
Treatment of episodes of hereditary angioedema with C1 inhibitor: serial assessment of observed abnormalities of the plasma bradykinin-forming pathway and fibrinolysis

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Background: Hereditary angioedema (HAE) is typically the result of a deficiency of C1 inhibitor (C1-INH) with gene defects that lead to diminished plasma levels or the production of a dysfunctional protein. Replacement therapy with C1-INH has been shown to be effective in ameliorating episodes of swelling. We have reported elevated baseline levels of bradykinin, C4a, and plasmin- α_2 -antiplasmin complexes in the plasma of patients with HAE compared with the plasma of healthy controls. The production of factor XII fragment on in vitro activation of plasma with HAE has also been observed.

Objective: To perform serial assessment of abnormalities of the bradykinin-forming pathway and fibrinolysis in patients with HAE after treatment of episodes of swelling with intravenous C1-INH.

Methods: We obtained samples of plasma from 9 patients with HAE at a quiescent period (baseline), during an attack of swelling, and at 1, 4, and 12 hours after termination of an infusion of C1-INH. Factor XIIa, kallikrein, and plasmin were each measured by cleavage of synthetic substrates specific for each item.

Results: Each enzyme was strikingly elevated at baseline compared with the levels in pooled healthy plasma, and there was a progressive decline of activity to normal for factor XIIa and plasmin. Kallikrein decreased in 7 of the 9 patients at 1 hour and then decreased in all patients. Bradykinin levels were elevated at the outset in all patients, increased prominently during an attack of swelling, decreased to baseline after 1 hour, and then decreased toward normal by 4 and 12 hours.

Conclusion: The plasma levels of factor XIIa, kallikrein, and bradykinin decreased when measured serially subsequent to the infusion of nanofiltered C1-INH.

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INTRODUCTION

C1 inhibitor (C1-INH) deficiency, whether hereditary or acquired, results in a markedly augmented generation of bradykinin.¹ This can be observed even if plasma is incubated in an ordinary test tube without the addition of any exogenous substance to activate the factor XII-dependent pathways.² Thus, the plasma is unstable if C1-INH is deficient. Bradykinin is thought to be the mediator of the swelling characteristic of these disorders.^{2–4} Angioedema typically affects the face, extremities, genitalia, lips, tongue, and throat. Patients may have laryngeal edema, a major cause of mortality in those with this disorder. They may also experience gastroin-

testinal attacks (ie, severe abdominal pain with or without nausea, vomiting, or diarrhea) that can resemble an acute abdomen and lead to unnecessary surgery.

A major advance in the treatment of C1-INH deficiency is replacement therapy using purified C1-INH.^{5,6} This can theoretically ameliorate short-term acute episodes and, given prophylactically, it can prevent occurrences of angioedema. A recent study⁷ examined plasma of patients with hereditary angioedema (HAE) in vitro, and demonstrated augmented activation of factor XII and prekallikrein and marked digestion of high-molecular-weight kininogen (ie, the substrate from which bradykinin is produced) compared with healthy plasma. We also demonstrated abnormal fibrinolysis, with baseline elevations of plasmin- α_2 -antiplasmin (PAP) complexes, and an augmented rate of fibrinolysis once plasma activation ensues. In addition, the formation of factor XII fragment (FXII_f), an active fragment derived from activated factor XII, was observed, which is a link with complement because FXII_f activates the C1_r subcomponent of C1.^{7–9} Consistent with this observation is production of C4a, a cleavage product derived from C4 once C1 has become activated. Thus, we can connect factor XII activation with the diminished levels of C4; the latter determination is a useful diagnostic test for C1-INH deficiency, with low baseline levels in 95% of patients.^{10,11}

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We have extended these studies to the plasma of patients with HAE at baseline, during attacks of swelling, and during treatment of such patients with purified C1-INH. Herein, we present the results of sequential assays of each component of the plasma bradykinin-forming cascade and the fibrinolytic enzyme plasmin. We confirm activation of each parameter to levels above baseline during attacks of swelling and serially evaluate these abnormalities on treatment with C1-INH.

