

## TREATMENT OF HEREDITARY ANGIOEDEMA WITH A VAPOR-HEATED C1 INHIBITOR CONCENTRATE

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**Abstract** *Background.* Hereditary angioedema results from a congenital deficiency of functional C1 inhibitor and is characterized by episodic bouts of edema, which may be life-threatening when they involve the larynx. We evaluated the effectiveness of a C1 inhibitor concentrate in the prevention and treatment of attacks of hereditary angioedema. The concentrate was vapor-heated to inactivate hepatitis and human immunodeficiency viruses.

*Methods.* We conducted two double-blind, placebo-controlled studies. The first was a crossover study consisting of two 17-day trials in which prophylactic infusions of either C1 inhibitor (25 plasma units per kilogram of body weight) or placebo were given intravenously every third day to six patients with hereditary angioedema. The second study was conducted in patients with acute attacks of hereditary angioedema and assessed the length of time to a clinical response after infusions of either 25 plasma units of C1 inhibitor per kilogram (55 in-

fusions in 11 patients) or placebo (49 infusions in 11 patients).

*Results.* The infusions of C1 inhibitor concentrate resulted in close to normal functional levels of C1 inhibitor and C4. As compared with placebo, prophylactic infusions of C1 inhibitor resulted in significantly lower daily symptom scores for the severity of edema of the extremities ( $P < 0.01$ ), larynx ( $P < 0.05$ ), abdomen ( $P < 0.05$ ), and genitourinary tract ( $P < 0.05$ ). Likewise, during the treatment study the time from the start of an infusion to the beginning of improvement in symptoms was shorter for the C1 inhibitor infusions than the placebo infusions (55 vs. 563 minutes,  $P < 0.001$ ). There was no evidence of toxicity.

*Conclusions.* Infusions of a vapor-heated C1 inhibitor concentrate are a safe and effective means of both preventing attacks of hereditary angioedema and treating acute attacks. (N Engl J Med 1996;334:1630-4.)

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**H**EREDITARY angioedema, first reported by Quincke in 1882<sup>1</sup> and described by Osler in 1888,<sup>2</sup> is characterized by episodic bouts of well-circumscribed, nonpitting subepithelial edema that primarily involve the extremities, larynx, face, and abdomen.<sup>3,4</sup> The condition is inherited as an autosomal dominant trait and characterized by functional levels of C1 inhibitor activity in the blood that are approximately 30 percent of normal values.<sup>5</sup> About 15 percent of patients, however, have normal levels of antigenic C1 inhibitor, but most of it is nonfunctional.<sup>6</sup> As a result of the failure of C1 inhibitor to block the enzymatic activity of C1, levels of the early-acting complement components C4 and C2 are low.<sup>7,8</sup> The role of C1 inhibitor in the pathogenesis of hereditary angioedema has been reviewed in detail.<sup>9</sup>

Attacks of hereditary angioedema generally last one to four days. Swelling of the extremities is typically painless and resolves without harm. Abdominal attacks from edema in the submucosa and serosa of the bowel wall are often associated with nausea, vomiting, and pain severe enough to necessitate the use of narcotic medications. Edema of the upper airway may result in asphyxiation; before modern prophylactic therapy approximately 25 percent of patients died of this complication.<sup>10</sup>

Prophylactic administration of either androgens<sup>11-13</sup> or antifibrinolytic agents<sup>14,15</sup> has proved useful in reducing the frequency or severity of attacks. Some patients,

however, are resistant to or cannot tolerate these drugs, and their use in children and pregnant women is fraught with difficulty. The availability of purified C1 inhibitor from human plasma led to several investigations of the usefulness of these concentrates in treating hereditary angioedema.<sup>16-18</sup> Unfortunately, their use was associated with a significant risk of transmission of the human immunodeficiency virus (HIV) and hepatitis. A lyophilized C1 inhibitor concentrate, which is vapor-heated to 60°C for 10 hours under pressure, conditions that effectively inactivate HIV and hepatitis B and C viruses,<sup>19</sup> was subsequently developed and was found to be useful in the treatment of hereditary angioedema.<sup>20</sup>

The symptoms of hereditary angioedema have a strong psychological component, as is evidenced by the fact that up to 30 to 40 percent of attacks are precipitated by emotional stress.<sup>4</sup> Therefore, blinded, controlled studies are critical in the evaluation of the effectiveness of any treatment for this disease. We performed two randomized, placebo-controlled, double-blind studies. One was a crossover study in which the efficacy and safety of multiple prophylactic infusions of C1 inhibitor concentrate and placebo were evaluated in six patients with hereditary angioedema whose symptoms could not be controlled with standard therapy. In the other study, 22 patients with acute attacks of hereditary angioedema were treated with C1 inhibitor concentrate or placebo.

