Arteriosclerosis, Thrombosis, and Vascular Biology

INVITED REVIEW

Therapeutic Potential of C1-Inhibitor in Vascular Diseases and Beyond

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ABSTRACT: C1INH (C1-inhibitor) is a multifunctional SERPIN (serine protease inhibitor) that functions as a major negative regulator of the complement, coagulation, and kallikrein-kinin systems. C1INH products were originally developed for the treatment of hereditary angioedema associated with C1INH deficiency. A growing body of literature indicates that C1INH products may find utility in the management of several other disease states. In this review, we detail the key biological activities of C1INH and consider the pathophysiological role of C1INH targets in many conditions. The therapeutic potential of exogenous C1INH is highlighted in the settings of thromboembolism, ischemia-reperfusion injury, sepsis, transplantation, and coronavirus disease 2019.

Key Words: angioedemas, hereditary ■ blood coagulation ■ complement C1 inhibitor protein ■ thrombosis ■ vascular diseases

Serine proteases represent a large class of enzymes that proteolytically cleave target proteins at specific serine residues.[1] Serine protease–based cascades have evolved to enable rapid activation of physiological processes in response to internal and external stimuli.[2] Such serine protease cascades are involved in the activation of several key physiological processes that impact vascular integrity, including coagulation and the immune response.[3–6] Serine proteases are directly inactivated by SERPINs (serine protease inhibitors) that function as vital negative regulators of serine protease–dependent processes.[7,8]

The SERPIN C1INH (C1-inhibitor), encoded by the *SERPING1* gene, was first identified by Ratnoff and Lepow[9] through its ability to inhibit, as its name implies, the first C (complement component) 1. C1INH is a 105-kDa protein primarily expressed and secreted from the liver and is highly abundant in blood with a plasma concentration of ≈250 µg/mL.[10,11] As is typical of SERPINs, C1INH is a globular protein that contains a C-terminal reactive center loop (RCL) that governs substrate selectivity.[7,12,13] The RCL of SERPINs is recognized by target serine proteases that, when engaged, undergo a dramatic conformational change.[14] This conformational change results in inactivation of the target serine protease and formation of an irreversible covalently bound complex in a process known as inhibition by deformation.[14]

A growing body of literature suggests that exogenous C1INH has protective effects in a wide range of pathologies. In this review, we outline key biological activities of C1INH and highlight the ability of C1INH to afford protection in a broad range of pathologies, including several with strong vascular components (Figure 1).

BIOLOGICAL FUNCTIONS OF C1INH

C1INH is perhaps unique among the SERPIN family in its function as the primary endogenous negative regulator of initiating proteases in 3 major biological pathways: the complement system, the contact pathway of coagulation, and the kallikrein-kinin system.[9] C1INH inhibits C1r and C1s of the classical, or immune complex-activated, complement pathway and is also the major endogenous inhibitor of the lectin, or microbial surface-activated, complement pathway, inhibiting MASP (mannose-binding lectin-associated protease) 1 and MASP2 (Figure 2A).[15–19] The coagulation system is a serine protease cascade that facilitates hemostasis after vascular injury or, when inappropriately activated, thrombosis. In the contact, or surface-activated, pathway of

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Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

BK	bradykinin
C	complement component
C1INH	C1-inhibitor
COVID-19	coronavirus disease 2019
F	factor
HAE	hereditary angioedema
HLA	human leukocyte antigen
HMWK	high-molecular-weight kininogen
IRI	ischemia-reperfusion injury
MASP	mannose-binding lectin-associated protease
MI	myocardial infarction
pd	plasma-derived
PKa	plasma kallikrein
r	recombinant
RCL	reactive center loop
SERPIN	serine protease inhibitor
VTE	venous thromboembolism

What Are the Clinical Implications?

C1INH (C1-inhibitor) is a multifunctional serine protease inhibitor with potent anticomplement and anticoagulant activity. Exogenous C1INH shows broad vascular-protective potential beyond hereditary angioedema in preclinical models. While C1INH-based therapies are clinically established for hereditary angioedema, their wider application remains limited.

coagulation, C1INH functions as a major endogenous inhibitor of F (factor) XIIa, its reciprocal activator PKa (plasma kallikrein), and its downstream activation product FXIa (Figure 2B). In the kallikrein-kinin system, PKa-mediated cleavage of the vasoactive peptide BK (bradykinin) from HMWK (high-molecular-weight kininogen) increases vascular permeability. Inhibition of PKa by C1INH limits BK generation and, thus, regulates vascular permeability (Figure 2C).[20–22] While C1INH is a potent inhibitor of these abovementioned proteases, it also inhibits, albeit to a lesser extent, both plasmin and tissue-type plasminogen activator of the fibrinolytic system.[23,24]

The inhibitory activity of C1INH can be enhanced by glycosaminoglycans, sulfated polysaccharides present on cell surfaces and in extracellular matrices.[25,26] Inhibition of complement C1s by C1INH is enhanced over 50-fold by heparin, a highly sulfated glycosaminoglycan, over 130-fold by dextran sulfate, a synthetic heparin analogue, and 20- to 30-fold by the less sulfated glycosaminoglycans, heparan sulfate, and dermatan sulfate.[27,28] While glycosaminoglycans do not seem to potentiate inactivation of PKa or FXIIa by C1INH, inhibition of FXIa by C1INH is enhanced 117-fold by dextran sulfate, 47-fold by heparin, and to a lesser extent by heparan sulfate and dermatan sulfate.[29–31] By comparison, FXIa inhibition by antithrombin is potentiated by heparin by a factor of 14, while FXa and thrombin inactivation are enhanced 600- and 2000-fold, respectively, by heparin.[32,33] Structural studies suggest that glycosaminoglycans enhance the inhibitory activity of C1INH by directly or indirectly facilitating interactions that are favorable for the formation of the inhibitory complex.[34,35] Specifically, this may be due to neutralization of the positively charged F1 helix by negatively charged polysaccharides, facilitating interaction with the positively charged binding regions of the target protease.[34,35] The clinical impact of the enhancement of C1INH function by therapeutic or endogenous glycosaminoglycans has not been determined.