METHODS

Patients

A total of 9 patients with HAE and 1 “healthy” control were studied; the control used was plasma pooled from 10 participants. The plasma samples of patients with HAE were collected to include a baseline sample taken during an asymptomatic period, a sample obtained during a major episode of swelling, and 3 sequential samples obtained after treatment with purified C1-INH (per Lev Pharmaceuticals Inc, New York, New York). The latter time points were 1, 4, and 12 hours after the infusion was terminated. The protocol for the study was approved by a central external institutional review board for Lev Pharmaceuticals Inc, and informed consent for the administration of drugs and the collection of samples was obtained in writing from the participants. After collection, the plasma samples were fractionated and stored at -80°C until use.

Assay Descriptions

Kallikrein activity assay. Kallikrein activity was measured by incubating plasma diluted in the assay buffer at room temperature for 1 hour with a kallikrein-specific chromogenic substrate (S2302), and kallikrein activity was determined by color development, as described previously.¹²

Factor XIIa activity assay. Factor XIIa activity measurement was similar to that of the kallikrein activity assay. The samples were incubated for 2 hours in assay buffer with a factor XIIa-specific chromogenic substrate (S2222), and the activity was determined by color development (optical density [OD], 405 nm).

Assay for plasmin. The activity of plasmin was measured using a chromogenic substrate (Biophen CS-41[03]; ANI-ARA Corporation, Mason, Ohio). The samples were incubated in the assay buffer with the substrate for 1 hour at room temperature, and the absorbance (OD) was measured at 405 nm.

Bradykinin assay. For quantitation of bradykinin, the proteins in the samples were precipitated with ice-cold ethanol and centrifuged for 1 hour at 10,000 rpm in a microcentrifuge at 4°C and the supernatant containing free bradykinin was collected. The supernatant was then evaporated to dryness using a centrifugal concentrator, and the residue was resuspended in the enzyme immunoassay buffer. A bradykinin enzyme immunoassay kit was used and supplied by Peninsula Laboratories, San Carlos, California. The concentration of bradykinin in the samples was calculated using a standard curve (range, 10–10,000 pg/mL).

PAP assay. The quantitative determination of PAP complex in control and HAE plasma samples was performed using a commercially available assay kit (PAP Micro ELISA kit; ALPCO Diagnostics, Salem, New Hampshire). Samples were incubated in a microtiter plate that was coated with monoclonal antibodies to PAP. The unbound fragments were removed by washing. Peroxidase-conjugated antibodies to plasminogen (which cross-react with plasmin) were added and incubated for 15 minutes. The unbound conjugates were removed by washing, and the substrate was added for color development. The color intensity is proportional to the concentration of PAP that was calculated using a standard curve.

Complement C4a assay. The complement C4a was quantitated based on its conversion to C4a des Arg by plasma carboxypeptidases. A competitive immunoassay kit obtained from Assay Designs, Ann Arbor, Michigan, was used. Using this method, a polyclonal antibody to human C4a des Arg is used to bind in a competitive manner the human C4a des Arg in the sample, a standard, or an alkaline phosphatase molecule that has human C4a des Arg attached to it. After incubation at room temperature, the excess reagents were washed and the substrate was added for color development. The intensity of the color is inversely proportional to the concentration of human C4a des Arg in either standards or samples. The measured OD was used to calculate the concentration of human C4a des Arg.

