

The thrombogenicity of C1 esterase inhibitor (human): Review of the evidence

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ABSTRACT

Thromboembolic events associated with human plasma-derived C1 esterase inhibitor (C1-INH) use in patients with hereditary angioedema (HAE) have been reported in the U.S. Food and Drug Administration (FDA) Adverse Event Reporting System database. The purpose of this article is to review and assess the strength of available evidence regarding the thrombogenicity of human plasma-derived C1-INH. A PubMed search was conducted of English language articles from January 1990 to December 2013 reporting the thrombogenicity of C1-INH. Original research articles were selected if the following criteria were met: (1) C1-INH was the focus of the study and (2) the authors addressed the pro- or antithrombotic potential of C1-INH. Additional articles on the clinical use of C1-INH in disease states other than HAE were obtained using reference lists of selected articles. Pivotal studies and prescribing information for C1-INH products were also reviewed. Limited animal and clinical data suggest that C1-INH, particularly at high doses of up to 500 U/kg (compared with the U.S. FDA-approved 20-U/kg dose), may be prothrombotic. In contrast, C1-INH has been used in some patients with myocardial infarction, ischemic stroke, sepsis, and capillary leak syndrome at off-label supratherapeutic doses (up to 100 U/kg) without evidence of a thrombogenic effect. Based on our review, thromboembolic events reported with C1-INH use are rare and patients with HAE who experienced such events often have underlying thromboembolic risk factors.

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C1 esterase inhibitor (C1-INH) is a serine protease inhibitor, deficiency of which causes an overproduction of vasoactive peptides, particularly bradykinin, and leads to subcutaneous or submucosal swelling, including potentially fatal swelling of the upper airway.¹ Hereditary angioedema (HAE) with C1-INH deficiency is a rare, autosomal-dominant disease and is characterized by recurrent episodes of edema at various body sites.² Consensus guidelines indicate that plasma-derived C1-INH is an appropriate and effective option both for acute treatment of individual attacks and for long-term prophylaxis for HAE with C1-INH deficiency.³

There have been reports of thromboembolic complications associated with the administration of C1-INH. The purpose of this article was to review and assess available evidence regarding the thrombogenicity of human plasma-derived C1-INH.

BACKGROUND OF C1-INH

C1-INH interacts with the plasmatic cascade systems to reduce the proinflammatory phase of acute-phase response (Fig. 1).¹ C1-INH regulates both the classic pathway and the mannan-binding lectin pathway of complement by inhibition of C1s and C1r subcomponents and mannan-binding lectin-associated serine proteases, MASP-1 and MASP-2.¹ C1-INH plays a role in the contact system by inactivation of two forms of activated factor XII, *viz.*, factor XIIa and factor XII_f, and kallikrein, which activates more factor XII. Factor XIIa can activate intrinsic coagulation, factor XII_f can activate the classic complement pathway, and both forms of activated factor XII can initiate fibrinolysis. C1-INH also decreases the amplification loop of the intrinsic coagulation cascade by inhibiting factor XIa. C1-INH has also been shown to inhibit leukocyte-endothelial cell adhesion by binding to selectins,^{4–7} which may contribute to its anti-inflammatory effect. In contrast, C1-INH has the potential to inhibit fibrinolysis by inhibiting plasmin to a minor extent; C1-INH inhibits <1% of generated plasmin, whereas α_2 -antiplasmin inhibits 92%.⁸

C1-INH products are approved for treatment, routine prevention, and/or preprocedure prevention of HAE attacks in adolescents and/or adults in the United States and Europe.³ Available formulations of human plasma-derived C1-INH in the United States include Berinert (C1-INH [human]; CSL Behring LLC, Kankakee, IL) and Cinryze (C1-INH [human]; C1 INH-nf; ViroPharma, Inc., a part of the Shire group of com-

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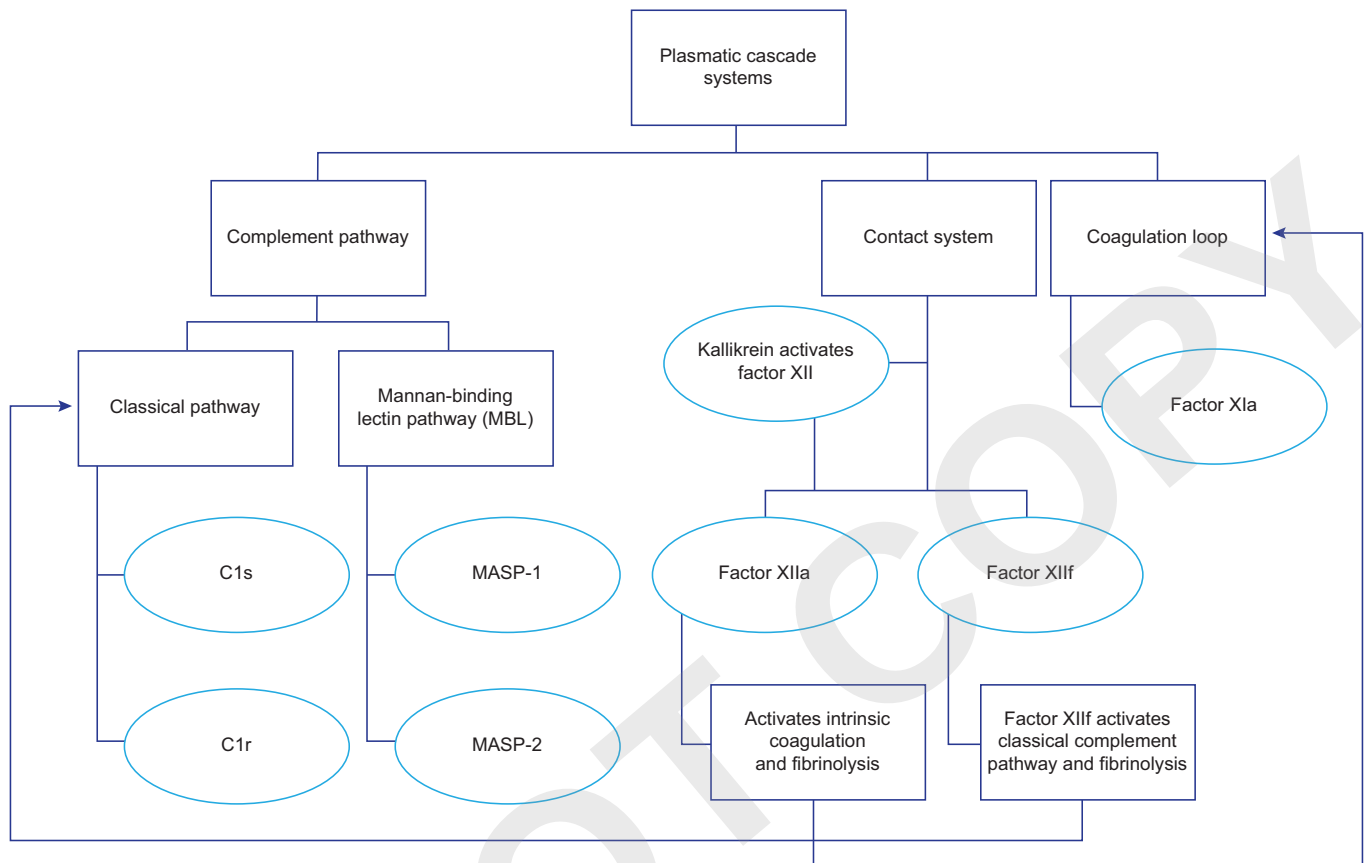