C1INH also has important biological functions that are independent of protease inhibitory functions. The best described of these is the ability of C1INH to bind and neutralize bacterial endotoxins, specifically the Gram-negative bacterial endotoxin lipopolysaccharide (Figure 2D). Lipopolysaccharide binds to C1INH through interaction with amino acids in the noninhibitory N-terminal domain.[36] Binding of C1INH to lipopolysaccharide is facilitated by a series of positively charged amino acids in the N-terminal domain and is also dependent on glycosylation of this region.[37,38] By binding to lipopolysaccharide, C1INH prevents interactions with lipopolysaccharide binding protein, which are required for presentation of lipopolysaccharide to monocytes and activation of downstream proinflammatory signaling.[39–42] This function of C1INH has particular relevance to its vascular-protective role in the setting of endotoxemia and bacterial sepsis.

C1INH can also interact with a number of cell surface and extracellular proteins associated with the vascular endothelium and circulating blood cells (Figure 2D). C1INH binds to the adhesion molecules P- and E-selectin on endothelial cells.[43,44] This interaction is likely supported by Sialyl-Lewis X present on glycosylated C1INH residues.[43,45] Binding of C1INH to P- or E-selectin inhibits leukocyte-endothelial cell interactions and inhibits leukocyte rolling.[45]

The binding of C1INH to the endothelial cell surface, likely through interactions with resident glycosaminoglycans, may also play an important role in localizing activity. Endothelial cell–derived and surface-bound C1INH retains potent inhibitory activity toward several targets, including PKa, C1s, thrombin.[46–48] C1INH is also reported to bind to collagen, laminin, and nidogen-1, components of the vascular basement membrane.[49,50] While the interaction between collagen and C1INH was reported to have a limited effect on the ability of C1INH to inhibit C1s and kallikrein, inhibition of FXIIa by C1INH was potently reduced by this interaction.[49,50] While some studies, detailed in the following, have sought to address the

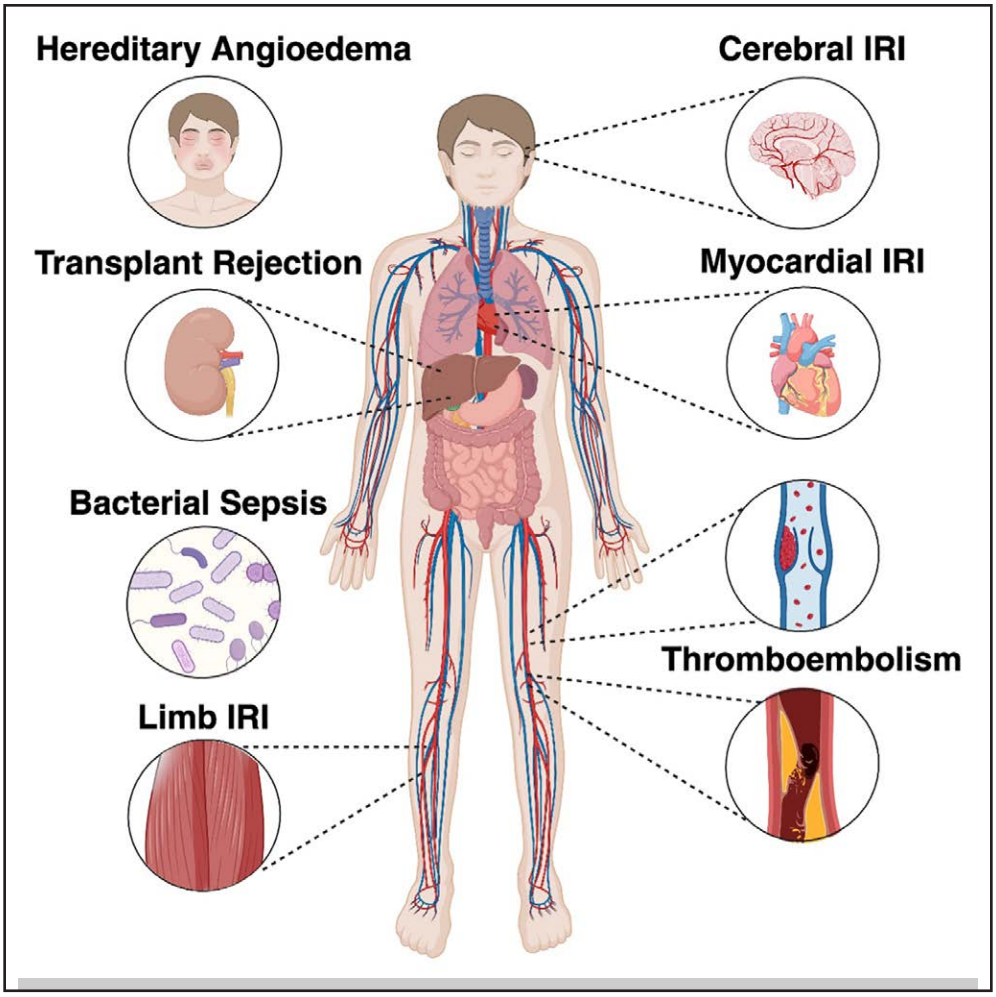

Figure 1. Established and emerging indications for C1INH (C1-inhibitor) treatment.
Treatment with C1INH products is established in the management of patients with hereditary angioedema and shows potential therapeutic utility in a number of other indications. Created with BioRender. IRI indicates ischemia-reperfusion injury.

contribution of these noncanonical C1INH activities, our understanding of this aspect of C1INH biology remains relatively limited.

