

Critical role of kallikrein in hereditary angioedema pathogenesis: A clinical trial of ecallantide, a novel kallikrein inhibitor

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Background: Hereditary angioedema (HAE) is a rare, autosomal-dominant disorder caused by C1 inhibitor gene mutation. Patients with HAE experience intermittent attacks of edema affecting the oropharynx, abdomen, gastrointestinal tract, and limbs. C1 inhibitor is the primary endogenous inhibitor of the kallikrein-kinin (contact) cascade.

Unregulated kallikrein activation generates bradykinin, the likely mediator of the swelling and pain characterizing HAE attacks. Ecallantide, a novel, recombinant protein, potently inhibits kallikrein. This is the first placebo-controlled assessment in human beings of a therapeutic intervention to improve symptoms of HAE attacks under the hypothesis that the contact cascade is the putative pathway responsible for HAE pathology.

Objective: To determine the safety and efficacy of ecallantide in patients with HAE.

Methods: This double-blind, placebo-controlled, ascending-dose study assessed efficacy and tolerability of ecallantide

(5, 10, 20, or 40 mg/m² intravenously) in individuals experiencing acute HAE attacks (N = 49). Twelve patients were assigned to each dose level: 10 to ecallantide and 2 to placebo, per cohort.

Results: Ecallantide treatment ameliorated the symptoms of HAE attacks: 72.5% (29/40) of patients treated with ecallantide versus 25.0% (2/8) of placebo patients reported significant improvement in symptoms within 4 hours ($P = .0169$).

Ecallantide was well tolerated at all doses.

Conclusion: Ecallantide, a potent, specific inhibitor of plasma kallikrein, significantly improved HAE symptoms over placebo. The trial provides strong support for the role of the kallikrein-kinin cascade and its end product, bradykinin, in the pathophysiology of HAE. Further clinical trials are underway. **Clinical implications:** Ecallantide is a promising new therapy for HAE attacks. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Ecallantide, bradykinin, C1 inhibitor, C1-INH, contact, hereditary angioedema, HAE, kallikrein, kinin

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Hereditary angioedema (HAE) is a rare genetic disorder with a prevalence of 1 in 10,000 to 50,000.^{1,2} Patients with HAE experience acute attacks of nonpitting, nonpruritic edema involving nearly every body area, most notably the larynx, oropharynx, face, gastrointestinal mucosa, limbs, or genitalia.^{3,4} Clinical presentations of HAE attacks can vary widely. HAE attacks can be painful and dangerous, usually last 1 to 5 days, and often occur with highly variable frequency.⁵ Laryngeal attacks may be life-threatening, with variable speed and degree of progression and with the potential for asphyxiation.^{6,7} Abdominal attacks may mimic acute abdomen, prompting unnecessary exploratory laparotomy.⁸

An approved, safe, and effective treatment for HAE does not exist currently in the United States. Available active and prophylactic medical treatments for HAE attacks carry significant risks and side effects.⁹⁻¹⁵ These treatments include fresh frozen plasma, attenuated androgens, and antifibrinolytics. Heparin provides no treatment benefit.¹⁶ C1 esterase inhibitor (C1-INH) concentrate is a safe and effective treatment for acute attacks of HAE but has not been approved by the US Food and Drug Administration.¹⁷⁻²⁰

Hereditary angioedema is characterized by a mutation in a single allele of C1-INH, located on chromosome 11.²¹

Abbreviations used

AE:	Adverse event
APTT:	Activated partial thromboplastin time
C1-INH:	C1 esterase inhibitor
CTC:	National Cancer Institute Common Toxicity Criteria
HAE:	Hereditary angioedema