Figure 1. C1 esterase inhibitor (C1-INH) reduces inflammation by inhibiting components (in oval shapes) of the plasmatic cascade systems. C1s and C1r, subunits of the C1 enzyme; MASP, MBL-associated serine proteases.

panies, Exton, PA). U.S. Food and Drug Administration (FDA)–approved doses are Berinert at a 20-U/kg intravenous (i.v.) injection (4 mL/min) for treatment of HAE attacks⁹ and Cinryze at a 1000-U i.v. injection (1 mL/min) every 3 or 4 days for routine prophylaxis of HAE attacks.¹⁰ Additional formulations of C1-INH are available or are in development in other countries. Bicizar (BioGenius LLC, Moscow, Russia) is another human plasma–derived C1-INH. Ceter (Sanquin Blood Supply Foundation, Amsterdam, The Netherlands; previously Central Laboratory of The Netherlands Red Cross Blood Transfusion Service [CLB]) is a similar product to Cinryze; Sanquin Blood Supply Foundation manufactures Cinryze on behalf of ViroPharma, Inc. Ruconest (Pharming group N.V., Leiden, The Netherlands) is a recombinant C1-INH product. In a biochemical comparison of Berinert, Cinryze, Ceter, and Ruconest, the purities using size exclusion chromatography were determined to be 97.0, 89.5, 88.6, and 98.6%, respectively.¹¹

REPORTS OF THROMBOGENICITY OF C1-INH

A total of 38 studies from the literature and manufacturer data are included in this review; 20 of these studies were from a PubMed search of English lan-

guage articles from January 1990 to December 2013. The search terms “(hereditary angioedema AND thrombotic) OR (C1 esterase inhibitor AND [thrombotic OR coagulation OR fibrinolysis])” yielded 165 articles. Titles and abstracts, if available, were reviewed to select original research articles that fulfilled the following criteria: (1) C1-INH was the focus of the study and (2) authors addressed the pro- or antithrombotic potential of C1-INH. Review articles and single case reports were excluded. Also excluded were articles that focused on gene mutations, *in vitro* research, health outcomes (e.g., quality-of-life measurements), characterizing new C1-INH formulations, or measuring C1-INH levels to diagnose or evaluate other disease states (e.g., diabetes and preeclampsia). Using reference lists of the 20 selected articles, 10 additional articles on the clinical use of C1-INH in myocardial infarction, ischemic stroke, sepsis, and capillary leak syndrome were reviewed for thromboembolic adverse events. In addition to the literature search, eight pivotal studies and prescribing information for Cinryze and Berinert were reviewed. Supplementary data on thrombogenic adverse events associated with C1-INH were provided by ViroPharma, Inc., the manufacturer of Cinryze.

Of the 38 total studies, 30 evaluated C1-INH in various dosages; Table 1 provides a comparison of these studies.^{12–40} The other eight studies^{41–48} investigated the effects of HAE on the coagulation system; these studies are not included in Table 1 and are discussed in the text.

Animal Data Suggesting Thrombogenicity of C1-INH

Data from animal studies suggest that high doses of C1-INH could be prothrombotic. A pharmacology/toxicology review memorandum of C1-INH (Cinryze) submitted for FDA review evaluated clot formation with the established Wessler *in vivo* model.¹² The Wessler stasis model infuses thrombin-free mammalian serum systemically to produce thrombi in rabbits and has been validated for measuring thrombogenic activity.⁴⁹ Doses ranging from 20 to >200 U/kg were administered to 24 rabbits.¹² The rabbit model showed that clot formation occurred at doses of 100–200 U/kg of C1-INH. Macroscopic fibrin threads were seen in one of four animals who received 20- to 50-U/kg doses, suggesting a potential risk at these doses. In a porcine model of coronary occlusion, 27 pigs were randomized to a single dose of C1-INH (Berinert; $n = 15$) or saline administered before coronary reperfusion, or sham operation.¹³ C1-INH at 40 U/kg reduced myocardial injury, whereas 100- and 200-U/kg doses had no beneficial effects. In four animals (three received 200 U/kg and 1 received 100 U/kg), blood could not be aspirated from the i.v. catheter because clots had formed. The authors of this study hypothesized that factor XIIIa and kallikrein, major activators of the fibrinolytic system, are inactivated by C1-INH, which could cause procoagulant effects.¹³ However, factor XII and kallikrein are minor contributors to the fibrinolytic cascade¹; major activators are tissue plasminogen activator and urokinase.⁵⁰