C1INH IN HEREDITARY ANGIOEDEMA

Congenital deficiency in C1INH results in a rare disorder called hereditary angioedema (HAE) that has a prevalence of ≈1 in 50 000 in the general population.[51] C1INH deficiency–associated HAE (HAE-C1INH) can be caused by quantitative (type I) or functional (type II) defects in C1INH resulting from mutations in the *SERPING1* gene.[51] In some instances, HAE can be caused by mutations in other genes, including, for example, *F12*, *KNG1*, and *PLG*, and is referred to as HAE with normal C1INH.[51] HAE primarily presents with episodes of subcutaneous and submucosal swelling that can affect a number of different tissues.[51] Swelling attacks in patients with HAE-C1INH are caused by excess PKa-mediated BK generation. BK and its degradation product des-Arg9-BK bind to the B2 and B1 BK receptors, respectively, mediating intracellular signaling that leads to enhanced vascular permeability.[52]

PKa-mediated cleavage of intact HMWK results in the release of BK and generation of cleaved HMWK. Consistent with dysregulation of the kallikrein-kinin system in patients with HAE-C1INH, plasma levels of cleaved HMWK are significantly elevated in this population compared with controls.[53–55] While formation of cleaved HWMK is a key feature of HAE-C1INH and a potential diagnostic marker, elevated cleaved HMWK levels have also been reported in other populations, including patients with acute liver injury and hemodialysis.[56,57]

Purified preparations of C1INH were developed as a targeted treatment for patients with HAE-C1INH and have been used as replacement therapy since 1986 when they were first approved for on-demand treatment.[58] Subsequently, C1INH products have also been approved as prophylactic therapies for the prevention of attacks.[59] Plasma-derived (pd) C1INH products include the pasteurized and nonfiltered products: Berinert and Cinryze for intravenous use and Haegarda for

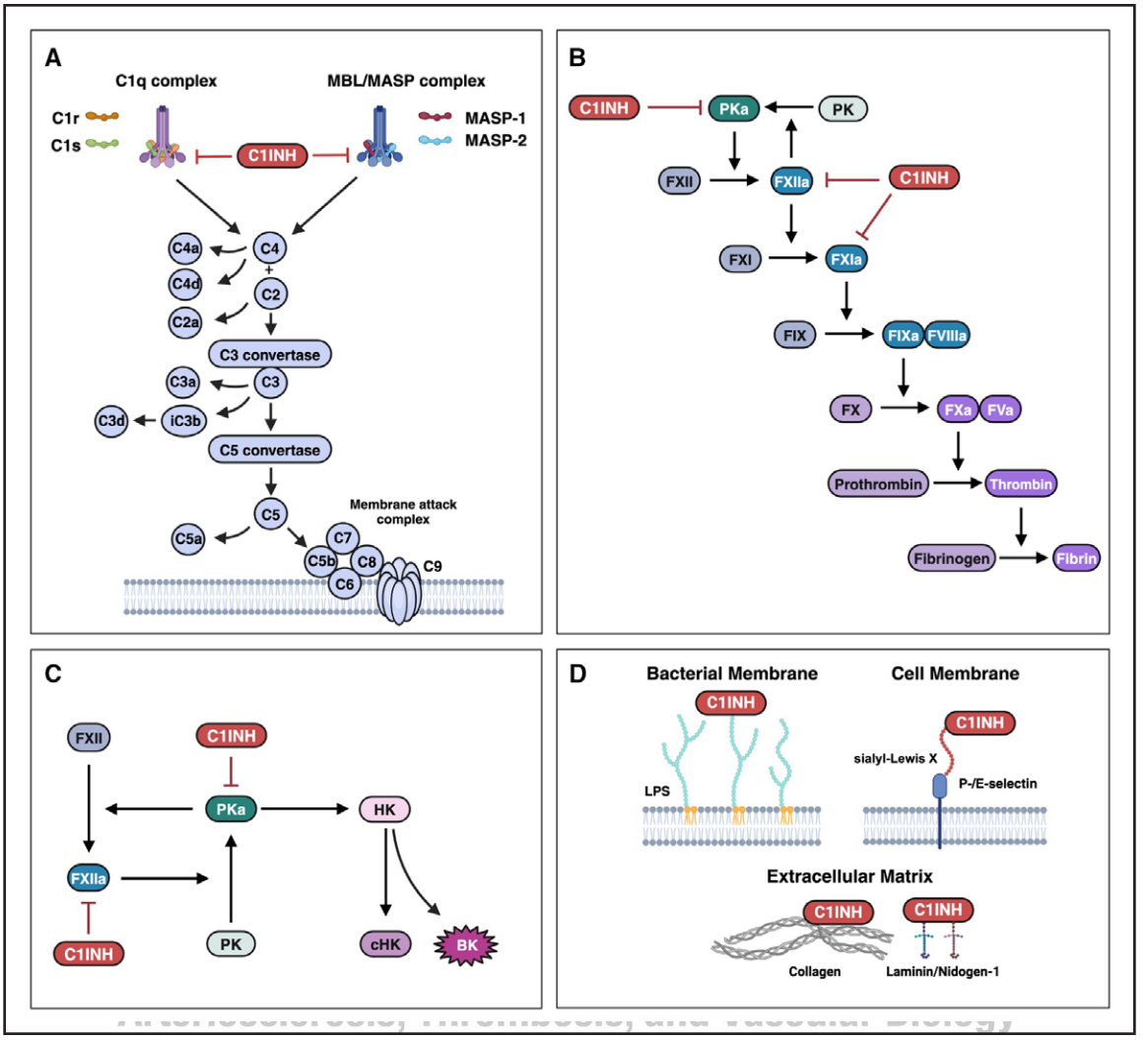

Figure 2. Biological functions of C1INH (C1-inhibitor).
C1INH functions as an important endogenous negative regulator of (**A**) the classical and lectin complement pathways, (**B**) the contact pathway of coagulation, and (**C**) the kallikrein-kinin system by exerting classical serine protease inhibitor activity. **D**, C1INH also possesses other nonserine protease inhibitor activities, including binding to lipopolysaccharide, adhesion molecules, and extracellular matrix components. Created with BioRender. BK indicates bradykinin; C, complement component; F, factor; MASP, mannose-binding lectin-associated protease; and PKa, plasma kallikrein.