## METHODS

### Patients

To be eligible for the study patients had to have an established diagnosis of hereditary angioedema, with functional C1 inhibitor levels of less than 30 percent of normal before the study and a history of more than five attacks of hereditary angioedema in the previous year. Participation in the prophylaxis study also required the lack of an ad-

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equate response to conventional treatment with androgens or antifibrinolytic agents or the occurrence of adverse reactions.

### C1 Inhibitor Concentrate

The C1 inhibitor preparation used (Immuno, Vienna, Austria) is a freeze-dried, sterile, human plasma fraction containing the inhibitor of the first component of complement in a concentrated and purified form. The moistened product was subjected to vapor heating at a mean temperature of 60°C (range, 55.5°C to 60.5°C) and a pressure of 190±20 millibars for 10 hours to inactivate blood-borne viruses. Each vial, when reconstituted with 10 ml of sterile water, contained approximately 55 plasma units per milliliter, with 1 plasma unit being equivalent to the functional activity of C1 inhibitor in 1 ml of fresh average human plasma. The lyophilized material was reconstituted immediately before the infusion and was administered at a dose of 25 plasma units per kilogram of body weight. Placebo (5 percent human albumin), which was identical in appearance to the C1 inhibitor concentrate, was administered in the same volume, at the same rate, and according to the same schedule.

### Study Designs

In the prophylaxis study, which was approved by the National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Subpanel, the director of the National Institutes of Health Clinical Center, and the Food and Drug Administration (FDA), each patient was admitted to the clinical center for two 17-day periods that were separated by at least 3 weeks. Six patients were randomly assigned to receive five intravenous infusions of either C1 inhibitor or placebo during the first admission and the alternate preparation during the second. Plasma and serum were collected for measurements of complement before and 2, 24, and 72 hours after each infusion. Functional measurements of C1 inhibitor were performed with a microtiter adaptation of the Immunochrom C1 inhibitor diagnostic kit.<sup>21</sup> Levels of C4 activity in plasma (sodium citrate) were determined with a hemolytic assay.<sup>22</sup> The results are expressed as a hemolytic titer, with the mean normal level being 168,000 units per milliliter (range, 98,000 to 308,000).

Each time a patient reported a new or increasingly severe attack of hereditary angioedema or requested medications for hereditary angioedema (including narcotics), an NIAID medical-staff fellow, who had no knowledge of the patient's therapy, would evaluate the symptoms; document the date, time, and location of the attack; and objectively score the severity of the attack from 0 for no relevant symptoms to 3 or 4 for symptoms of greatest severity. The following symptoms were evaluated: abdominal edema (score, 0 to 3), edema of the extremities (0 to 3), laryngeal edema (0 to 4), and genitourinary edema (0 to 3). The highest scores recorded for each system during a given six-hour period were used for comparative purposes, with the daily symptom score for each system representing the average of the four consecutive six-hour periods. This provided a combined measurement of the severity, as well as the frequency and persistence, of symptoms of hereditary angioedema. The episodic nature of this disease, with swelling episodes typically being separated by symptom-free periods lasting days or weeks, is such that a typical patient can be expected to have one or two mild-to-moderate attacks per month. This would result in mean daily symptom scores of about 0.1 to 0.2. Daily symptom scores were compared with a repeated-measures Kruskal-Wallis analysis of variance.<sup>23</sup> In cases in which there was no variance (i.e., no symptoms in one treatment group), two-way contingency tables were analyzed and the mid P values were determined.<sup>24</sup>

In the treatment study, which was approved by the Children's Hospital and Brigham and Women's Hospital institutional review boards and the FDA, patients were required to report for an infusion within five hours after the beginning of an attack of hereditary angioedema. The time from the start of the infusion to the beginning of symptom abatement was used as a measure of clinical efficacy. Open-label C1 inhibitor was available for use as rescue therapy if the physician felt that the symptoms were life threatening. Only one patient was treated immediately with C1 inhibitor because of life-threatening laryngeal edema; this patient did not qualify for statistical evaluation. In other instances open-label C1 inhibitor was administered if there was no