Clinical Data Suggesting Thrombogenicity of C1-INH

Thirteen neonates who did not have HAE underwent cardiac surgery with extracorporeal circulation.¹⁴ High-dose (up to 500 U/kg) C1-INH (Berinert HS) was administered to the 13 neonates during and after cardiopulmonary bypass surgery to prevent capillary leak syndrome. All patients developed venous thrombosis and nine died of emboli. The authors suggested that the temporal association between administration of C1-INH and development of thrombi may be explained by inhibition of the fibrinolytic system.

An analysis of the FDA Adverse Event Reporting System database has identified case reports of thromboembolic events associated with C1-INH in patients with HAE.¹⁵ Information component (IC) values were

estimated using the Bayesian confidence propagation neural network methodology,^{51–53} which considers the number of reports on the particular drug, adverse event in question, drug–adverse event combination, and total number of reports in the database. A positive IC value indicates the combination of the drug and adverse event is reported more frequently than statistically expected. Ten cases were identified in patients receiving recommended doses of C1-INH (Cinryze): two deep-vein thromboses, two transient ischemic attacks, one myocardial infarction, two jugular vein thromboses, two pulmonary emboli, and one embolic stroke/intracardiac thrombus. In this case, the IC_{025} value was 2.91, suggesting a signal. Because of limitations of the database, the Adverse Event Reporting System could not establish causality and detailed descriptions of cases were not provided, making expert medical assessments impossible.

There have been postmarketing reports of thromboembolic events (basilar artery thrombosis, pulmonary microemboli, and thrombosis) with recommended doses of Berinert for the treatment of HAE.⁹ However, no information was available on patients' medical histories or underlying risk factors.

FURTHER ASSESSMENT OF THROMBOGENICITY OF C1-INH

Incidence of Thromboembolic Adverse Events in Clinical Studies

In a 2.6-year-long open-label prevention study ($n = 146$) of Cinryze³⁴ five serious thromboembolic events (myocardial infarction, deep-vein thrombosis, pulmonary embolism, and two cerebrovascular accidents) occurred, but all patients had underlying risk factors (Table 2; data on file).³⁴ No thromboembolic events were seen in the four other clinical studies^{35–37} in the Cinryze development program ($n = 262$ unique subjects in five studies). Similarly, in various clinical studies of Berinert ($n = 284$) at doses of 20 U/kg^{38,39} and 500 U,⁴⁰ no thromboembolic events were reported. Based on available clinical data, thromboembolic events associated with C1-INH appear to be rare.

Role of Underlying Thromboembolic Risk Factors

Thromboembolic events have been reported in patients receiving recommended doses of Cinryze. These patients often had underlying thromboembolic risk factors, such as presence of an indwelling venous catheter or access device, prior history of thrombosis, atherosclerosis, oral contraceptive or androgen use, morbid obesity, and immobility,¹⁰ suggesting patients with known risk factors for thromboembolic events should be monitored during and after C1-INH administration.

Approximately 70–80% of all upper-extremity thrombosis and 10% of all venous thromboembolic

Table 1 Studies on various dosages of C1-INH evaluated to determine evidence of thrombogenicity of C1-INH*

Study	n#	Study Type	Product	Dosage Regimen	Disease state	Evidence of Thrombogenicity
Buehler ¹²	24	Animal (rabbits)	Cinryze	20- to 200-U/kg single dose	None—thrombosis model	Y
Horstick <i>et al.</i> ¹³	15	Animal (pigs)	Berinert	40-, 100-, 200-U/kg single dose	Coronary occlusion	Y
Drug Commission ¹⁴	13	Human	Berinert	Up to 500-U/kg single dose	Capillary leak syndrome after cardiopulmonary bypass	Y
Gandhi <i>et al.</i> ¹⁵	71§	Human	Cinryze	1000 U every 3–4 days	HAE	Y
Baig <i>et al.</i> ¹⁶	32	Animal (neonatal pigs)	Berinert	500-, 1000-, and 1500-U¶ (600 U/kg) single dose	Cardiopulmonary bypass	N
Duehrkop <i>et al.</i> ¹⁷	6	Animal (rats)	Berinert	50-U/kg single dose	Hind limb ischemia	N
Heydenreich <i>et al.</i> ¹⁸	369 (mice) ; 33 (rats)	Animal (mice and rats)	Berinert	7.5- or 15.0-U single dose (mice); 20-U/kg single dose (rats)	Ischemic stroke	N
Horstick <i>et al.</i> ¹⁹	6	Animal (pig)	Berinert	20-U/kg single dose	Coronary occlusion	N
Lu <i>et al.</i> ²⁰	6	Animal (mice)	Berinert	400-U/kg single dose	Myocardial ischemia and reperfusion injury	N
Salvatierra <i>et al.</i> ²¹	6	Animal (dog)	Berinert	500-U single dose	Lung transplantation	N
Fattouch <i>et al.</i> ²²	38	Human	Brand not specified	500 U of bolus before surgery; 500-U infusion for 3 hr after surgery	Myocardial ischemia and reperfusion injury	N
Thielmann <i>et al.</i> ²³	28	Human	Berinert	40 U/kg of bolus before surgery; 20-U/kg infusion 6 hr after surgery	Myocardial ischemia and reperfusion injury	N
de Zwaan <i>et al.</i> ²⁴	22	Human	Cetor	48-hr continuous infusion (50 or 100 U/kg loading dose, followed by 1.25 or 2 U/kg per hr)	Myocardial ischemia and reperfusion injury	N
Caliezi <i>et al.</i> ²⁵	20	Human	Berinert	6000, 3000, 2000, and 1000 U at 12-hr intervals	Severe sepsis or septic shock	N