subcutaneous injection.[59] In addition, a r (recombinant) C1INH product, Ruconest, isolated from the milk of transgenic rabbits, has also been developed.[59] Ruconest has a shorter half-life compared with pdC1INH products, of ≈3 hours, likely owing to the different glycosylation pattern of rC1INH compared with pdC1INH.[59]

The therapeutic benefit of C1INH products in the setting of HAE-C1INH has been demonstrated extensively in clinical studies.[60] Placebo-controlled clinical trials of intravenous pdC1INH have demonstrated that treatment significantly shortens attack duration in patients with HAE-C1INH when used for on-demand treatment.[61,62] In separate arms of these trials, intravenous pdC1INH was also found to be effective as prophylaxis, significantly reducing the frequency of attacks in patients with HAE-C1INH.[61,62] Similar findings have been made with rC1INH in clinical trials of HAE-C1INH.[63,64] Subcutaneous pdC1INH, developed to provide a longer therapeutic half-life compared with intravenous administration, has also been shown to be effective in reducing attack frequency in patients with HAE-C1INH when used for prophylaxis.[65] New prophylactic therapies for HAE-C1INH are being developed at a rapid pace with inhibitory targets, including PKa, FXIIa, and BK receptors.[66] However, C1INH replacement remains the only therapeutic approach that fully reconstitutes the biological activity, including anticomplement activity, of C1INH absent in patients with HAE-C1INH.

Evidence from HAE-C1INH indicates that endogenous C1INH likely plays an important role as an endogenous anticoagulant. Several clinical studies have shown that patients with HAE-C1INH have a systemic

Table. Pathologies in Which C1INH Administration Has Been Clinically Evaluated

Indication	Treatment	Dose	Frequency (duration)	Outcomes	References
HAE (treatment)A	pdC1INH or rC1INH	20 IU/kgB IV 1000 IUC IV 50 IU/kgR IV	On-demand	Reduction and resolution of symptoms	62,63,210–213
HAE (short-term prophylaxis)A	pdC1INH	1000 IU	Once, within 24 h	Reduced attack incidence	214–216
HAE (long-term prophylaxis)A	pdC1INH	60 IU/kgH SC 1000 IUC IV	2×/wk (indefinite)	Reduced attack frequency	62,65,217
Sepsis	pdC1INH	Cumulative dose: 10 000–12 000 IUB IV	2×/d (2–5 d)	Improved kidney function Reduced capillary leak Reduced complement activation Reduced Inflammation	186,188–190,218
Myocardial infarction IRI	pdC1INH	Cumulative dose: 1000–10 000 IU IV	Perioperatively and postoperatively, continuous, or 2×/d (3–48 h)	Reduced cardiac injury markers	128–130
Kidney transplant (delayed graft function)	pdC1INH	50 IU/kg IV	Perioperatively and postoperatively, 1×/d (24 h)	Improved renal function Reduced graft failure	155,156
Kidney transplant (antibody-mediated rejection)	pdC1INH	20 IU/kg IV	2–3×/wk (2 wk–6 mo)	Improved renal function Reduced complement activation	152–154
COVID-19	rC1INH	Cumulative dose: 21 000–41 800 IU IV	2–3×/d (48–72 h)	Decreased inflammatory markers	198,199

A indicates approved indication; B, Berinert; C, Cinryze; C1INH, C1-inhibitor; COVID-19, coronavirus disease 2019; H, Haegarda; HAE, hereditary angioedema; IRI, ischemia-reperfusion injury; IU, international units; pdC1INH, plasma-derived C1-inhibitor; R, Ruconest; and rC1INH, recombinant C1-inhibitor.

procoagulant state with increased baseline plasma levels of coagulation activation markers, including prothrombin fragments 1+2, thrombin antithrombin complexes, and D-dimer, compared with healthy controls.[67–71] These markers are further elevated in patients with HAE-C1INH during attacks.[67,68] Importantly, elevated levels of these markers have all been independently associated with an increased risk of venous thromboembolism (VTE).[72–75] Furthermore, recent epidemiological evidence has demonstrated that patients with HAE-C1INH have an increased risk of VTE.[76–78] The effect of HAE-C1INH on arterial thromboembolism risk is less clear. HAE-C1INH is associated with reduced coronary artery flow and systemic endothelial dysfunction that is itself associated with future cardiovascular events.[79,80] In a case-control study, no clear association between HAE-C1INH and risk of myocardial infarction (MI) or ischemic stroke was apparent, while a significantly increased risk of peripheral artery disease was noted.[76,81] However, in a recent study, predicted loss-of-function variants in the *SERPING1* were associated with a significantly increased risk of both VTE and arterial thromboembolism, including peripheral artery disease and ischemic stroke.[82]

There has been some concern about the potential thrombogenicity of C1INH products. In a small study of neonates with severe congenital heart defects undergoing bypass surgery receiving high doses of pdC1INH off-label, a number of thrombotic events were reported.[83] However, the extent to which C1INH contributed to these events is unclear. A small number of thrombotic events have also been reported in clinical studies and postapproval monitoring of C1INH products in patients with HAE-C1INH.[83–85] In an open-label study of pdC1INH, ≈3% of patients with HAE-C1INH developed serious thrombotic events, including VTE, MI, and ischemic stroke; however, the majority of these individuals had independent cardiovascular disease risk factors.[86] Subsequent systematic analyses have failed to reveal any significant association between C1INH usage and thrombotic events.[83,84,87]

In a series of controlled clinical studies, the protective effects of C1INH administration have been observed. In patients with HAE-C1INH, administration of a single dose of rC1INH significantly prolonged activated partial thromboplastin times and reduced prothrombin fragments 1+2 levels compared with vehicle controls.[88] Similarly, analysis of samples from clinical trials evaluating pdC1INH in HAE-C1INH found that treatment durably prolonged activated partial thromboplastin times and reduced plasma levels of prothrombin fragment 1+2 and D-dimer.[89] Critically, in a retrospective cohort study, patients with HAE-C1INH receiving pdC1INH for short-term and intermittent prophylaxis had a 10-fold lower incidence of VTE compared with untreated controls.[90]