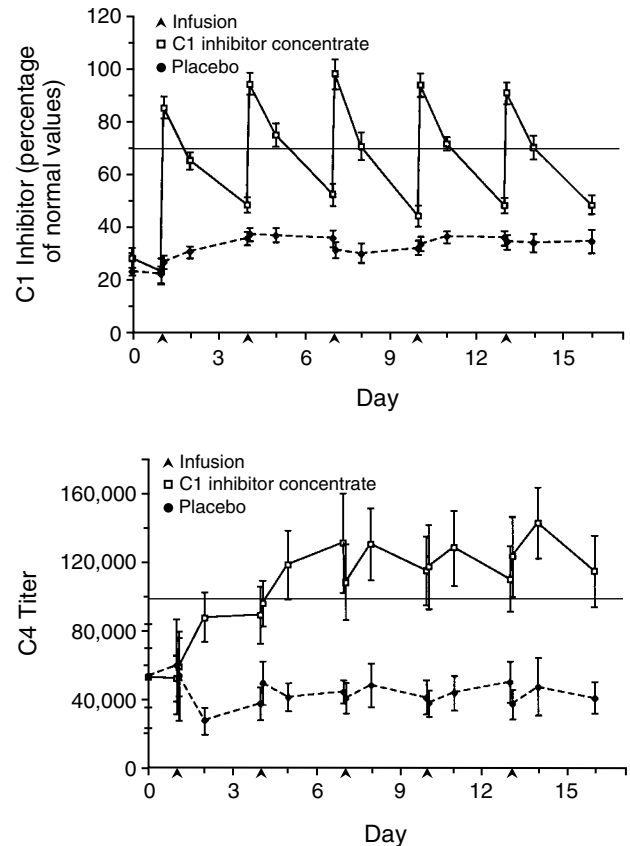


Figure 1. Mean Functional C1 Inhibitor Levels and C4 Levels in Patients with Hereditary Angioedema after Infusions of C1 Inhibitor Concentrate or Placebo.

Each point represents the mean plasma level of six recipients. The bars show the range. The lower limits of normal are represented by the solid horizontal lines.

abatement of symptoms after 240 minutes; patients with these attacks remained eligible for statistical evaluation. Significance testing was performed with repeated-measures analysis of variance to compare the mean response time between treatment groups.<sup>25</sup> Pre-infusion and post-infusion levels of functional C1 inhibitor were determined as described above.<sup>21</sup>

## RESULTS

### Prophylaxis Study

Of the six patients enrolled in the prophylaxis study, four continued to take androgens (8 mg of stanozolol per day or 400 to 800 mg of danazol per day) throughout the study, which had been prescribed at the maximal tolerated dose, but with suboptimal results. The remaining two patients had been receiving no daily medications for hereditary angioedema, because previous treatments had not been effective. All six patients had previously received antifibrinolytic agents, which either were ineffective or caused serious adverse effects.

The mean functional C1 inhibitor and C4 levels in the six patients are shown in Figure 1. Infusions of C1 inhibitor concentrate, but not placebo, resulted in a dramatic increase in plasma levels of C1 inhibitor and C4. C1 inhibitor levels fell to approximately the lower

limit of normal by 24 hours, but were still above base line by 72 hours.

Levels of C4 at base line were not significantly different from those measured two hours after the first infusions of either C1 inhibitor concentrate or placebo. Beyond that time, however, the levels of functional C4 after the C1 inhibitor infusions were markedly higher than those after placebo. Unlike C1 inhibitor levels, which increased immediately, C4 levels tended to increase more slowly, not reaching the normal range until 24 hours after the first infusion. Although C4 levels declined between infusions, they did not have the marked "sawtooth" pattern of C1 inhibitor levels.

As shown in Table 1, the mean daily scores for edema of the extremities, larynx, abdomen, and genitourinary tract revealed that there was significantly less disease activity — over 60 percent less — during treatment with C1 inhibitor concentrate than during placebo infusions. Abdominal discomfort was by far the most common side effect and was also the most difficult to evaluate objectively, since some patients were suspected of feigning symptoms in order to receive narcotic medications. Not one patient receiving the C1 inhibitor concentrate had objective evidence of either laryngeal or genitourinary edema, whereas four of the six patients receiving placebo had disease-related edema of one or both of these systems.

### Treatment Study

Eleven patients randomly assigned to receive C1 inhibitor concentrate were treated for a total of 55 attacks. An identical number of patients were given placebo for a total of 49 attacks. The number (and percentage) of attacks in each treatment group that began to improve within 30 minutes after the start of the infusion is shown in Table 2. Sixty-nine percent of the attacks treated with C1 inhibitor responded within this period, as compared with 2 percent of the placebo-treated attacks. Two hundred forty minutes after the start of infusion, 95 percent of the attacks treated with C1 inhibitor had begun to improve, as compared with 12 percent of those treated with placebo. Laryngeal, abdominal, and facial attacks are the most likely to become severe or life-threatening and thus represent situations in which effective therapy is most critical. All at-

Table 2. Length of Time to the Response to C1 Inhibitor Concentrate or Placebo.