Continued

Table 1 Continued

Study	n#	Study Type	Product	Dosage Regimen	Disease state	Evidence of Thrombogenicity
Igonin <i>et al.</i> ²⁶	41	Human	Bicizar	Initial 6000-U infusion, total of 12,000 over 48 hr**	Sepsis	N
Hack <i>et al.</i> ²⁷	5	Human	Beriner	2000 U; 1000 U every 12 hr thereafter for 5 days	Septic shock	N
Tassani <i>et al.</i> ²⁸	12	Human	Beriner	100-U/kg single dose	Capillary leak syndrome after cardiopulmonary bypass	N
Nürnberg <i>et al.</i> ²⁹	15	Human	Product from Centeon, now CSL Behring	60-U/kg dose, followed by two 30-U/kg doses and four 15-U/kg doses every 12 hr	Capillary leak syndrome after bone marrow transplantation	N
Ogilvie <i>et al.</i> ³⁰	6	Human	Brand not specified	2000 U, followed by 500 or 1000 U twice daily for 4 days	IL-2-induced toxicity	N
Herzog <i>et al.</i> ³¹	Not specified	Animal (rabbit)	Beriner	Cumulative doses of 100, 400, and 800 U/kg	None—thrombosis model	N
Bernstein <i>et al.</i> ³²	20	Human	Cinryze	1500 U every 3–4 days for 12 wk (with possible escalation to 2000 or 2500 U for another 12 wk)	HAE	N
Levy <i>et al.</i> ³³	N/A	Thromboelastometry	Cinryze	0.14, 0.7, 1.4, 2.8, and 7.0 U/mL##	None	N
Zuraw and Kalfus ³⁴	146	Human	Cinryze	1000 U every 3–7 days for up to 2.6 yr	HAE	Y
Zuraw <i>et al.</i> ³⁵	27	Human	Cinryze	1000 U alone or followed by second 1000-U dose 60 min later	HAE	N
Zuraw <i>et al.</i> ³⁶ (placebo-controlled treatment study)	35§§	Human	Cinryze	1000 U (as needed over 2 yr)	HAE	N
Zuraw <i>et al.</i> ³⁶ (placebo-controlled prevention study)	22§§			1000 U every 3–4 days (24-wk crossover period)		N

Continued

Table 1 Continued

Study	#	Study Type	Product	Dosage Regimen	Disease state	Evidence of Thrombogenicity
Riedl <i>et al.</i> ³⁷	113	Human	Cinryze	1000 U (as needed)	HAE	N
Craig <i>et al.</i> ³⁸	83	Human	Berinert	10 or 20 U/kg (as needed)	HAE	N
Craig <i>et al.</i> ³⁹	57	Human	Berinert	20 U/kg (as needed)	HAE	N
Farkas <i>et al.</i> ⁴⁰	61	Human	Berinert	500 U as needed (or at 5-day intervals for prevention in pregnant women)	HAE	N

*The following eight human studies did not evaluate dosages of C1-INH but assessed the effect of HAE on the coagulation system and were included in this review: Coppola *et al.*,⁴¹ Cugno *et al.*,⁴² Cugno *et al.*,⁴³ Joseph *et al.*,⁴⁴ Relan *et al.*,⁴⁵ van Geffen *et al.*,⁴⁶ and Nussberger *et al.*⁴⁸.

#Number of subjects who received C1-INH.

§Seventy-one case reports of patients with HAE using C1-INH products; 2458 reports of thrombotic events (total number of case reports in the U.S. Adverse Event Reporting Systems: 841,126).

¶Equivalent to 600 U/kg.

||Total number of animals in the study.

**Daily dose based on de Zwaam *et al.*²⁴

##Equivalent to 10-fold higher concentration than clinical dosing.

§§Eighty-eight subjects subsequently enrolled in an open-label treatment extension; there was overlap in subjects enrolled in the two studies (treatment study and prevention study) and extension phase.

C1-INH = C1 esterase inhibitor; HAE = hereditary angioedema; N/A = not available; PK = pharmacokinetic; Y = yes; N = no.

Table 2 Thrombotic events in the C1-INH (Cinryze) open-label prevention study (data on file)

Serious Adverse Events	Last Dose of C1-INH* in Relation to Adverse Event	Relevant Medical Condition(s)	Action(s) Taken	Causality	Outcome
Myocardial infarction	2 days prior	Hepatocellular carcinoma, coronary artery disease (previous myocardial infarction with stent placement), hypertension, and dyslipidemia	C1-INH continued; heparin	Not related	Death
Deep-vein thrombosis (right leg)	2 days prior	Right leg fibroma, excision of fibroma, and immobilization	None; enoxaparin and warfarin	Not related	Recovered
Cerebrovascular accident	6 days prior	Bacterial endocarditis	C1-INH continued; aortic valve replaced	Not related	Recovered
Foreign body pulmonary embolism	3 days prior	Foreign body injection	None	Not related	Death
Cerebrovascular accident	3 days prior	Cerebrovascular disease, type 2 diabetes mellitus, and Pickwickian (steroid use)	C1-INH continued	Not related	Recovered

Source: Ref. 34.

*One thousand units of C1-INH over 10 min.