In C1INH-deficient mice, that model key aspects of HAE-C1INH, administration of pdC1INH at 15 µg/g normalized the enhanced venous thrombus formation phenotype observed in these mice.[70] Preclinical studies outside of the setting of HAE-C1INH also indicate that exogenous C1INH exerts antithrombotic effects. Exogenous pdC1INH selectively and significantly prolonged contact pathway–initiated coagulation, but not extrinsic pathway–initiated coagulation, in a dose-dependent manner.[91] Interestingly, exogenous pdC1INH also inhibited thrombin-mediated platelet aggregation.[91] Consistent with these actions, exogenous C1INH at

a dose of 800 IU/kg significantly attenuated arterial thrombosis in a rabbit carotid artery ferric chloride model.[91] Furthermore, in a mouse model of atherosclerotic lesion progression, exogenous pdC1INH significantly reduced neointimal hyperplasia accompanied by reductions in lesional macrophage and T-cell content, as well as reduced complement activation.[92] The exact mechanisms by which C1INH exerts antithrombotic effects in these models have yet to be established; however, plasma levels of the C1INH targets, FXIa and MASP2, have both been identified as predictive biomarkers of VTE risk, suggesting that both anticoagulant and anticomplement activities of C1INH may be involved.[93]

Available evidence from clinical studies has demonstrated that C1INH replacement is safe and effective for the management of patients with HAE-C1INH. Moreover, C1INH replacement in this setting normalizes coagulation and VTE. However, further studies are required to assess the impact of exogenous C1INH on pathological activation of coagulation and VTE outside of the setting of HAE-C1INH.

C1INH IN ISCHEMIA-REPERFUSION INJURY

Ischemia results from impaired blood flow to tissues and organs that can be caused by thrombotic, embolic, vasculitic, vasospastic, depositional, or mechanical occlusion of blood vessels. Deprivation of blood and oxygen to ischemic tissues and organs causes hypoxia that can induce metabolic stress and activation of cell damage or death pathways.[94] Furthermore, restoration of blood flow to ischemic tissues and organs, while reestablishing the essential supply of blood and oxygen, can result in additional tissue injury, referred to as ischemia-reperfusion injury (IRI).[95] During ischemia, hypoxic cells release damage-associated molecular patterns and expose neoantigens on their surface that activates the innate immune system.[96] Damage-associated molecular patterns and immune complexes present on the surface of damaged cells lead to recruitment of neutrophils and macrophages to the injury site.[96] Recruited neutrophils and macrophages release proinflammatory cytokines, chemokines, and histamines and produce reactive oxygen species that drive microvascular alterations and increased vascular permeability.[96] Sudden reintroduction of oxygen into cells upon reperfusion can also elicit the generation of reactive oxygen species that cause further oxidative damage and damage-associated molecular pattern release.[97]

IRI is a common complication of numerous cardiovascular diseases and their management, including MI and ischemic stroke. In these ischemic pathologies, reperfusion injury occurs when blood flow is mechanically or pharmacologically restored to these key organs. IRI can also affect other tissues and organs, including the intestines and skeletal muscle tissue of the lower limbs. A number of preclinical models have been established to study IRI in these tissues and organs. Currently, despite being a relatively prevalent phenomenon, no approved drugs are available for the treatment or prevention of IRI. Here, we will highlight the potential utility of C1INH in mitigating IRI in a range of organs.

Preclinical studies have extensively evaluated the therapeutic potential of exogenous C1INH in myocardial IRI. In rat models of myocardial IRI, exogenous human pdC1INH, administered before reperfusion at doses of 40-100 IU/kg, significantly reduced myocardial injury, cardiomyocyte apoptosis, and neutrophil infiltration.[98–101] Similar findings have been reported in murine, feline, and porcine models of myocardial IRI across a range of doses between 20 and 400 IU/kg.[102–105] Inhibition of both classical and lectin complement pathway activation by exogenous C1INH may play a role in the observed protective effects of this treatment. Inhibition of C1s or mannose-binding lectin has been shown to significantly reduce myocardial IRI, whereas alternative pathway inhibition had no significant effect.[99,105–108] Complement-independent functions likely also contribute to the observed protective effect of exogenous C1INH. Exogenous C1INH was found to reduce myocardial IRI in C3-deficient mice to a similar extent as wild-type mice.[102] Intriguingly, RCL-cleaved C1INH, which cannot inhibit target proteases, was found to significantly reduce cardiomyocyte apoptosis and reduce myocardial injury in a mouse IRI model.[100,102] This indicates that some of the protective effects associated with exogenous C1INH are independent of its protease inhibition function. C1INH can also disrupt E- and P-selectin–mediated interactions between leukocytes and the endothelium.[43,45] Disruption of such interactions may contribute to reduced neutrophil recruitment associated with exogenous C1INH.

The protective effects of exogenous C1INH have also been evaluated in preclinical models of cerebral IRI. In a mouse model of transient middle cerebral artery occlusion, administration of exogenous human pdC1INH at doses of 300 to 600 IU/kg before reperfusion significantly reduced the cerebral infarct volume, reduced leukocyte recruitment, improved functional outcomes, and improved survival.[109–113] Similar effects of exogenous C1INH at doses of 20 to 50 IU/kg have been observed in rat transient middle cerebral artery occlusion models.[112,114] Consistent with the neuroprotective effects of exogenous C1INH, preserved blood-brain barrier integrity, reduced cerebral edema, and reduced thromboinflammation have also been observed.[111,112] Neuroprotection afforded by exogenous C1INH in C1q-deficient mice indicates that the protective effects are not dependent on inhibition of the classical complement pathway.[110] While, in the majority of studies, C1INH was evaluated when administered

before reperfusion in one study, delayed administration of C1INH after reperfusion still significantly improved functional outcomes.[112] Interestingly, no effect of exogenous C1INH was observed in a murine model of cerebral hemorrhage–induced brain injury, indicating that the protective effects of this intervention may be limited to the setting of IRI.[115]

