protease activated receptor-2, suggesting that FXa inhibitors will likely reduce viral infectivity [74]. Together, direct FXa inhibitors have been shown to promote a substantial (in)direct antiviral activity against a range of RNA

and DNA viruses by blocking the viral entry stage [69] and possibly by other mechanisms [28, 70–74]. Such antiviral activity provides additional therapeutic benefit while using FXa inhibitors in COVID-19 patients.

5 Pharmacokinetics, Drug–Drug Interactions, and Adverse Effects of Direct FXa Inhibitors

All approved FXa inhibitors are orally used. With the exception of betrixaban, FXa inhibitors are hepatically metabolized to predominantly inactive metabolites. Renal elimination of rivaroxaban, apixaban, and edoxaban, and/or their metabolites, is significant. However, betrixaban is predominantly eliminated in feces [17]. Therefore, dose adjustment of rivaroxaban, apixaban, and edoxaban is needed in the case of renal impairment, while their use is to be avoided in patients with moderate or severe hepatic impairment. They all have moderate to high plasma protein binding profiles. This is of enormous significance in the case of rivaroxaban, which has the highest potential of plasma protein binding, and thus, should be cautiously used in COVID-19 patients with hypoalbuminemia [75]. The corresponding pharmacokinetic parameters of approved FXa inhibitors are listed in Table 1 [14–17, 76].

All FXa direct inhibitors are potentially associated with significant drug–drug interactions because they are metabolized by hepatic CYP450 enzymes (CYP450 3A4 for rivaroxaban, apixaban, and edoxaban) and/or they are substrates for p-glycoprotein [77]. With respect to their use in combination with currently used anti-COVID-19 therapeutics, recent analysis indicated that coadministration of rivaroxaban, apixaban, or edoxaban with lopinavir/ritonavir is associated with a high risk of serious drug–drug interactions that require dose adjustment. The same high risk exists between edoxaban and azithromycin [78]. The study also indicated mild or moderate potential interactions between rivaroxaban or apixaban and tocilizumab or sarilumab, as well as between edoxaban and hydroxychloroquine. A very low risk

of drug–drug interactions is expected between direct FXa inhibitors and remdesivir, ribavirin, methylprednisolone, or anakinra [78].

The most common complication in using these drugs is bleeding, although bleeding risk may vary among the different agents. Reversing bleeding can be achieved by the use of andexanet alfa. If andexanet alfa is not available, four-factor prothrombin complex concentrate can be used [79, 80]. Although andexanet alfa has not been evaluated to reverse the anticoagulant effects of betrixaban or edoxaban in humans, nevertheless, since both are similar FXa inhibitors as rivaroxaban and apixaban, it is likely that andexanet alfa will also be effective in reversing their actions [81].

6 Conclusion

Clinically available direct FXa inhibitors potentially hold a significant promise in treating COVID-19 because of their anticoagulant, anti-inflammatory, and antiviral activities. The diversity of their pharmacological effects will likely improve the overall clinical outcome of COVID-19 treatment. Currently, there are about 10 clinical studies that appear to include rivaroxaban, apixaban, or edoxaban in trials for COVID-19 patients (Table 2). Regarding the appropriate dosage regimens of FXa inhibitors to be used in COVID-19 patients, the reported protocols in the ongoing trials have considered dosage regimens similar to those being used in thrombotic patients, taking into account the status of their renal function. Some trials reported the drugs to be used for 21–30 days. Nevertheless, it remains to be determined whether the treatment protocols will yield the desired outcomes.

Importantly, an issue that may arise while using direct FXa inhibitors in treating hospitalized COVID-19 patients is the difficulty of administering oral solid pharmaceutical preparations, particularly to critically ill patients. However, this obstacle can potentially be overcome by the use of a crushed tablet in an aqueous suspension to be administered via nasogastric tube. In fact, a recent phase I, randomized,

Table 1 Pharmacokinetic profiles of direct FXa inhibitors

Drug	Oral BA (%)	T_{max} (h)	V_d	$t_{1/2}$ (h)	Protein binding (%)	Metabolism	Excretion (%)
Rivaroxaban	80–100 (food) ~60 (fasting)	2–4	50 L	5–9 11–13 (elderly)	92–95 (albumin-dependent)	Hepatic CYP3A4/5 and CYP2J2	66 (urine) 28 (feces)
Apixaban	~50	3–4	21 L	8–15	87	Hepatic CYP3A4/5	27 (urine)
Edoxaban		62 1–2	107 L	10–14	55	Hepatic CYP3A4/5	50 (urine)
Betrixaban		34 3–4	32 L/kg	19–27	60	Minimal	85 (feces) 11 (urine)

BA bioavailability, CYP cytochrome P450, FXa factor Xa, $t_{1/2}$ terminal elimination half-life, T_{max} time to peak plasma concentration, V_d apparent volume of distribution