C1-INH = C1 esterase inhibitor.

events are related to catheters.⁵⁴ In a worldwide survey of physicians who treat patients with HAE, thromboembolic events occurred in 0.6% (5/856) of patients receiving C1-INH and in 18% (3/17) of patients with a central catheter.⁵⁵

Effects of HAE on Coagulation System

Another confounding factor is the effect of the HAE disease state on the coagulation system. In a study of effects of a single 15-U/kg C1-INH infusion on platelet activity ($n = 6$),⁴¹ platelet aggregation decreased significantly after 60 minutes and was still low compared with baseline values after 7 days. The authors suggested that C1-INH deficiency may contribute to increased platelet activity in patients with HAE. In a *post hoc* analysis ($n = 25$) of an HAE study,⁴⁶ coagulation and fibrinolytic systems were activated in patients during an HAE attack. Patients had elevated levels of prothrombin fragment 1 + 2 (F1 + 2), thrombin-anti-thrombin complex, D-dimer, and plasmin- α_2 -antiplasmin complexes. Treatment with recombinant C1-INH at 50 or 100 U/kg did not affect coagulation and fibrinolytic parameters except for an expected prolongation in activated partial thromboplastin time and a decrease in F1 + 2. No thromboembolic adverse events were reported up to 90 days after drug administration. In a study of 43 patients with HAE and 20 control subjects,⁴⁷ increased thrombin generation and F1 + 2 levels were seen in patients with C1-INH deficiency during HAE attacks. Thrombin generation correlated inversely with C1-INH levels. An assessment of the abnormalities of the bradykinin-forming pathway and fibrinolysis in patients with HAE found that factor XIIa, kallikrein, and bradykinin decreased after C1-INH treatment (Cinryze).⁴⁵ During HAE attacks, patients have high F1 + 2 and D-dimer levels,⁴² high bradykinin levels,⁴⁸ and increased thrombin generation.⁴³ Release of vasoactive mediators during attacks is associated with activation of the fibrinolytic system.⁴⁴ In HAE, this is likely because of release of tissue plasminogen activator from endothelial cells stimulated by bradykinin interaction with the B2-receptor^{45,56} rather than any direct fibrinolytic effect of kallikrein or factor XIIa.

Use of C1-INH in Other Disease States

Animal Data. In a porcine model of neonatal cardiopulmonary bypass,¹⁶ C1-INH improved cardiac and pulmonary function. In the study, 52 neonatal pigs were randomized to receive a single dose of C1-INH (Berinert) at 0 ($n = 22$), 500 ($n = 15$), 1000 ($n = 8$), or 1500 U ($n = 7$) before surgery. The pigs had a mean weight of 2.54 kg, so the highest dose (1500 U) was equivalent to ~600 U/kg, which exceeds that given in the German neonates in the previously discussed arti-

cle.¹⁴ C1-INH protected against capillary leak syndrome and improved pulmonary function. Baig and colleagues¹⁶ hypothesized that the effects were related to the inhibition of kallikrein and subsequent decreased bradykinin production. Three pigs in the 0-U dose group and two in the 1500-U dose group did not survive the surgery. C1-INH supplementation improved systolic function; however, the highest dose appeared to decrease diastolic function. Further investigation is needed because diastolic dysfunction may be associated with increased risk of thrombosis.⁵⁷

C1-INH has successfully prevented reperfusion injury in animal models by regulating inflammatory and thromboembolic processes.^{17–21} In a rat hind-limb ischemia model,¹⁷ C1-INH (Berinert) at a dose of 50 U/kg reduced edema formation and fibrin deposition in muscle and lung tissue. In a mouse model of ischemic stroke ($n = 369$),¹⁸ a single i.v. injection of 15 U of C1-INH (Berinert), but not 7 U, had a strong anti-inflammatory and antithrombotic effect. C1-INH reduced infarct volumes by 60%, mitigated clot formation by counteracting factor XIIIa activity and inflammation, and reduced intracerebral fibrin formation. Blood–brain barrier damage, edema formation, and inflammation decreased with C1-INH compared with controls. Results were confirmed with doses of 20 U/kg of C1-INH in a rat model ($n = 33$). Similarly, in a canine model of lung transplantation,²¹ 500 U of C1-INH (Berinert) prevented hypoxemia, complement activation, and endothelial leukocyte adhesion. C1-INH acts by inhibiting both the complement pathway and the neutrophil accumulation in ischemic tissue.^{19,20}

Clinical Data. Treatment with C1-INH was able to reduce myocardial ischemia–reperfusion injury after emergency coronary artery bypass grafting for acute ST-elevation myocardial infarction in three randomized controlled trials.^{22–24} C1-INH was administered at different doses: 1000 U total (brand not specified; $n = 38$),²² 60 U/kg total (Berinert P; $n = 28$),²³ and a 48-hour continuous infusion (50 or 100 U/kg loading dose, followed by 1.25 or 2 U/kg per hour; $n = 22$ (Cetor; CLB [now Sanquin], Amsterdam, The Netherlands).²⁴ No drug-related adverse events were observed in any patients. In these disease states, C1-INH is administered as a single dose or a short-term treatment.