Evidence from preclinical studies also indicates that exogenous C1INH is protective in skeletal muscle IRI. These models are relevant to revascularization in critical limb ischemia, a potentially limb-threatening condition caused by arterial occlusion and subsequent skeletal muscle necrosis. In mouse and rat infrarenal arterial clamping models, exogenous human pdC1INH, evaluated across a broad dose range of 50 to 1000 IU/kg, significantly reduced muscle injury as indicated by reduced muscle edema, increased muscle viability, and reduced plasma levels of creatinine kinase.[116–118] No consistent reduction in immunoglobulin or complement factor deposition was observed in reperfused muscle after C1INH treatment.[117,118] This is somewhat surprising given the ability of C1INH to inhibit complement. Exogenous C1INH was found to significantly reduce fibrin deposition in reperfused muscle.[118] It is interesting to consider if the protective effects of exogenous C1INH could be related to the anticoagulant activity of this model. In an ex vivo porcine limb IRI model, exogenous C1INH significantly reduced deposition of immunoglobulins, complement factors, and fibrin while preserving endothelial integrity and quiescence.[119]

Occlusion of blood vessels perfusing the intestines and subsequent revascularization can lead to intestinal IRI. A large body of preclinical literature has implicated complement activation in the pathogenesis of intestinal IRI.[120] Accordingly, several studies have evaluated the therapeutic potential of exogenous C1INH in preclinical models of intestinal IRI. Pretreatment with exogenous C1INH at doses of 100 to 800 IU/kg before reperfusion significantly reduced mucosal injury, preserved microcirculatory perfusion, reduced leukocyte adhesion, prevented metabolic acidosis, and improved survival.[121–123] Some of the protective effects associated with exogenous C1INH likely occur independently of complement inhibition as exogenous C1INH was equally protective in C3-deficient mice.[123] Moreover, RCL-cleaved C1INH was equally protective as intact C1INH, suggesting that the observed protection may be afforded by noninhibitory functions.[123]

There is limited evidence for the protective effects of exogenous C1INH in renal IRI. One study reported that exogenous C1INH at a relatively high dose of 750 IU/kg preserved renal function in a mouse renal IRI, and this was associated with reduced complement activation and leukocyte recruitment.[124] Exogenous C1INH appeared to have durable protective effects in this model, reducing chronic kidney fibrosis.[125] In a clinical trial of contrast-induced kidney injury, rC1INH treatment resulted in significant reductions in kidney injury markers, urinary neutrophil gelatinase-associated lipocalin, and cystatin C but was not associated with a reduction in endothelial cell injury or the incidence of acute kidney injury.[126,127] The protective effects of C1INH in renal IRI are particularly relevant to kidney transplantation, which is discussed in the following.

The therapeutic potential of exogenous C1INH has been evaluated in several clinical studies. Exogenous C1INH was first evaluated in a small series of patients with MI undergoing coronary artery bypass graft surgery and was considered promising based on the survival of all patients.[128] In a subsequent small study of patients with MI, revascularized by thrombolytic therapy or percutaneous coronary interventions, C1INH treatment, initiated within 6 hours of the precipitating event, significantly lowered plasma levels of the cardiac injury markers troponin T and creatinine kinase.[129] In a pair of controlled studies of patients with MI revascularized by coronary artery bypass graft surgery, C1INH treatment significantly reduced plasma cardiac troponin I, improved cardiac function, and reduced the intensity and duration of medical support.[130,131] Exogenous C1INH has yet to be clinically evaluated in the setting of cerebral or limb IRI.

In summary, evidence from preclinical models indicates that C1INH administration protects against IRI in a range of tissues. It is likely that the protective effect of C1INH occurs independently of protease inhibitory activity. However, further studies are required to determine the exact molecular mechanism that underpins the protective effects of C1INH. Available clinical evidence, particularly in the setting of exogenous C1INH administration for MI-associated IRI, provides further support for the protective effects of this intervention. However, C1INH-based therapies have yet to be approved for this indication.

C1INH IN ALLOTRANSPLANTATION

Allotransplantation represents a lifesaving or life-altering intervention in which diseased organs or tissues are replaced by healthy material from a genetically nonidentical donor. Mismatch between donor and recipient is associated with worse outcomes and can lead to acute or chronic rejection of transplanted material.[132] In addition, IRI occurring on reperfusion of transplanted organs can also contribute to worse outcomes and acute rejection.[133]

Complement plays a key role in antibody-mediated rejection of transplanted material as mismatched HLA (human leukocyte antigen) in the donor material can be recognized by anti-HLA antibodies expressed by the recipient, forming immune complexes that activate the classical pathway leading to microvascular injury.[134]

Complement also plays an important role in transplant IRI.[135] Preclinical studies have demonstrated that inhibition of complement activation can reduce both antibody-mediated rejection and IRI-mediated rejection.[136–142]

Activation of coagulation occurs in the peritransplant period due to IRI and other complications inherent to the harvesting and implantation process.[143,144] Thrombotic microangiopathy, in the graft microvasculature, represents a serious complication of solid organ transplantation with an incidence as high as 14%. It is thought that thrombotic microangiopathy results from mutual activation of the complement and coagulation systems.[144]

The therapeutic potential of exogenous C1INH has been evaluated in preclinical models of transplantation, in particular, kidney transplantation. In a pair of studies using nonhuman primate kidney transplantation models, rC1INH administered to recipients at doses of 100 to 500 IU/kg post-transplantation significantly reduced acute antibody-mediated rejection and renal injury while improving renal function.[145,146] In a complementary nonhuman primate study, administration of rC1INH to donors pretransplant significantly reduced recipient complement activation and improved renal function.[147] In a porcine model of kidney transplantation, exogenous C1INH administered at a dose of 500 IU/kg peritransplant improved long-term kidney function and reduced chronic fibrosis, suggesting that this treatment has durable benefits.[148] C1INH administration was also found to have potential benefits in cross-species transplantation of porcine kidneys into nonhuman primates, preventing acute vascular rejection.[149] Exogenous C1INH has also been evaluated in other models of organ transplantation. In ovine and canine models of lung transplantation, C1INH administration at doses of 20 to 200 IU/kg was associated with improved lung function.[125,150] In the canine model, C1INH administration also reduced contact and complement pathway activation.[125] In a mouse model of cardiac transplantation that was associated with lectin pathway-mediated IRI-induced rejection, C1INH administration significantly improved graft survival.[151] Together, these studies indicate that exogenous C1INH has broadly protective effects in organ transplantation.