More than 100 C1-INH mutations have been found, many of which are spontaneous first-generation mutations.^{22,23} C1-INH is a member of the serpin family and is the primary regulatory suppressor of the kallikrein-kinin (also known as the contact) cascade.²⁴ C1-INH also suppresses the coagulation and complement cascades (Fig 1). Because C1-INH regulates multiple cascades, the identification of the enzymes responsible for the pathophysiology of HAE has been challenging.²⁵ Recently, researchers have concluded that attacks are a result of overactivation of the kallikrein-kinin cascade and subsequent generation of bradykinin.²⁶⁻²⁸ Complement and coagulation cascade enzymes may be involved in HAE but are no longer thought to be the primary mediators of HAE symptomatology.^{29,30} Trauma or stress, common precipitants of HAE attacks, are thought to decrease endogenous C1-INH levels below a critical point, allowing relative overactivation of the kallikrein cascade. Kallikrein then cleaves high-molecular-weight kininogen, releasing bradykinin, which is thought to cause the localized edema, swelling, and pain that characterize HAE attacks.

There is considerable evidence for the activation of the kallikrein cascade and the production of bradykinin during HAE attacks. Investigation of plasma during HAE attacks has demonstrated activation of the kinin system and increases in bradykinin.³¹⁻³³ Genetic studies have demonstrated that a disruption in C1-INH's interaction with kallikrein (but not C1-INH's interaction with the complement cascade) is crucial to the development of HAE.^{34,35} Finally, in a knockout mouse model, C1-INH-deficient mice show evidence of increased vascular permeability that is reversed by ecallantide, a kallikrein inhibitor.³⁶

Ecallantide, also referred to as DX-88 (Dyax Corp, Cambridge, Mass) is a potent and specific kallikrein inhibitor that blocks generation of kallikrein and its byproduct, bradykinin. Ecallantide is a 60-amino acid recombinant protein produced in the *Pichia pastoris* strain of yeast and was discovered through phage display technology.^{37,38} Ecallantide is highly specific for plasma kallikrein, whereas C1-INH has similar specificities for multiple enzymes.³⁹ We hypothesize that the potency of ecallantide will make it a rapid-acting, effective inhibitor of plasma kallikrein, whereas its specificity should limit side effects. Further, as a recombinant protein, ecallantide carries little risk of the viral contamination associated with blood-derived products.

The current study (Evaluation of DX-88's Effect in Mitigating AngioedemaSM) is the first placebo-controlled assessment in human beings of a therapeutic intervention based on the hypothesis that the kallikrein cascade is the

causative factor in HAE and that treatment with ecallantide will improve symptoms of the recurrent attacks.

METHODS

Study design

The EDEMA1 study was a multicenter, randomized, placebo-controlled, double-blind, ascending-dose trial evaluating 4 doses of ecallantide in patients experiencing acute attacks of HAE. Patients were randomized to treatment with a single dose of ecallantide (5, 10, 20, or 40 mg/m² intravenously) or placebo (PBS) by intravenous infusion. Twelve patients were assigned to each dose cohort, with 10 patients randomized to receive ecallantide and 2 to placebo. On the basis of the patient's calculated body surface area, ecallantide (or placebo) was diluted in saline to 20 mL and administered as a single intravenous infusion over a period of 10 minutes. Patients were monitored in the clinic for at least 8 hours after dosing; follow-up visits occurred at 1, 2, and 4 weeks after infusion.

The trial was conducted at 26 study sites located in the United States, Belgium, and Israel. The protocol and all amendments were reviewed and approved by hospital Institutional Review Boards or Ethics Committees. All patients enrolled in the study provided freely written informed consent in accordance with the United States Food and Drug Administration regulations, the current version of the Declaration of Helsinki, and the International Conference on Harmonization Guidelines. An unblinded Data Monitoring Safety Board regularly reviewed and assessed safety and efficacy data during the study.

Patients

Patients were required to be at least 10 years of age and to have confirmed HAE. Diagnosis was established by the presence of at least 1 clinical and 1 laboratory criterion. Clinical criteria included recurrent, self-limited, noninflammatory angioedema lasting more than 12 hours, without significant urticaria; recurrent, self-limited, abdominal pain lasting more than 6 hours without organic disease; recurrent laryngeal edema; and a family history of recurrent angioedema, abdominal pain, or laryngeal edema. The laboratory criteria included C1-INH functional levels less than 50% of normal or genetic confirmation of C1-INH mutation.