Statistical Analysis

Statistical analysis was performed using the *t* test. $P < .05$ was considered significant. We used the preinfusion value during an attack of swelling as the starting value and compared it to postinfusion values for factor XIIa, kallikrein, and plasmin (Fig 1 and Fig 2), as well as values for bradykinin and PAP complexes (Fig 3). For C4a, we included compar-

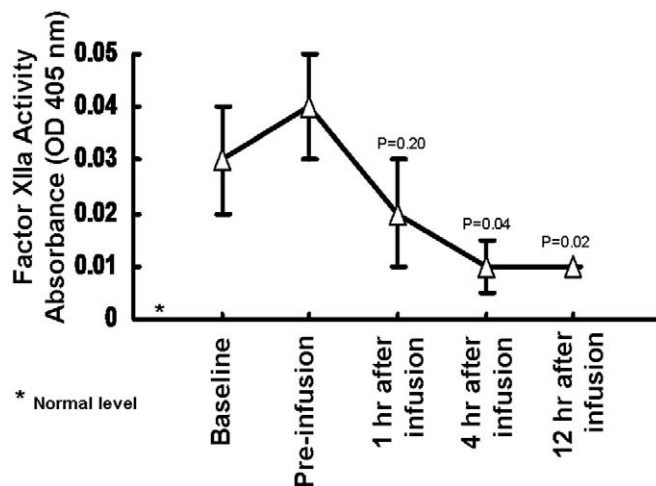


Figure 1. Activity of factor XIIa in the plasma of patients with hereditary angioedema before and after treatment with purified C1 inhibitor (C1-INH). *P* values compare C1-INH infusion values before and after the infusion. OD indicates optical density.

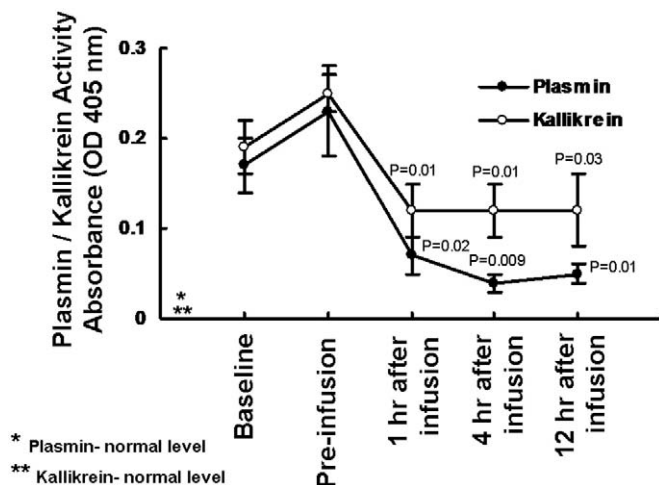


Figure 2. Activity of kallikrein and plasmin in the plasma of patients with hereditary angioedema before and after treatment with purified C1 inhibitor (C1-INH). *P* values compare C1-INH infusion values before and after the infusion. OD indicates optical density.

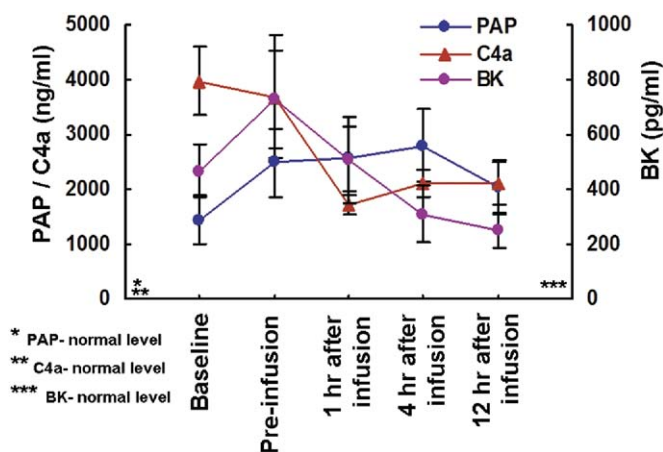


Figure 3. Activity of C4a, plasmin α_2 -antiplasmin complex (PAP), and bradykinin (BK) in the plasma of patients with hereditary angioedema before and after treatment with purified C1 inhibitor (C1-INH). *P* values are significant for BK at 4 hours after the infusion ($P = .04$) and 12 hours after the infusion ($P = .01$). The *P* values for PAP complexes are not significant at any point. The *P* values for C4a are significant if compared with the "screen" value (1 hour, $P = .01$; 4 hours, $P = .03$; 12 hours, $P = .04$) but is not significant when compared with the preinfusion value because of the increased SE of this determination.

ison of the 1-, 4-, and 12-hour postinfusion values with both the preinfusion value and the baseline value.