In the clinical setting, exogenous C1INH administration has primarily been evaluated in the context of kidney transplantation. In a small single-arm study of kidney transplant recipients, C1INH administration was safe and associated with reduced complement deposition and improved kidney function.[152] In a series of placebo-controlled trials, C1INH administration after kidney transplantation was well tolerated, significantly reducing complement activation and graft failure while showing a trend toward improved graft function.[153–155] On long-term follow-up of C1INH administration after kidney transplant, a significant reduction in graft failure has been reported.[156] Interestingly, in a recent randomized double-blind placebo-controlled trial, direct administration of C1INH to the donor organ before implantation significantly improved long-term kidney function.[157] Studies are limited outside of the setting of kidney transplant, with one multiarm study of lung transplantation–associated primary graft dysfunction, finding that C1INH significantly shortened time on mechanical ventilation.[158] Further small case series indicate that exogenous C1INH may have utility in preventing graft failure in lung transplant.[159,160]

Despite promising preclinical and clinical data, particularly for kidney transplant, exogenous C1INH is not used in the transplant setting. This is likely due to the limited size and scope of completed clinical trials.

C1INH IN BACTERIAL INFECTION AND SEPSIS

Bacterial infections are common in humans and are a major cause of mortality, associated with ≈13.7 million deaths per year worldwide.[161] Mortality from bacterial infections is primarily driven by severe sepsis, a condition characterized by multiorgan failure due to a dysregulated immune response.

The complement system is a key component of the innate immune response to bacterial infection.[162] The classical complement pathway is activated by direct or antibody-mediated recognition of bacteria by C1q. The lectin complement pathway is activated by the binding of MASP-associated lectins and ficolins to bacterial membranes. Complement activation can either facilitate direct lytic bacterial killing or phagocytic bacterial killing by immune cells. The products of complement activation, including the membrane attack complex (C5b-9) and anaphylatoxins (C3a, C4a, and C5a), have a range of effects on the vasculature, promoting inflammation and inflammatory cell recruitment, endothelial injury, and increased vascular permeability, leading to shock and organ hypoperfusion.[163,164] Several clinical studies have indicated that increased activation of the complement system is associated with poor outcomes in patients with sepsis. Significantly higher plasma levels of C3a, C4a, and C5a and significantly lower plasma levels of C3 and C4 have been observed in fatal sepsis cases compared with nonfatal cases[165–169]

Contact pathway factors, including prekallikrein and FXII, can also be activated by bacterial components, such as lipopolysaccharide, polyphosphate, peptidoglycan, and components of the innate immune system, such as neutrophil extracellular traps.[170–172] Excessive activation of coagulation in sepsis leads to a condition known as disseminated intravascular coagulation, a form of consumptive coagulopathy that presents with widespread microvascular thrombosis, systemic elevation of markers of coagulation activation, and thrombocytopenia. Disseminated intravascular coagulation is associated with

mortality in sepsis caused by tissue ischemia and organ failure from microvascular thrombosis, as well as bleeding due to consumption of coagulation factors.[173] Elevated plasma levels of FXIIa-like activity, derived from residual amidolytic activity of FXIIa-alpha-2-macroglobulin complexes, have been reported in patients with sepsis-associated disseminated intravascular coagulation and have been associated with poor prognosis.[174] The importance of contact pathway activation in the morbidity and mortality of sepsis is also evident from preclinical models. In a baboon model of *Staphylococcus aureus*–induced sepsis, inhibition of FXII or FXI activation significantly reduced markers of coagulation activation, including C1INH complexes, and resulted in reduced organ failure and mortality.[175,176]

A number of preclinical studies have evaluated the therapeutic potential of exogenous C1INH in models of sepsis. In a primate model of *Escherichia coli*–induced sepsis, exogenous C1INH administered at 500 IU/kg significantly reduced markers of activation of complement, coagulation, and inflammation.[177] In mouse and rat models of *Streptococcus pneumonia*–induced meningitis, exogenous C1INH at 500 to 2000 IU/kg reduced neurological impairment and cerebral inflammation.[178] In a mouse model of cecal ligation puncture–induced polymicrobial sepsis, exogenous C1INH at 600-µg/mouse reduced dissemination of bacteria and significantly improved survival.[179] Interestingly, the reactive center–cleaved C1INH, which retains the ability to bind to lipopolysaccharide, also reduced bacterial dissemination and improved survival in the cecal ligation puncture model, indicating that the protective effects of C1INH may occur independent of its protease inhibitory activity.[179]

Based on the ability of C1INH to directly interact with the endotoxin lipopolysaccharide, the effect of exogenous C1INH has also been evaluated in preclinical models of endotoxemia. Exogenous C1INH, evaluated across a broad range of doses, was found to reduce hypercoagulability and pulmonary dysfunction in rat, rabbit, and canine models of lipopolysaccharide-induced endotoxemia.[180–184] Consistent with the anticoagulant potential of C1INH, supplementation in whole blood significantly reduced lipopolysaccharide-induced prothrombin fragment generation.[185] In murine models of lipopolysaccharide-induced endotoxemia, exogenous C1INH administered at 200 µg/mouse significantly improved survival and reduced vascular permeability.[36,37] RCL-cleaved C1INH provided similar protection as active C1INH in murine lipopolysaccharide models, again suggesting that the protective effects of C1INH are independent of protease inhibitory functions.[36] The importance of lipopolysaccharide binding was further supported by the loss in efficacy of C1INH preparations subject to deglycosylation at N-linked sites critical for lipopolysaccharide binding.[36,37] Together, these preclinical studies suggest that C1INH may have beneficial effects in the setting of bacterial sepsis through limiting induction of the hypercoagulable and hyperinflammatory states associated with this pathology with an intriguing role for direct neutralization of lipopolysaccharide.