Exclusion criteria included the following: serious intercurrent illness, active infection, serum creatinine >10% above the upper limit of normal, and liver transaminase levels greater than or equal to twice the upper limit of normal. Patients were also excluded if they had received an investigational drug or device within 30 days before entering the study or if they had any previous treatment with ecallantide. Patients with acquired angioedema and women who were pregnant or breast-feeding were ineligible.

Efficacy assessments

The primary efficacy endpoint of the study was the percentage of patients reporting significant improvement at the primary attack location (abdominal, laryngeal, or peripheral) by 4 hours after drug infusion. The primary attack location was defined by the site of most severe symptoms, as identified by the patient. Time to significant improvement was calculated, in minutes, as the time from the end of the treatment infusion to the time of the first indication of significant improvement of the symptom affecting this primary location. A successful outcome was defined as patient report of significant improvement within 4 hours after the end of treatment infusion.

Safety assessments

Safety evaluations included adverse event (AE) monitoring, coagulation parameters, and development of non-IgE antiecallantide

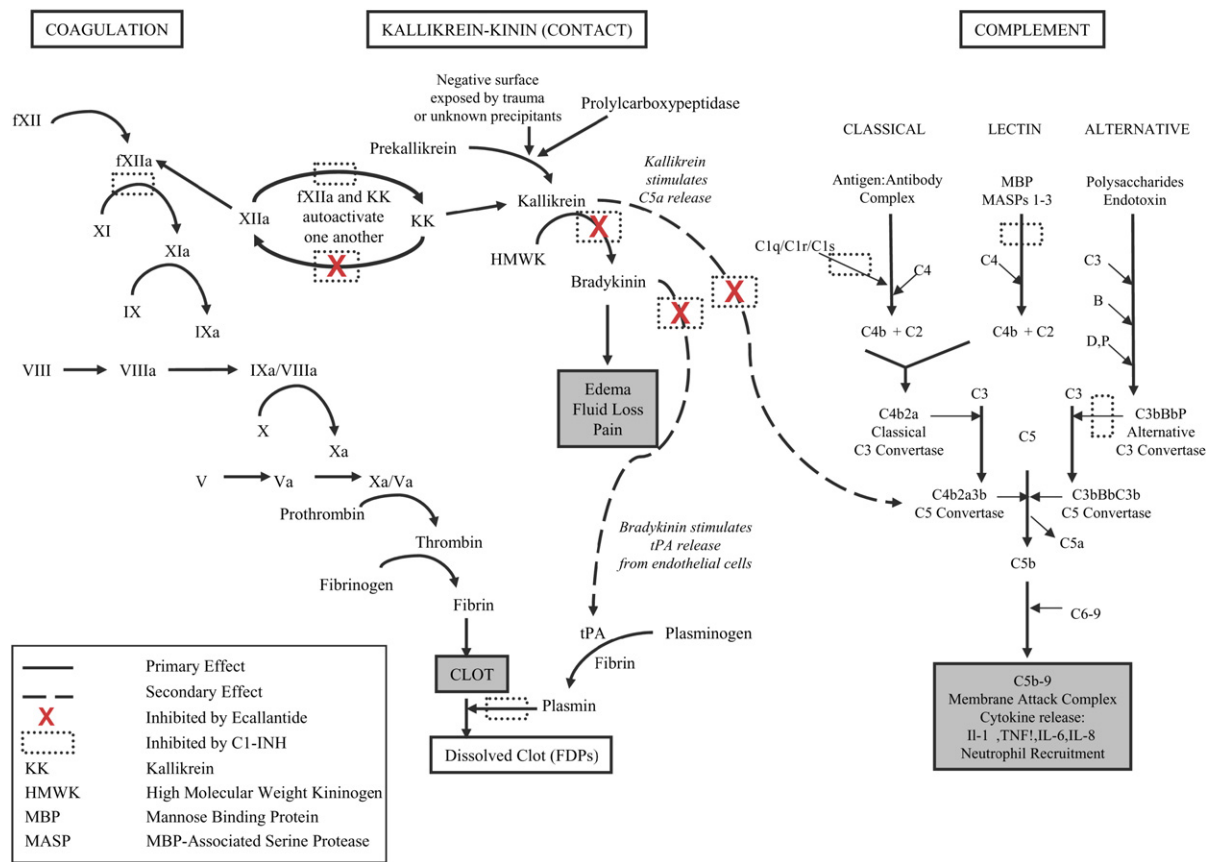


FIG 1. Kallikrein, coagulation and complement cascades: role of C1-INH regulation. C1-INH is the primary regulatory suppressor of the kallikrein-kinin (also known as the contact) cascade, and also suppresses the coagulation and complement cascades. Reprinted with permission from Levy J, O'Donnell P. The therapeutic potential of a kallikrein inhibitor for treating hereditary angioedema. *Expert Opin Investig Drugs* 2006; 15:1077-90.³⁹

antibodies. Serum samples were collected pretreatment and 2 and 4 weeks posttreatment and analyzed for antiecallantide IgG antibody by using a validated ELISA method developed and validated by MDS Pharma Services (Montreal, Quebec, Canada) in compliance with Good Laboratory Practices. In this assay, antiecallantide IgG antibodies in human serum samples and rabbit antiecallantide antiserum (positive control) were captured by bound ecallantide, followed by peroxidase-conjugated goat antirabbit IgG or donkey antihuman IgG secondary antibodies. Bound peroxidase was detected by a color reaction measured at OD 460 to 650 nm. Presence of antiecallantide IgG antibodies was deemed positive when OD was > 2-fold the mean OD of 50 normal human samples. Low and high positive control references were run on each assay plate. Positive samples were confirmed for specificity to ecallantide by a loss of