RESULTS

Factor XIIa Activity

Factor XIIa activity was strikingly elevated at baseline compared with the activity of control plasma, and there was a progressive decline of activity toward normal with therapy

(Fig 1). Of the 9 patients, elevated baseline factor XIIa was evident in 6, this level increased further at the time of an attack of swelling in 6 patients, and there was a progressive decline with levels below baseline at 12 hours in 7 patients. A statistically significant decrease in factor XIIa was noted at 1, 4, and 12 hours after infusion.

Kallikrein Activity

A small amount of factor XIIa will lead to exponential conversion of prekallikrein to kallikrein, followed by digestion of high-molecular-weight kininogen to release bradykinin. In vivo bradykinin levels, however, are difficult to determine accurately because of "spontaneous" activation on handling of plasma when C1-INH is absent^{2,3} and degradation of bradykinin by kininases. Thus, the most reliable reflection of activation of the plasma kinin-forming cascade is quantitation of kallikrein.

Elevated baseline levels of kallikrein are seen in all 9 patients (Fig 2) compared with the healthy control. The levels increase further during the episode of swelling in 6 patients. Kallikrein activity increased even further at the 1-hour postinfusion point in 2 patients and decreased by the 12-hour time point in 6 patients. Nevertheless, a statistically significant decrease in kallikrein activity was noted at 1, 4, and 12 hours after infusion.

Bradykinin Levels

We next assayed bradykinin levels in each plasma sample, and the results are shown in Figure 3. An increase with the episode of swelling, a decrease to baseline at 1 hour after infusion, and a further decline at 4 and 12 hours are seen, with the latter *P* values ($P = .04$ and $P = .01$, respectively) reaching statistical significance.

Fibrinolysis

The fibrinolytic enzyme plasmin is released during episodes of swelling in patients with HAE,^{7,13} and treatment with antifibrinolytic agents has efficacy, albeit less, than with androgenic compounds. We measured plasmin as an active enzyme, using a synthetic substrate (Fig 2), in addition to quantitating PAP complexes (Fig 3). The results depict the mean (SD) for all 9 patients. A prominent decline in plasmin activity is seen at all 3 time points after treatment with C1-INH (Fig 2), and all are statistically significant. In contrast, PAP complex levels increase at the time of an episode of swelling and remain elevated in the circulation throughout the study period, although some decline is noted at the 12-hour time point (Fig 3). None of these values indicates a statistically significant change.

C4a Activity

The marked elevation of C4a levels in HAE plasma at baseline, but not in healthy plasma, indicates either in vivo activation in the patient or in vitro activation even as frozen plasma. However, the values may truly reflect the in vivo situation because C4 levels are depressed even when patients are asymptomatic.^{10,14} There is a trend toward a statistically

significant decline when compared with the baseline C4a level, but not when compared with the preinfusion value.

DISCUSSION

Hereditary angioedema is the result of a mutant *C1-INH* gene that is transmitted as an autosomal-dominant trait and leads to angioedema that typically affects the face (including the tongue and pharynx), the extremities, and the gastrointestinal tract. It is also one of the known causes of laryngeal edema, which can lead to fatalities by asphyxiation. The swelling has been shown to be the result of the generation of bradykinin²⁻⁴ because of the activation of factor XII, the conversion of prekallikrein to kallikrein, and the digestion of high-molecular-weight kininogen. There is also conversion of plasminogen to plasmin, although the pathway involved is less clear; possibilities include activation of plasminogen by kallikrein,¹⁵ activation of plasma prourokinase by kallikrein,¹⁶ or liberation of tissue plasminogen activator from endothelial cells as a result of bradykinin interaction with the B2 receptor.¹⁷ Plasmin may augment the generation of bradykinin, particularly when C1-INH levels are diminished (or absent), by cleaving factor XII to generate FXIIa¹⁸ and by digesting high-molecular-weight kininogen.¹⁹ Plasmin also digests and inactivates C1-INH and may, therefore, lower levels even further in patients whose C1-INH synthesis is already compromised.²⁰