C1-INH has been used in high doses for the treatment of other disorders, such as sepsis and capillary leak syndrome, without drug-related side effects. In a randomized, double-blind, placebo-controlled study ($n = 40$), C1-INH (Berinert HS) was administered as a 1-hour i.v. infusion regimen at 6000, 3000, 2000, and 1000 U at 12-hour intervals to 20 patients with severe sepsis or septic shock.²⁵ At 90 days, mortality rate was similar between groups (C1-INH group, seven deaths;

placebo group, six deaths), respectively, all related to septic shock, with the exception of one patient in the C1-INH group who died from an intraoperative aortic rupture during cardiac surgery. Adverse events included six major bleeds in the C1-INH group and two in the placebo group, all of which were attributed to sepsis. In an open-label, randomized, controlled trial, 41 patients with sepsis received 12,000 U of C1-INH (Bicizar) over 48 hours in addition to conventional treatment compared with standard sepsis therapy alone ($n = 20$).²⁶ All fatalities (8% in C1-INH group versus 45% in standard therapy group; $p = 0.001$) were caused by sepsis or surgical complications, and there were no serious treatment-related adverse events. Another small study in five patients showed similar findings.²⁷

A randomized, placebo-controlled, double-blind study ($n = 24$) was conducted administering 1 dose of C1-INH (100 U/kg; Berinert) to 12 neonates before cardiac surgery with cardiopulmonary bypass to prevent capillary leak.²⁸ C1-INH decreased inflammatory response in the neonates, and all 24 neonates were discharged home without complications; no other information on adverse events was provided. In two other studies ($n = 21$ total), capillary leak syndrome was successfully treated with a cumulative dose of 180 U/kg of C1-INH (C1-INH preparation from Centeon, Liederbach, Germany; now CSL Behring) in patients after bone marrow transplantation ($n = 15$)²⁹ and with 2000 U of C1-INH (C1-INH preparation from CLB [now Sanquin]) followed by 500 or 1000 U every 12 hours for 4 days in patients with IL-2–induced toxicity ($n = 6$),³⁰ without evidence of thrombogenic activity of C1-INH. Overall, C1-INH appeared to be well tolerated in these patients, even at doses higher than those approved for patients with HAE.

Investigation into Dose-Escalation Effects and Ex Vivo Hemostatic Effects

In response to recent reports of thromboembolic adverse events in humans, the modified Wessler model was used to assess venous thrombosis in rabbits administered cumulative C1-INH (Berinert) doses of 100, 400, and 800 U/kg.³¹ Dose-dependent inhibition of coagulation factors XI and XII and thrombin was seen. No thrombus formation occurred. At 800 U/kg, C1-INH inhibited arterial occlusion 2.2-fold compared with isotonic saline, prolonged activated partial thromboplastin time, and decreased platelet aggregation. The rabbit model showed no prothrombotic risk in association with C1-INH treatment at supratherapeutic doses.

In a dose-escalation study for prophylaxis in patients with HAE, 1500 U of Cinryze was administered every 3 or 4 days for 12 weeks.³² At 12 weeks, adequate

disease control (one or less attack per month or at the investigator's discretion) was assessed to determine if dose escalation was needed. Of 20 subjects started at 1500 U of C1-INH, 13 were escalated to 2000 U, and 12 were subsequently escalated to 2500 U. Most adverse events (86/91; 95%) were considered unrelated to study treatment and 85% (77/91) were mild to moderate in intensity. Up to 2500 U of C1-INH administered every 3 or 4 days for 6 months did not result in any thromboembolic events, with the exception of one patient who experienced a blood clot in a catheter port while receiving 1500 U of C1-INH.

An *ex vivo* evaluation of hemostatic effects using thromboelastometry with the ROTEM system (Tem Innovations GmbH, Munich, Germany) was conducted in blood samples from 10 healthy volunteers.³³ C1-INH concentrations of 0.14, 0.7, 1.4, 2.8, and 7.0 U/mL were added to whole blood samples and were compared with blood samples without C1-INH, samples with procoagulants, and samples with anticoagulants. Platelet-poor samples were used for measurement by a calibrated, automated thrombin generation system. C1-INH was not a procoagulant at any concentration studied; in fact, the two highest concentrations (2.8 and 7.0 U/mL) reduced maximum clot firmness ($p < 0.05$). The 7.0-U/mL concentration is 10-fold higher than levels achieved with clinical dosing. C1-INH in increasing concentrations did not affect coagulation time, angle of the thromboelastometric curve, and fibrinolysis, and did not significantly affect thrombin generation lag time, peak thrombin generation, or thrombin generation rate. Procoagulant and anticoagulant controls showed expected hemostatic effects; however, C1-INH did not show procoagulant activity.

CONCLUSION

The cases of thromboembolic adverse events associated with C1-INH are not common and appear to occur predominantly in patients at high risk of thromboembolic complications. C1-INH has been used in various disease states, often at doses higher than those approved for patients with HAE, and thromboembolic complications have been rarely reported. *Ex vivo* models provide conflicting results of the effect of these products on coagulation. Based on the totality of evidence, thromboembolism associated with C1-INH administration appears to be rare, and a cause-and-effect relationship has not been established. Additional long-term evaluation is needed.