signal in an immunodepletion assay following sample incubation with exogenous ecallantide. No testing for IgE antibodies to ecallantide was performed in the EDEMA1 study. Standard symptoms of HAE that were considered part of a subject's normal course of an HAE attack were not considered AEs. All toxicities and adverse events were graded using the National Cancer Institute Common Toxicity Criteria (CTC), version 2.0.

Statistical analysis

The Fisher exact test was used to assess significance of differences in categorical data and declared statistical significance at the .05 level.

Kaplan-Meier techniques were applied to estimate the median times to beginning of significant improvement and significant improvement in HAE symptoms.

RESULTS

Treatment assignments, patient demographics, and baseline characteristics

Forty-eight patients were randomized to intravenous treatment with ecallantide ($n = 40$, 83.3%) or to receive placebo ($n = 8$, 16.7%). Ten patients each were randomized to be treated with a dose of 5, 10, 20, or 40 mg/m² ecallantide. As a result of the simultaneous HAE attacks of 2 patients at different study sites, an additional patient (40 mg/m² ecallantide cohort) was enrolled beyond the protocol-designated population of 48 patients. Only 1 of these patients was randomized to treatment. The 49th patient was enrolled and treated; however, efficacy data were not analyzed, although safety data are presented.

Despite minor differences in patient demographics and disease characteristics, the pooled ecallantide patients and pooled placebo patients were generally similar at the baseline of the study. The majority of the patients were

female (77.6%) and white (87.8%). Disease activity, frequency of attacks, and interval from last episode were comparable between the groups.

Efficacy

The primary efficacy endpoint of the study was achieved with statistical significance: a greater proportion of patients treated with ecallantide for their HAE attacks achieved a successful outcome compared with patients who received placebo ($P = .0169$). Of patients treated with ecallantide, 72.5% (29/40) reported significant improvement in symptoms within 4 hours of treatment compared with 25.0% (2/8) of patients who received placebo (Table I). Overall, patients treated with ecallantide trended toward shorter median times to improvement in symptoms. Kaplan-Meier estimates of the median time to the beginning of improvement were 30.5 minutes for patients treated with ecallantide and 71.5 minutes for patients who received placebo. Estimates of the median time to significant improvement were 75.0 minutes for patients treated with ecallantide and 303.0 minutes for patients who received placebo.