Effective therapy of HAE in the United States relies primarily on androgenic compounds, such as danazol or stanozolol,^{21,22} although antifibrinolytic agents, such as ϵ -amino caproic acid²³ and tranexamic acid,²⁴ can be used as well. The most logical therapy would be replacement therapy with a C1-INH^{5,6,25} or use of agents that suppress the formation of bradykinin (eg, a kallikrein inhibitor)²⁶ or block the effects of bradykinin (eg, a B₂-receptor antagonist).²⁷ In fact, a C1-INH preparation is now Food and Drug Administration approved for prophylactic treatment of HAE. In this study, we examined the effects of a purified C1-INH preparation, administered as therapy for brief attacks of angioedema, on the plasma bradykinin-forming cascade and the fibrinolytic enzyme plasmin.

Activated factor XII, kallikrein, plasmin, and bradykinin levels were elevated in patients at baseline, as has been observed repeatedly in the past.^{28,29} These may reflect in vivo levels, but it is also clear that activation of HAE plasma can occur in vitro as samples are handled, although precautions have been taken to avoid this. Most patients demonstrated further activation beyond the baseline level during an episode of swelling, and in all cases in which activation was observed, therapy with C1-INH reduced the levels below baseline. Statistically significant decreases in values after C1-INH infusion were noted for factor XIIa, kallikrein, and plasmin at all 3 time points and for bradykinin at the last 2 time points. These data are consistent with the putative ability of intravenously administered C1-INH to ameliorate brief episodes of angioedema in patients with HAE. C1 inhibitor is the major inhibitor of the activated forms of factor XII (factor XIIa and

FXIIa), accounting for more than 90% of the total plasma inhibitory activity,^{30,31} and it inhibits factor XII autoactivation.³² It is a major inhibitor of plasma kallikrein, the other inhibitor being α_2 -macroglobulin,³³ and it is a contributor to plasmin inhibition,³⁴ although α_2 -antiplasmin accounts for most total plasma antifibrinolytic activity.³⁵ Kallikrein-(α - α_2 -macroglobulin complexes retain about 30% enzymatic activity on synthetic low molecular weight substrates which may account for remaining activity at 1, 4, and 12 hr (Fig. 2).

We were unable to include a similarly obtained group of patients who were treated with placebo. Such samples were not available; in addition, many of these patients were rescued by subsequent infusion of C1-INH because of increasing symptoms. Nevertheless, we believe the changes described herein, within the first 12 hours of treatment, reflect infusion of C1-INH because of the rapidity of the response and the observation that changes observed in each patient all trended in the same direction with the same time course (despite individual variation), an unlikely scenario in an untreated group of patients.

Patients with HAE represent a heterogeneous group with differing mutations within the *C1-INH* gene^{36,37} and a range of residual C1-INH activity typically from 0% to 40% of normal at the time of disease activity. Therefore, the parameters we measured demonstrate variability when patients are compared individually or collectively. Some of the observed variability might also be the result of changes in enzyme activities or bradykinin levels that occur during the processing and storage of samples. Nevertheless, the common trend noted is observed in the entire group for all parameters involving the bradykinin-forming cascade and plasmin. It is likely that PAP complexes were not cleared sufficient to lower levels during the 12-hour observation period, whereas C4a levels showed a trend suggesting a decline. However, a larger sample size would be needed to be sure. We conclude that infusion of C1-INH as treatment for brief episodes of angioedema in patients with HAE contributes to decrease of abnormalities of the plasma bradykinin-forming cascade and fibrinolysis.

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