LOCATION OF EDEMA	RESPONSE IN ≤30 MINUTES		RESPONSE IN <240 MINUTES	
	C1 INHIBITOR	PLACEBO	C1 INHIBITOR	PLACEBO
	<i>no. of responses/no. of attacks (% responding)</i>			
Abdomen	25/35 (71)	0/34	35/35 (100)	2/34 (6)
Larynx	3/4 (75)	0/4	4/4 (100)	1/4 (25)
Face	7/7 (100)	0/8	7/7 (100)	1/8 (12)
Extremities	9/16 (56)	1/16 (6)	13/16 (81)	3/16 (19)
First 3 locations*	33/44 (75)	0/40	44/44 (100)	4/40 (10)
All locations*	38/55 (69)	1/49 (2)	52/55 (95)	6/49 (12)

\*For single attacks involving more than one location, the location with the earliest response was used for statistical analysis.

tacks involving these three areas improved within 240 minutes after C1 inhibitor treatment, 75 percent of them within 30 minutes. In contrast, only 10 percent responded within 240 minutes after the administration of placebo, and none within 30 minutes (Table 2).

As shown in Table 3, the mean interval between the start of the infusion and the beginning of an effect for all attacks treated with C1 inhibitor was 55 minutes, as compared with 563 minutes for those treated with placebo ( $P < 0.001$ ). Only four episodes of laryngeal edema were treated in each group. Although the mean time to relief of laryngeal edema after the administration of C1 inhibitor (35 minutes) appeared to be much shorter than that observed after placebo (512 minutes), the difference was not statistically significant because of the small sample. At 240 minutes, 21 of the attacks not responding to the initial infusion were treated with open-label C1 inhibitor. All were subsequently determined to have been treated with placebo initially, and all responded to the C1 inhibitor. Excluding these attacks from the analysis increased the mean response time of placebo-treated attacks to 799 minutes (Table 3). Seven additional attacks (all treated with placebo) were of such severity that open-label C1 inhibitor was given before 240 minutes, and thus, they did not qualify for evaluation.

Functional C1 inhibitor levels were measured during 34 attacks subsequently determined to have been treated with C1 inhibitor concentrate. The mean level rose from 11.0 percent of normal before the infusion to 69.4 percent of normal after the infusion. The mean level of C1 inhibitor achieved after the treatment of abdominal, laryngeal, and facial attacks with the concentrate was 66.0 percent of normal, with no differences in either pre-infusion or post-infusion levels between attacks that responded within 30 minutes and those that did not. The C1 inhibitor levels in 49 attacks that were subsequently determined to have been treated with placebo remained at approximately 10 percent of normal before and after the infusion.

### DISCUSSION

We assessed the efficacy of a vapor-heated C1 inhibitor preparation in a double-blind, placebo-controlled setting in patients with hereditary angioedema. Levels

Table 1. Daily Symptom Scores in Six Patients with Hereditary Angioedema Who Were Receiving C1 Inhibitor Concentrate or Placebo.\*

LOCATION OF EDEMA	INHIBITOR C1	PLACEBO	P VALUE
	<i>mean (±SE) daily score</i>		
Extremities	0.022±0.011	0.125±0.037	<0.01†
Larynx	0.000±0.000	0.036±0.022	<0.05‡
Abdomen	0.169±0.041	0.308±0.057	<0.05†
Genitourinary tract	0.000±0.000	0.031±0.123	<0.05‡
Total	0.191±0.044	0.500±0.089	<0.001†

\*Ninety measurements were made during each treatment period.

†By analysis of variance.

‡Mid P values determined with two-way contingency tables.<sup>24</sup>

Table 3. Interval between the Start of the Infusion and the Beginning of an Effect.\*

LOCATION OF EDEMA	C1 INHIBITOR (ALL ATTACKS)			PLACEBO (ALL ATTACKS)				PLACEBO (ALL ATTACKS BUT THOSE INVOLVING RESCUE THERAPY)†			
	NO. OF PATIENTS	NO. OF ATTACKS	MINUTES TO RESPONSE	NO. OF PATIENTS	NO. OF ATTACKS	MINUTES TO RESPONSE	P VALUE‡	NO. OF PATIENTS	NO. OF ATTACKS	MINUTES TO RESPONSE	P VALUE‡
Abdomen	9	35	29±4	11	34	556±92	<0.001	9	13	1055±166	<0.001
Larynx	3	4	35±23	4	4	512±161	NS	4	4	512±161	NS
Face	5	7	14±5	4	8	694±163	0.005	4	7	757±174	0.005
Extremities	7	16	120±53	5	16	691±136	0.023	5	14	754±148	0.020
First 3 locations§	10	44	28±4	11	40	547±81	0.001	11	19	878±135	<0.001
All locations§	11	55	55±16	11	49	563±72	<0.001	11	28	799±105	<0.001

\*Plus-minus values are means ±SE.