Although not included in the efficacy results analysis, the additional patient treated with 40 mg/m² ecallantide reported onset of improvement in abdominal attack symptoms 50 minutes postdose and significant improvement by 110 minutes postdose. This patient can be considered an additional successful outcome.

During the study, 5 patients treated with ecallantide and 3 patients who received placebo also received medication (primarily opioid analgesics and/or antiemetics) for HAE symptoms on the date of ecallantide dosing that, in the opinion of the medical monitor, could have contributed to the resolution of the attack symptoms. Of the 5 patients treated with ecallantide, 1 patient (20 mg/m²) improved, 2 patients (10 mg/m²) significantly improved, 1 patient (20 mg/m²) worsened, and 1 patient (5 mg/m²) had no change in symptoms. Of the 3 patients who received placebo, 1 patient improved, 1 patient significantly improved, and 1 patient worsened. As a reasonable, statistically conservative measure, these 8 patients were classified as treatment failures in the efficacy analyses, despite their actual treatment outcomes.

The highest ecallantide dose cohort (40 mg/m²) demonstrated a statistically significantly greater proportion of successful outcomes compared with the pooled placebo group ($P = .013$; Table II). When successful outcomes were compared by attack location, a statistically significant difference in response rates was observed between patients who experienced an HAE attack in a peripheral location ($P = .0172$; Table III).

Safety

Overall, the incidence of AEs (79.6%, 39/49 patients) was similar between the ecallantide and the placebo groups, with no pattern discernible between individual dose cohorts. Of patients treated with ecallantide, 78.0% (32/41) experienced AEs, whereas 87.5% (7/8) of patients who received placebo experienced AEs. The majority

TABLE I. Proportion of successful outcomes

Ecallantide		Placebo		P value
Response	Percentage	Response	Percentage	
29/40	72.5%	2/8*	25.0%	.0169*

*Statistically significant ($P < .05$), Fisher exact test.

(89.5%, 111/124) of the AEs reported were mild (CTC grade 1) or moderate (CTC grade 2) in severity. Overall, the frequency of AEs was low, each usually occurring in 1 or 2 patients.

Among patients treated with ecallantide, the most frequently occurring AEs included headache (6 patients); diarrhea, nausea, upper respiratory infection, and vomiting (4 patients each); and abdominal pain, allergic rhinitis, cough, and sinusitis (3 patients each). The events of allergic rhinitis experienced by 3 patients were considered to be related to treatment with ecallantide; the events of 2 patients are discussed as acute dosing reactions. The third patient experienced allergic rhinitis of moderate severity (CTC grade 2). All of the remaining frequently occurring AEs were unrelated to treatment and were mild or moderate in severity (CTC grade 1 or 2).

A total of 4 patients, naive to treatment with ecallantide, experienced acute dosing reactions. Such reactions were defined as AEs that occurred during study drug administration or during the observation period after the dosing and that were considered by the investigator to be severe (CTC grade 3) or life-threatening (CTC grade 4) in severity and related to treatment. The first patient (20 mg/m²) experienced sneezing, itchy throat, congestion, rhinorrhea, and shortness of breath beginning approximately 3 minutes after initiation of ecallantide infusion, followed by throat edema approximately 25 minutes after the infusion was completed. Infusion of ecallantide was not interrupted or altered, and the patient tolerated the treatment. During the reactions, the patient was administered antihistamines, corticosteroids, and epinephrine that successfully treated the symptoms. The second patient (20 mg/m²) experienced sneezing, runny nose, itchy eyes, and shortness of breath approximately 5 minutes after initiation of ecallantide infusion. The infusion was not interrupted or altered, and the patient tolerated the treatment. Within 10 minutes of completing the infusion, the patient's symptoms worsened, although other vital signs and cutaneous signs remained normal. The patient was treated with antihistamines and asthma medication that significantly resolved the respiratory symptoms within 5 minutes. This patient had a known severe allergy to ragweed and had traveled 2 hours to the clinic along a route known to be highly populated with ragweed. Neither of these patients experienced fever or showed elevations in white blood cell counts or liver function enzymes that might suggest nonantibody pathways of inflammation. Both of these patients underwent skin prick and intradermal testing to ecallantide followed by a graded challenge. Skin testing was negative, and both patients tolerated the rechallenge without adverse effects. Overall, clinical