Table 2 Clinical trials testing rivaroxaban, apixaban, or edoxaban in patients with COVID-19^a

Study title (acronym; ClinicalTrials.gov unique identifier)	N	Type (sponsors)	Intervention
Rivaroxaban			
Effect of anticoagulation therapy on clinical outcomes in COVID-19 (COVID-PREVENT; NCT04416048)	400	Multicenter, prospective, randomized, event-driven, open-label, interventional trial (Charite University, German Centre for Cardiovascular Research, and Bayer)	20 mg (15 mg for subjects with an eGFR \geq 30 mL/min/1.73 m ² and < 50 mL/min/1.73 m ²) once daily for at least 7 days. Further description is available
Anti-coronavirus therapies to prevent progression of COVID-19 trial (ACT COVID19; NCT04324463)	4000	Open-label, parallel group, factorial, randomized controlled, interventional trial (Population Health Research Institute and Bayer)	2.5 mg twice daily for 28 days
Austrian coronavirus adaptive clinical trial (COVID-19) (ACOVACT; NCT04351724)	500	Multicenter, randomized, active-controlled, open-label, interventional trial (Medical University of Vienna)	2.5 or 10 mg for 29 days
Full anticoagulation versus prophylaxis in COVID-19: COALZAO ACTION Trial (ACTION; NCT04394377)	600	Randomized, interventional trial (Brazilian Clinical Research Institute)	20 mg/day for 30 days (15 mg if CrCl = 30–49 mL/min and/or concomitant use of azithromycin); followed by enoxaparin/unfractionated heparin as needed
Preventing cardiac complication of COVID-19 disease with early acute coronary syndrome therapy: a randomized controlled trial (C-19-ACS; NCT04333407)	3170	Prospective, multicenter, randomized, open-label, controlled, interventional trial (Imperial College London)	2.5 mg for 30 days
A trial to evaluate safety and efficacy of rivaroxaban (COVID-19) (NCT04504032)	600	Randomized, controlled, phase IIb, blinded, interventional trial (Bill & Melinda Gates Medical Research Institute)	10 mg tablet by mouth, once daily for 21 days
A study of rivaroxaban to reduce the risk of major venous and arterial thrombotic events, hospitalization and death in medically ill outpatients with acute, symptomatic coronavirus disease 2019 (COVID-19) infection (PREVENT-HD; NCT04508023)	4000	Multicenter, randomized, placebo-controlled, double-blinded, pragmatic phase III, interventional trial (Janssen Research & Development, LLC)	10 mg tablet orally, once daily for 35 days
Apixaban			
FREEDOM COVID-19 anticoagulation strategy (FREEDOM COVID; NCT04512079)	3600	Randomized, open-label, interventional trial (Icahn School of Medicine at Mount Sinai)	5 mg every 12 h; 2.5 mg every 12 h for patients with at least two of three of the following: age \geq 80 years, weight \leq 60 kg, or serum creatinine \geq 1.5 mg/dL
COVID-19 positive outpatient thrombosis prevention in adults aged 40–79 (NCT04498273)	7000	Multicenter, adaptive, randomized, placebo-controlled, double-blinded, interventional trial (University of Pittsburgh and National Heart, Lung, and Blood Institute)	2.5 or 5 mg twice daily
Edoxaban			
CorONa Virus edoxaban ColchicinE COVID-19 (CONVINCE; NCT04516941)	420	A randomized, open-label, interventional study (University Hospital Inselspital and Daiichi Sankyo Europe, GmbH)	Edoxaban 60 mg once daily, or 30 mg once daily in patients with CrCl \leq 50 mL/min or body weight \leq 60 kg from randomization to end of the study visit at day 25

COVID-19 coronavirus disease 2019, eGFR estimated glomerular filtration rate, CrCl creatinine clearance

^aAll three factor Xa inhibitors are also included in the NCT04518735 clinical trial

single-dose, crossover study presented results supporting the use of 60 mg crushed edoxaban tablets for administration as an apple puree oral preparation or an aqueous suspension via a nasogastric tube [82]. One can be skeptical about the use of a nasogastric tube given the hemodynamic instability of critically ill patients. In this arena, betrixaban was approved for use in adult patients hospitalized for an acute medical illness who are at risk for thromboembolic complications because of moderate or severe restricted mobility and other risk factors for venous thromboembolism [83]. Overall, the development of a novel parenterally administered formulation or a parenteral delivery system for the currently available direct FXa inhibitors may further facilitate the realization of their full potential in treating hospitalized COVID-19 patients.

Acknowledgements Rami A. Al-Horani is supported by a grant from the National Institute of General Medical Sciences (NIGMS) under award number SC3GM131986. The content is the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health.

Declarations

Conflicts of interest Rami A. Al-Horani declares no competing financial conflicts of interest.

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