†In 21 instances patients who had no response after four hours were given C1 inhibitor concentrate as rescue therapy.

‡For the comparison of mean response times between treatment groups by repeated-measures analysis of variance. NS denotes not significant.

§For single attacks involving more than one location, the location with the earliest response was used for statistical analysis.

of C1 inhibitor increased dramatically after the initial infusions of concentrate, to a mean of 85 percent of normal values, confirming that the activity of the infused protein, as assessed by an *in vitro* assay, remained intact after vapor heating. These increases compare favorably with those in other studies, in which the mean post-infusion levels were 70 percent of normal (range, 54 to 110 percent).<sup>17,18,20</sup> Direct comparisons of these studies cannot be made, however, because of differences in the amount of C1 inhibitor infused. Twenty-four hours after the infusion, the mean levels of C1 inhibitor were approximately 70 percent of normal, which is the lower limit of normal values, and fell to about 48 percent of normal — a value still significantly above the base-line level — by 72 hours.

Plasma C4 is extremely sensitive to the protective effects of C1 inhibitor, and levels are often very low in patients with hereditary angioedema, especially during attacks. After the administration of C1 inhibitor concentrate, the functional levels of C4 rose well into the normal range. In contrast to the sharp rise and fall seen with C1 inhibitor, levels of C4 increased more slowly, as reported elsewhere.<sup>18</sup>

Some of the patients in the study were suspected of feigning symptoms in an effort to be given narcotics, a factor that sometimes made clinical assessments difficult. Nevertheless, objective determinations of symptoms during each phase of the study demonstrated the effectiveness of the C1 inhibitor concentrate, even in patients who had no response to any other therapy. This underscores the critical importance of double-blind studies in attempting to evaluate the clinical response of patients with hereditary angioedema.

On the basis of the clinical benefit seen in these patients, there may be a role for short-term or long-term prophylactic use of C1 inhibitor concentrates in some children and pregnant women with particularly severe hereditary angioedema. The availability of a C1 inhibitor concentrate to reverse the pain of abdominal attacks quickly may, in fact, be an important adjunct in reducing the narcotic dependence of some patients with hereditary angioedema. Of much greater importance, however, is the use of this concentrate as a safe and effective treatment for acute attacks of hereditary angio-

edema, particularly those involving the larynx and abdomen. In the treatment study, the submucosal swelling in these two areas responded better to infusions of C1 inhibitor concentrates than did subcutaneous edema of the extremities, as has been noted previously.<sup>17</sup> A clinical response was noted within 30 minutes after most infusions of C1 inhibitor, as compared with many hours after the infusions of placebo.

All patients with hereditary angioedema are at increased risk for attacks of laryngeal edema during and after procedures involving manipulation of the oral cavity and upper airway. Of particular concern are dental surgery, including tooth extractions, and surgical procedures requiring general anesthesia and intubation. It would be reasonable to consider the use of C1 inhibitor concentrate for short-term prophylaxis in these circumstances. The mean plasma levels of C1 inhibitor protein more than tripled, from 26 percent to 85 percent of normal levels, after the initial prophylactic infusions of the C1 inhibitor concentrate. This response compares favorably with the 1.5-fold increase seen after a series of seven prophylactic infusions of fresh-frozen plasma, which prevented complications of hereditary angioedema resulting from dental procedures.<sup>26</sup>

Eighty-five blinded and 21 compassionate infusions of C1 inhibitor, delivering a total of more than 200,000 plasma units, were administered over the course of this study. All were tolerated well, with no evidence of serious immediate or short-term adverse effects. The patients were monitored for up to four years for HIV and hepatitis B and C seroconversion. All remained seronegative. Serum from all patients in the prophylaxis study and 13 patients in the treatment study was screened for evidence of autoantibodies to C1 inhibitor that could have resulted from exposure to the concentrates. All samples were negative.

This study demonstrates the safety and efficacy of this viral-inactivated C1 inhibitor concentrate in the treatment and prophylaxis of hereditary angioedema.

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