TABLE II. Proportion of successful outcomes by dose level

Dose level	Ecallantide		Pooled placebo		P value
	Response	Percentage	Response	Percentage	
5 mg/m ²	8/10	80.0%	2/8	25.0%	.0536*
10 mg/m ²	5/10	50.0%	2/8	25.0%	.3665
20 mg/m ²	7/10	70.0%	2/8	25.0%	.1534
40 mg/m ²	9/10	90.0%	2/8	25.0%	.0128†

*Approaching statistical significance.

†Statistically significant ($P < .05$), Fisher exact test.**TABLE III.** Proportion of successful outcomes by primary attack location

Primary attack location	Ecallantide		Placebo		P value
	Response	Percentage	Response	Percentage	
Abdominal	13/19	68.4%	1/3	33.3%	.5273
Laryngeal	3/3	100.0%	1/1	100.0%	—
Peripheral	13/18	72.2%	0/4	0.0%	.0172*

*Statistically significant ($P < .05$), Fisher exact test.

laboratory results and vital sign measures for all patients were unaffected by ecallantide treatment during the study.

The third patient (10 mg/m²) with an acute dosing reaction was hospitalized for an HAE attack that occurred 21 days after study treatment. The temporal relationship of this event to treatment with ecallantide was tenuous given the half-life of the drug (approximately 2 hours); further, this patient had a history of requiring emergency intervention and hospitalization for HAE attacks. The fourth patient (40 mg/m²) experienced 2 severe AEs: prolonged prothrombin and thrombin times on the day of study treatment. In addition, at 4 hours postinfusion and at time of discharge (same day as study treatment), the patient's thrombin times were abnormally high (≥ 120 seconds; normal range, 1-18 seconds) and were assessed as clinically significant, but not as AEs. At all other visits, the patient's coagulation parameters were not clinically significant. Importantly, for this and all other patients treated with ecallantide, no events involving abnormal bleeding were observed. Further, antiecallantide antibodies were not detected in any of these patients.

Four patients were observed with posttreatment activated partial thromboplastin times (APTTs) that were considered as abnormal by the investigator. Of these, 3 patients (5 mg/m² cohort) were observed with abnormally low APTT immediately before treatment (24.0-26.3 seconds; normal range, 28-38 seconds). In the hours after treatment, APTT for these 3 patients showed little change (approximately ± 1 second) but remained below normal. The fourth patient (40 mg/m² cohort) with APTT of 22.9 seconds immediately before treatment experienced an abnormally high APTT 1 hour posttreatment (49.1 seconds; normal range, 25-37 seconds). By 4 hours posttreatment, this patient's APTT returned to near baseline (23.7 seconds). APTTs for this patient at all other

time points of the study were not considered abnormal. No patients in the placebo group experienced abnormal APTT.

On the basis of results obtained by ELISA, no evidence of antibodies against ecallantide was observed in any of the patients during the study.

DISCUSSION

In this first placebo-controlled clinical trial of a kallikrein inhibitor to treat HAE, ecallantide was a safe and effective treatment. The primary efficacy endpoint of the study was achieved, with 72.5% of patients treated with ecallantide reporting significant improvement in symptoms within 4 hours compared with 25.0% of placebo patients ($P = .0169$). During the study, 5 patients treated with ecallantide and 3 patients who received placebo received additional medication for HAE symptoms on the date of dosing that could have contributed to attack symptom resolution (see Efficacy). For our analysis, all of these patients were considered treatment failures. However, outcomes became statistically insignificant if an exceptionally conservative analysis was performed, wherein the 5 ecallantide patients were considered as treatment failures and the 3 placebo patients were considered as treatment successes, despite actual outcomes.