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REFERENCES

1. Zeerleder S. C1-inhibitor: More than a serine protease inhibitor. *Semin Thromb Hemost* 37:362–374, 2011.
2. Zuraw BL. Clinical practice. Hereditary angioedema. *N Engl J Med* 359:1027–1036, 2008.
3. Cicardi M, Aberer W, Banerji A, et al, on behalf of HAWK (Hereditary Angioedema International Working Group) under the patronage of EAACI (European Academy of Allergy and Clinical Immunology). Classification, diagnosis, and approach to treatment for angioedema: Consensus report from the Hereditary Angioedema International Working Group. *Allergy* 69:602–616, 2014.
4. Birnbaum J, Klotz E, Spies CD, et al. The combinations C1 esterase inhibitor with coagulation factor XIII and N-acetylcysteine with tirilazad mesylate reduce the leukocyte adherence in an experimental endotoxemia in rats. *Clin Hemorheol Microcirc* 40:167–176, 2008.
5. Birnbaum J, Klotz E, Spies CD, et al. Impact of combined C1 esterase inhibitor/coagulation factor XIII or N-acetylcysteine/tirilazad mesylate administration on leukocyte adherence and cytokine release in experimental endotoxaemia. *J Int Med Res* 36:748–759, 2008.
6. Cai S, and Davis AE III. Complement regulatory protein C1 inhibitor binds to selectins and interferes with endothelial-leukocyte adhesion. *J Immunol* 171:4786–4791, 2003.
7. Zhang H, Qin G, Liang G, et al. Suppression of complement regulatory protein C1 inhibitor in vascular endothelial activation by inhibiting vascular cell adhesion molecule-1 action. *Biochem Biophys Res Commun* 358:1120–1127, 2007.
8. Levi M, Roem D, Kamp AM, et al. Assessment of the relative contribution of different protease inhibitors to the inhibition of plasmin in vivo. *Thromb Haemost* 69:141–146, 1993.
9. CSL Behring LLC. Berinert prescribing information. Kankakee, IL; 2012. (Last accessed February 2014).
10. ViroPharma Biologics, Inc. Cinryze prescribing information. Exton, PA; 2014. (Last accessed March 2014).
11. Feussner A, Kalina U, Hofmann P, et al. Biochemical comparison of four commercially available C1 esterase inhibitor concentrates for treatment of hereditary angioedema. *Transfusion* 2014. Epub ahead of print May 8, 2014. doi: 10.1111/trf.12678.
12. Buehler PW. www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/ApprovedProducts/LicensedProductsBLAs/FractionatedPlasmaProducts/UCM229783.pdf.
13. Horstick G, Berg O, Heimann A, et al. Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: Dose-related beneficial versus detrimental effects. *Circulation* 104:3125–3131, 2001.
14. Drug Commission of the German Medical Profession. Serious thrombogenesis following Berinert HS. *Dtsch Arztebl* 97:A-1016, 2000.
15. Gandhi PK, Gentry WM, and Bottorff MB. Thrombotic events associated with C1 esterase inhibitor products in patients with hereditary angioedema: Investigation from the United States Food and Drug Administration adverse event reporting system database. *Pharmacotherapy* 32:902–909, 2012.
16. Baig K, Nassar R, Craig DM, et al. Complement factor 1 inhibitor improves cardiopulmonary function in neonatal cardiopulmonary bypass. *Ann Thorac Surg* 83:1477–1482, 2007.
17. Duehrkop C, Banz Y, Spirig R, et al. C1 esterase inhibitor reduces lower extremity ischemia/reperfusion injury and associated lung damage. *PLoS One* 8:e72059, 2013.
18. Heydenreich N, Nolte MW, Göb E, et al. C1-inhibitor protects from brain ischemia-reperfusion injury by combined anti-in-