Exogenous C1INH has also been evaluated in a number of clinical studies. In initial single-arm studies of patients with sepsis, administration of exogenous C1INH was found to be safe with potential clinical benefit related to reduced complement activation and capillary leak syndrome.[186,187] In a pair of small, double-blind, placebo-controlled trials, treatment with C1INH did not improve survival but was associated with significantly reduced organ dysfunction accompanied by reduced complement and neutrophil activation.[188,189] Plasma C1INH antigen and activity were followed in one of these trials, and participants achieved a doubling of baseline C1INH antigen/activity at 24 hours, which remained significantly elevated compared with the placebo group at day 4.[188] In another similarly sized double-blind, placebo-controlled trial, treatment with C1INH using a similar protocol significantly improved survival and was associated with reduced inflammation.[190] Larger trials are likely needed to more thoroughly evaluate the potential clinical benefit of exogenous C1INH in patients with sepsis.

C1INH IN CORONAVIRUS DISEASE 2019

Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease caused by the SARS-CoV-2 virus.[191] In mild cases, COVID-19 is associated with symptoms including fever, cough, and muscle aches.[192] However, in severe cases, COVID-19 is associated with respiratory failure, multiple organ dysfunction, hyperinflammation, hypercoagulability, and death.[192] Dysregulation of the host immune response has been proposed as a key driver of the hyperinflammatory state associated with COVID-19.[193] The dysregulated host immune response in COVID-19 is evidenced by the persistent activation of complement.[194] Early in the pandemic, severe COVID-19 was associated with robust activation of coagulation and a high incidence of thromboembolic disease.[195] The kallikrein-kinin system was also reported to be activated in patients with COVID-19.[196] C1INH occupies a unique position as the negative regulator of the aforementioned pathways dysregulated in COVID-19.

A small number of clinical studies have sought to evaluate the effect of exogenous C1INH administration in patients with COVID-19.[197] In an early study, rC1INH administration to 5 patients with worsening COVID-19 was well tolerated with all patients recovering.[198] In a larger follow-up randomized placebo-controlled trial, rC1INH administration had no effect on clinical outcomes in patients at risk of progression to severe COVID-19.[199] Furthermore, in another small randomized controlled trial, pdC1INH had no significant effect on the clinical course of disease in patients with severe COVID-19 compared

with controls.[200] Together, these findings suggest that exogenous C1INH has limited therapeutic potential in the setting of COVID-19.

LIMITATIONS AND FUTURE DIRECTIONS

C1INH has a diverse array of biological activities that include canonical inhibition of the contact pathway of coagulation or the complement system and noncanonical interactions such as lipopolysaccharide binding. While exogenous C1INH has shown promise in preclinical and clinical studies in a range of different pathological settings, the opportunity to derive conclusive mechanistic insights has been limited due, in part, to the multifunctional nature of C1INH. RCL-cleaved C1INH preparations have been used in preclinical studies to evaluate the contribution of noncanonical C1INH activities.[36–38] However, RCL-cleaved C1INH may be an imperfect tool as it requires C1INH to be exposed to, and cleaved by, a target protease, such as trypsin.[36–38] Cleavage of C1INH results in insertion of the RCL into sheet A, as is observed with latent C1INH, which likely induces broader disruption of native C1INH structure and may alter noncanonical activity.[35]

The RCL amino acid sequence is essential in defining the activity and selectivity of C1INH toward target proteases.[201] Single amino acid substitution variants at key residues within the C1INH RCL have been shown to strongly alter both activity and selectivity.[202–205] We propose that these C1INH single amino acid substitution variants likely represent powerful mechanistic tools. By way of example, C1INH^{R466C}, a single amino acid substitution variant at the P1 position, present in some patients with HAE-C1INH, has no detectable protease inhibitory activity.[204] The C1INH^{R466C} variant would, therefore, represent an ideal tool with which to study protease inhibitory–independent activities of C1INH. The identification of substrate-selective C1INH variants would further facilitate the study of C1INH-mediated inhibition of specific proteases. Several recent studies have demonstrated the utility of manipulating SERPIN RCLs to modulate substrate selectivity.[206–208]

While the focus of this review primarily relates to the therapeutic potential of exogenous C1INH, it should also be noted that the role of endogenous C1INH, outside the setting of HAE-C1INH, also remains relatively understudied. Carefully conducted clinical case-control studies utilizing appropriately powered populations of HAE-C1INH cases may reveal further pathological consequences of congenital C1INH deficiency.[76,77,81] Furthermore, C1INH-deficient mice represent a powerful tool with which to study the effect of C1INH deficiency in established disease models and may be better suited for the derivation of mechanistic insights.[70,209]

CONCLUSIONS

C1INH is a broad-acting SERPIN that negatively regulates several key molecular and cellular processes, including activation of the coagulation and complement systems, while also exhibiting important noncanonical activities. A growing body of preclinical and clinical evidence indicates that exogenous C1INH exerts vascular-protective effects in a range of pathologies. This evidence suggests that C1INH-based therapies may find utility outside of their classical indications for HAE-C1INH.

ARTICLE INFORMATION

Received September 23, 2025; accepted December 3, 2025.

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Acknowledgments

The authors wrote and edited the manuscript. The authors would like to thank Dr Nigel Mackman (The University of North Carolina at Chapel Hill) for helpful discussions.

Sources of Funding

This work was supported by the Swedish Heart Lung Foundation (to L. Sundler Björkman), the Swedish Society of Medicine (to L. Sundler Björkman), an American Society of Hematology Scholar Award (to S.P. Grover), and the National Heart, Lung, and Blood Institute of the National Institutes of Health grants R01HL171042 (to S.P. Grover) and T32HL007149 (to H. Eswaran). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosures

L. Sundler Björkman received an unrestricted research grant from CSL Behring and honoraria from CSL Behring, BioCryst, and Takeda. S.P. Grover received an unrestricted research grant from CSL Behring and honoraria from CSL Behring and BioCryst. H. Eswaran reports no conflicts.

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