Kaplan-Meier analyses showed that patients treated with ecallantide tended to improve more quickly than those treated with placebo. Ecallantide was shown to be effective for attacks at all anatomic locations. Patients treated at the highest dose (40 mg/m²) and patients with peripheral attacks demonstrated a statistically significantly greater proportion of successful outcomes compared with the pooled placebo group. However, by design, the

EDEMA1 study was not sufficiently powered to demonstrate a statistical difference between treatment doses or attack sites. Adverse events were largely mild-to-moderate in severity and not related to treatment. Increases in the severity of adverse events did not appear to be related to dose level.

The demonstrable efficacy of ecallantide was an important step in the conceptual understanding of HAE. Because C1-INH regulates multiple cascades, it has been difficult to deduce the mediators responsible for the pathophysiology of HAE. Only recently, the kallikrein-kinin cascade has been identified as the likely mediator of HAE attack pathology.^{26,40,41} The EDEMA1 study used ecallantide to block kallikrein and the downstream production of bradykinin. By demonstrating efficacy in patients with HAE, the study offers the clearest clinical evidence to date of the central role of the kallikrein-kinin system in the pathogenesis of HAE.

In vitro studies of ecallantide have consistently shown elevated APTT; however, results of *in vivo* studies have varied. In the EDEMA1 study, expected abnormal elevations of APTT posttreatment were rarely observed, suggesting that protocol parameters (eg, blood draw timing) or physiological parameters in relation to peak plasma concentrations were involved. Additional investigation of the relationship between clotting parameters and ecallantide is the focus of further clinical studies. Importantly, elevated APTT in the EDEMA1 study did not pose bleeding risks as evidenced by normal prothrombin times and thrombin times. It is known that kallikrein inhibition blocks activation of fXIIa (Fig 1), causing prolongation of laboratory-based intrinsic clotting cascade measurements. The elevated APTT mimics that found in Hageman factor (fXII) deficiency and Fletcher factor (prekallikrein) deficiency, in which patients have increased APTT but no clinical bleeding abnormalities. In all 3 cases (Fletcher deficiency, Hageman deficiency, and kallikrein inhibition), hemostasis is maintained by factors further in the intrinsic clotting cascade (eg, fXI, fX) by the extrinsic cascade and by platelets. Therefore, evidence of intact clotting function is offered by normal thrombin times and bleeding times in patients with Hageman factor deficiency and Fletcher factor deficiency and in patients dosed with ecallantide.⁴²

It is well known that an immune response may occur after treatment with exogenous human proteins.⁴³ The development of antiecallantide antibodies was not detected in any patients during the EDEMA1 study, including those who experienced acute dose reactions. In an earlier European study, antiecallantide antibodies were detected.⁴⁴ In subsequent trials involving a total of more than 180 patients treated with ecallantide, only 3 patients tested positive for antiecallantide antibodies, 7 patients for anti-*P pastoris* antibodies, and 1 patient for both. The 1 patient with both antiecallantide and anti-*P pastoris* antibodies and 1 patient with antiecallantide antibodies experienced acute dosing reactions. These 2 patients were successfully rechallenged and continued with ecallantide treatment, showing clinical efficacy without

adverse effects.³⁹ Modifications to the manufacturing process have substantially reduced *P pastoris* host cell proteins to decrease potential antibody development. Therefore, we think it is unlikely that antibodies to *P pastoris* will be important in future adverse drug reactions. In ongoing studies with ecallantide, acute dose reactions and antiecallantide and anti-*P pastoris* antibodies are actively monitored. Overall, repeat dosing with ecallantide has shown no diminished treatment effect in patients, suggesting that the antibodies are nonneutralizing and do not interfere with response to treatment.

In this first clinical assessment of a kallikrein inhibitor, ecallantide produced clinically meaningful improvement in the symptoms of acute HAE attacks. This placebo-controlled trial provides evidence of the kallikrein cascade role in pathogenesis of HAE attacks. It further demonstrates the clinical benefit of ecallantide in a disease with very limited treatment options.

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