- flammatory and antithrombotic mechanisms. *Stroke* 43:2457–2467, 2012.
19. Horstick G, Heimann A, Götze O, et al. Intracoronary application of C1 esterase inhibitor improves cardiac function and reduces myocardial necrosis in an experimental model of ischemia and reperfusion. *Circulation* 95:701–708, 1997.
 20. Lu F, Fernandes SM, and Davis AE III. The effect of C1 inhibitor on myocardial ischemia and reperfusion injury. *Cardiovasc Pathol* 22:75–80, 2013.
 21. Salvatierra A, Velasco F, Rodriguez M, et al. C1-esterase inhibitor prevents early pulmonary dysfunction after lung transplantation in the dog. *Am J Respir Crit Care Med* 155:1147–1154, 1997.
 22. Fattouch K, Bianco G, Speziale G, et al. Beneficial effects of C1 esterase inhibitor in ST-elevation myocardial infarction in patients who underwent surgical reperfusion: A randomised double-blind study. *Eur J Cardiothorac Surg* 32:326–332, 2007.
 23. Thielmann M, Marggraf G, Neuhäuser M, et al. Administration of C1-esterase inhibitor during emergency coronary artery bypass surgery in acute ST-elevation myocardial infarction. *Eur J Cardiothorac Surg* 30:285–293, 2006.
 24. de Zwaan C, Kleine AH, Diris JH, et al. Continuous 48-h C1-inhibitor treatment, following reperfusion therapy, in patients with acute myocardial infarction. *Eur Heart J* 23:1670–1677, 2002.
 25. Caliezi C, Zeerleder S, Redondo M, et al. C1-inhibitor in patients with severe sepsis and septic shock: Beneficial effect on renal dysfunction. *Crit Care Med* 30:1722–1728, 2002.
 26. Igonin AA, Protsenko DN, Galstyan GM, et al. C1-esterase inhibitor infusion increases survival rates for patients with sepsis*. *Crit Care Med* 40:770–777, 2012.
 27. Hack CE, Voerman HJ, Eisele B, et al. C1-esterase inhibitor substitution in sepsis. *Lancet* 339:378, 1992.
 28. Tassani P, Kunkel R, Richter JA, et al. Effect of C1-esterase-inhibitor on capillary leak and inflammatory response syndrome during arterial switch operations in neonates. *J Cardiothorac Vasc Anesth* 15:469–473, 2001.
 29. Nürnberger W, Heying R, Burdach S, and Göbel U. C1 esterase inhibitor concentrate for capillary leakage syndrome following bone marrow transplantation. *Ann Hematol* 75:95–101, 1997.
 30. Ogilvie AC, Baars JW, Eerenberg AJ, et al. A pilot study to evaluate the effects of C1 esterase inhibitor on the toxicity of high-dose interleukin 2. *Br J Cancer* 69:596–598, 1994.
 31. Herzog E, Schuermann D, Raquet E, et al. Berinert (C1-esterase inhibitor concentrate) treatment is not associated with prothrombotic risk based on preclinical efficacy and safety investigations. Poster Presentation at the 2013 annual meeting of the American Academy of Allergy, Asthma and Immunology (AAAAI), San Antonio, TX, February 22–26, 2013.
 32. Bernstein JA, Manning ME, Li H, et al. Escalating doses of C1 esterase inhibitor (CINRYZE) for prophylaxis in patients with hereditary angioedema. *J Allergy Clin Immunol Pract* 2:77–84, 2014.
 33. Levy J, Szlam F, and Gelone S. An ex vivo evaluation of the hemostatic effects of plasma-derived C1 inhibitor. Presented at the XXIV Congress of the International Society on Thrombosis and Haemostasis, Amsterdam, The Netherlands, June 29–July 4, 2013. Available online at www.eventure-online.com/eventure/publicAbstractView.do?id=213980&congressId=6839; accessed December 27, 2013.
 34. Zuraw BL, and Kalfus I. Safety and efficacy of prophylactic nanofiltered C1-inhibitor in hereditary angioedema. *Am J Med* 125:938.e1–7, 2012.
 35. Zuraw B. Pharmacokinetics of C1 esterase inhibitor in hereditary angioedema subjects. <http://clinicaltrials.gov/ct2/show/NCT00432510>. (Last accessed March 2014).
 36. Zuraw BL, Busse PJ, White M, et al. Nanofiltered C1 inhibitor concentrate for treatment of hereditary angioedema. *N Engl J Med* 363:513–522, 2010.
 37. Riedl MA, Hurewitz DS, Levy R, et al. Nanofiltered C1 esterase inhibitor (human) for the treatment of acute attacks of hereditary angioedema: An open-label trial. *Ann Allergy Asthma Immunol* 108:49–53, 2012.
 38. Craig TJ, Levy RJ, Wasserman RL, et al. Efficacy of human C1 esterase inhibitor concentrate compared with placebo in acute hereditary angioedema attacks. *J Allergy Clin Immunol* 124: 801–808, 2009.
 39. Craig TJ, Bewtra AK, Bahna SL, et al. C1 esterase inhibitor concentrate in 1085 hereditary angioedema attacks: Final results of the I.M.P.A.C.T. 2 study. *Allergy* 66:1604–1611, 2011.
 40. Farkas H, Jakab L, Temesszentandrás G, et al. Hereditary angioedema: A decade of human C1-inhibitor concentrate therapy. *J Allergy Clin Immunol* 120:941–947, 2007.
 41. Coppola L, Guastafierro S, Verrazzo G, et al. C1 inhibitor infusion modifies platelet activity in hereditary angioedema patients. *Arch Pathol Lab Med* 126:842–845, 2002.
 42. Cugno M, Zanichelli A, Bellatorre AG, et al. Plasma biomarkers of acute attacks in patients with angioedema due to C1-inhibitor deficiency. *Allergy* 64:254–257, 2009.
 43. Cugno M, Cicardi M, Bottasso B, et al. Activation of the coagulation cascade in C1-inhibitor deficiencies. *Blood* 89:3213–3218, 1997.
 44. Cugno M, Hack CE, de Boer JP, et al. Generation of plasmin during acute attacks of hereditary angioedema. *J Lab Clin Med* 121:38–43, 1993.
 45. Joseph K, Tholanikunnel TE, and Kaplan AP. Treatment of episodes of hereditary angioedema with C1 inhibitor: Serial assessment of observed abnormalities of the plasma bradykinin-forming pathway and fibrinolysis. *Ann Allergy Asthma Immunol* 104:50–54, 2010.
 46. Relan A, Bakhtiari K, van Amersfoort ES, et al. Recombinant C1-inhibitor: Effects on coagulation and fibrinolysis in patients with hereditary angioedema. *BioDrugs* 26:43–52, 2012.
 47. van Geffen M, Cugno M, Lap P, et al. Alterations of coagulation and fibrinolysis in patients with angioedema due to C1-inhibitor deficiency. *Clin Exp Immunol* 167:472–478, 2012.
 48. Nussberger J, Cugno M, and Cicardi M. Bradykinin-mediated angioedema. *N Engl J Med* 347:621–622, 2002.
 49. Wessler S, Reimer SM, and Sheps MC. Biologic assay of a thrombosis-inducing activity in human serum. *J Appl Physiol* 14:943–946, 1959.
 50. Vassalli JD, Sappino AP, and Belin D. The plasminogen activator/plasmin system. *J Clin Invest* 88:1067–1072, 1991.
 51. Bate A, Lindquist M, Edwards IR, et al. A Bayesian neural network method for adverse drug reaction signal generation. *Eur J Clin Pharmacol* 54:315–321, 1998.
 52. Norén GN, Bate A, Orre R, and Edwards IR. Extending the methods used to screen the WHO drug safety database towards analysis of complex associations and improved accuracy for rare events. *Stat Med* 25:3740–3757, 2006.
 53. van Puijenbroek EP, Bate A, Leufkens HG, et al. A comparison of measures of disproportionality for signal detection in spontaneous reporting systems for adverse drug reactions. *Pharmacoepidemiol Drug Saf* 11:3–10, 2002.
 54. Kucher N. Clinical practice. Deep-vein thrombosis of the upper extremities. *N Engl J Med* 364:861–869, 2011.
 55. Kalaria S, and Craig T. Assessment of hereditary angioedema treatment risks. *Allergy Asthma Proc* 34:519–522, 2013.
 56. Smith D, Gilbert M, and Owen WG. Tissue plasminogen activator release in vivo in response to vasoactive agents. *Blood* 66:835–839, 1985.
 57. Feng D, Syed IS, Martinez M, et al. Intracardiac thrombosis and anticoagulation therapy in cardiac amyloidosis. *Circulation* 119: 2490–2497, 2009. □

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